

Annenberg/CPB Course Guide

Rediscovering Biology:

Molecular to Global Perspectives

A 13-part multi-media course for in-service high school biology teachers

Produced by Oregon Public Broadcasting

Rediscovering Biology: Molecular to Global Perspectives

is produced by
Oregon Public Broadcasting

© 2004 The Annenberg Foundation
All rights reserved.
ISBN: 1-57680-748-7

Funding for
Rediscovering Biology: Molecular to Global Perspectives
is provided by Annenberg/CPB.

Annenberg/CPB, a unit of The Annenberg Foundation, uses media and telecommunications to advance excellent teaching in American schools. Annenberg/CPB funds educational series and teacher professional development workshops for the Annenberg/CPB Channel. The Channel is distributed free by satellite to schools and other educational and community organizations nationwide, and streamed via broadband on the Web.

The notable series, workshops, and activities of Annenberg/CPB include *Cycles of Life: Exploring Biology, Earth Revealed, The Mechanical Universe, Reactions in Chemistry, Teaching High School Science, and The World of Chemistry*.

To purchase copies of our videos and guides, or to learn more about our other professional development materials and the Annenberg/CPB Channel, contact us by phone, by mail, or on the Web.



1-800-LEARNER

P.O. Box 2345
S. Burlington, VT 05407-2345

info@learner.org

www.learner.org

Table of Contents

Introduction	1
About the Course	1
Course Components	3
Helpful Hints for Facilitators	5
Materials Needed	5
About the Contributors	6
Unit 1. Genomics	9
Unit 2. Proteins and Proteomics	13
Unit 3. Evolution and Phylogenetics	19
Unit 4. Microbial Diversity	23
Unit 5. Emerging Infectious Diseases	27
Unit 6. HIV and AIDS	33
Unit 7. Genetics of Development	39
Unit 8. Cell Biology and Cancer	43
Unit 9. Human Evolution	47
Unit 10. Neurobiology	51
Unit 11. Biology of Sex and Gender	55
Unit 12. Biodiversity	61
Unit 13. Genetically Modified Organisms	65
Appendix	69
Activities, Handouts, Solutions, and Tips	71
Credits	341

About the Course

Overview

Designed as a professional development content series for in-service high school biology teachers, ***Rediscovering Biology*** is a set of graduate-level course materials covering new developments in the field of research biology.

The goal of *Rediscovering Biology* is to provide the biology content that teachers need to put emerging biological discoveries in context. This 13-part integrated media learning resource uses video, Web, and print to deliver content and activities sufficient to support a three-credit graduate-level course. The materials may also be used in in-service workshops or study groups.

The materials were designed to be used in various ways. Some individuals may want to learn about a single topic and study parts of one unit on their own. Some may join in small facilitator-led groups, such as professional development in-service sessions, to go over one or a group of related units. Others may choose to complete the entire course. For the latter group, graduate credit may be earned through Colorado State University. For information on earning credit, go to http://www.learner.org/channel/workshops/graduate_credit.html. See Course Components for more information about using the materials.

Learning Objectives

With *Rediscovering Biology*, teachers will attain the in-depth knowledge required to put concepts of high school biology—particularly new advances in the field—into context for themselves and their students.

Teachers will:

1. deepen their understanding of current advances in life science in a way that emulates how new knowledge is actually gained by researchers: through inquiry, questioning, investigating, analysis, and exposure to new ideas;
2. experience rich, interactive learning about the latest technological advances in the life sciences; and
3. enrich their understanding of the bioethical issues arising from advances in technology and research areas.

Unit Descriptions

Unit 1. Genomics

Having determined the complete DNA nucleotide sequence of humans and several other organisms, today's research has shifted to identifying genes and determining their function. This unit reviews the techniques used in BLAST searches, microarray experiments, and other genomics tools.

Unit 2. Proteins and Proteomics

Researchers know it is the proteins made by a cell that determine what that cell does. In this unit we explore the varying complements of proteins, their effects, structures, and interactions within the mechanism of cell function and introduce the larger picture of proteomics and systems biology.

Unit 3. Evolution and Phylogenetics

The ability to compare DNA sequences from different organisms is refining our perspective on evolution. This unit illustrates how molecular techniques are now combined with fossil evidence to explore relationships in organisms from whales to anthrax.

About the Course, cont'd.

Unit 4. Microbial Diversity

Microbial diversity far surpasses all other diversity on the planet. This unit examines recent studies of microbes including extremophiles, the comparisons of Bacteria and Archaea, and the formation and life cycle of biofilms.

Unit 5. Emerging Infectious Diseases

New diseases arise and old diseases, such as malaria and influenza, are returning with renewed vigor. In this unit we study the complex causes and far-reaching impacts of emerging infectious diseases around the globe.

Unit 6. HIV and AIDS

Studying natural resistance to HIV has led to insights into the infection process and may produce new treatments or a vaccine. This unit explores recent developments in the study of HIV and AIDS, the future global impact of the current infection levels, and the ethical issues surrounding current research and treatments.

Unit 7. Genetics of Development

Organisms as different as flies, fish, and humans share a set of genes, known as a genetic toolkit, which guides development. This unit explores new understanding of the remarkable similarity in these molecules and processes, and the ethical questions involved in this research.

Unit 8. Cell Biology and Cancer

Cancers result when genes required for normal cell function are mutated and the resulting cells undergo other changes ultimately leading to uncontrolled division. This program reveals new information on normal cell function, proto-oncogenes and tumor suppressor genes and their role in the cell cycle, and current research in drug design for specific cancers.

Unit 9. Human Evolution

Homo sapiens is now the only living representative of what was once a multi-branched bush of hominid species. In this unit we examine mitochondrial Eve and other fossil clues that increasingly point to Africa as the point of origin of our species. How did humans replace their hominid cousins, including Neanderthal, leaving the chimpanzee as our closest living relative?

Unit 10. Neurobiology

Neurons' electrical activity results in the release of neurotransmitters that account for everything from survival, to addiction, to learning and memory. This unit explains how neurons communicate to achieve all these functions.

Unit 11. Biology of Sex and Gender

Several genes help determine what makes a human embryo develop female or male sexual anatomies. This unit examines recent findings that have challenged previous beliefs about the roles of anatomy, environment, and genetics in the determination of gender, and the evolution of sexual determination.

Unit 12. Biodiversity

With current extinction rates exceeding those of previous mass extinctions, many biodiversity studies focus on efforts to count the Earth's species before they are lost. We explore in this unit current field experiments studying complex ecosystems, and how environmental and biodiversity changes might affect their functions.

About the Course, cont'd.

Unit 13. Genetically Modified Organisms

While genetic modification of organisms has occurred for millennia, we now have the tools to insert specific genes from one organism into cells of unrelated species. This unit illustrates the processes used, and how such genetically transformed organisms are increasingly common in agriculture, industry, and medicine, and introduces the ethical considerations of GMO research.

Course Components

Rediscovering Biology is a multi-media project. Each of the 13 units comprises a half-hour video, an online text chapter, and a chapter of this course guide that includes a set of learning activities.

For study of any of the content areas, you may view the videos or read the online text chapters independently, or you may choose to use both. For facilitated workshops or graduate-level courses for credit, you should use the videos, online text, and this course guide. The videos and online text cover content, and the course guide provides learning activities and a suggested structure for putting it all together.

The Videos

View videos of *Rediscovering Biology* on the Annenberg/CPB Channel or on-demand at our Web site. You can also order videocassettes from our Web site or by calling 1-800-LEARNER. For a broadcast schedule, video on-demand, or to order videocassettes online, go to www.learner.org.

Each video includes interviews with two or more expert scientists. Through these interviews, you will get a sense of how and why these scientists do their research, and will have a look at some of the equipment and techniques they use. In choosing experts to interview, we looked for those who are nationally and internationally recognized, regardless of their gender or ethnicity. Should you wish to know more about the work of a particular researcher featured in the videos, the full transcripts from the interviews with these experts are available on the Web site.

The Online Text

The online text chapters are not simply a repeat of what is in the video. Rather, they show how information from the video fits into the larger field. In other words, they provide context for the focused examples presented in the video. One central theme present in nearly all of the chapters of the online text is the role that genetics and genomic studies have had in increasing our understanding of the various fields of biology. Go to the course Web site at www.learner.org/channel/courses/biology for access to the online text and other course resources.

Each chapter was written by one of three authors, selected for his or her knowledge of biology and ability to write clearly about that knowledge. All of these authors have taught at the college level. The chapters vary somewhat in style and level of difficulty; these differences result both from the nature of the material itself and from differences among writers.

Course Components, cont'd.

Course Guide With Learning Activities

Several learning activities have been tailored to the information in each unit. These activities include simple review and discussion questions; exercises that demonstrate how data are generated, interpreted, and applied; explorations of ethical issues; and consideration of how the information relates to other fields. Most of the activities assume the participants are familiar with the unit's video and online text. This course guide includes a menu of the unit activities, helpful hints for the facilitator, the approximate length of time needed for each activity, and materials needed to complete the activities.

The Web Site

Go to the course Web site at www.learner.org/channel/courses/biology for access to the online text, a pdf version of this guide and all the activity materials, and additional resources, including:

- a glossary that serves as a navigational tool to other parts of the project
- interactive case studies
- transcripts from expert scientist interviews
- animations from the videos and case studies
- still images from the videos and online text

Channel-Talk

Channel-Talkbio is the email discussion list for *Rediscovering Biology: Molecular to Global Perspectives*. Share information and pose questions about the course and get to know your colleagues.

To subscribe to Channel-Talkbio, visit:

<http://www.learner.org/mailman/listinfo/channel-talkbio>

Helpful Hints for Facilitators

Successful Course Sessions

These guidelines will help you conduct successful course sessions.

Designate Responsibilities

Each week, someone should be responsible for facilitating the course sessions. This may be a professional facilitator or a volunteer from among the participants, or you may choose to divide and rotate duties among several participants.

Prepare for the Session and Bring the Necessary Materials

For each unit, the facilitator should review the menu of activities in this guide and select which activities to conduct. The facilitator is responsible for preparing and bringing enough materials for the participants to conduct the activities. The facilitator should also tell participants about any assignments that should be completed prior to arriving (for example, reading the appropriate online text chapter; send participants to the course Web site to download the online text chapters). If you will be viewing the video programs on videocassette, you may want to preview the programs.

Keep an Eye on the Time

We have suggested the amount of time you should spend on each question or activity. While these times are merely guidelines, you should keep an eye on the clock, particularly if you are watching a live broadcast. You may want to set a timer to ensure that you won't miss the beginning of the video. If you are watching the programs on videotape, you will have more flexibility if your discussions run longer.

Record Your Discussions

We recommend that someone take notes during each discussion, or even better, that you tape-record the discussions. The notes or audiotapes can serve as make-up materials in case anyone misses a session.

Share Your Discussions on the Web

The course sessions serve as a starting point to share and think about ideas. Encourage participants to continue their discussions with participants from other sites on Channel-Talk at the course Web site at www.learner.org/channel/courses/biology.

Materials Needed

Activity Materials

Each unit in this course guide contains a list of the materials needed for each activity. Consult the list prior to each session, and gather enough materials for the participants. Most of the written materials are provided in the Appendix of this guide.

About the Contributors

Advisors

In addition to determining the content of the units, our advisors and consultants have been actively involved in reviewing the material for all 13 units throughout the development of the course. Videos, animations, case studies, and text chapters have all been reviewed several times during their production for accuracy and to ensure that these materials are as useful as possible to the intended audience.

Our primary advisors and consultants consisted of a team of eight scientists involved in teaching, curriculum development, and research at the university and secondary levels.

Mark Bloom, Ph.D., is a science educator at Biological Sciences Curriculum Study (BSCS). He has developed print and Web-based curriculum materials for students in middle school, high school, and college. Previously, he was the assistant director of the Dolan DNA Learning Center, where he ran workshop programs for high school and college teachers. He developed the first educational kits using the polymerase chain reaction and co-authored the college lab manual *Laboratory DNA Science*. Mark was lead advisor for the Genomics, Proteins and Proteomics, Cell Biology and Cancer, and Biology of Sex and Gender units.

Steve Boyarsky is the coordinator of curriculum improvement at Staff Development at Southern Oregon Education Service District. Steve coordinates professional development in a three-county region in southern Oregon. He taught high school biology and human anatomy/physiology for 18 years. Steve has been involved with state and national level biology education through the National Science Teachers Association, a congressional fellowship, grants, and curriculum projects. Steve commented on appropriateness of content, level, and style of all project components.

Alan Dickman, Ph.D., is the biology curriculum director and an associate professor of biology at the University of Oregon. He has organized summer outreach programs in science for middle school, high school, and community college teachers, and has been involved in nationally funded programs to improve college-level biology education. Alan teaches introductory biology courses and an upper-division forest biology course. As lead scholar, Alan was responsible for final scholarly quality of all content of all project components.

Marion Field Fass, Sc.D., is an associate professor of biology at Beloit College. She has been involved in curriculum reform efforts in biology through the BioQUEST Curriculum Consortium and the SENCER (Science Education for New Civic Engagements and Responsibilities) project of AAC&U. In 2002 she traveled to Kenya and Tanzania to work with professors who were developing undergraduate courses about the epidemic of HIV/AIDS and about its impact in their communities. Marion was lead advisor for the Microbial Diversity, Emerging Infectious Diseases, HIV and AIDS, and Genetically Modified Organisms units.

Paula Henderson has taught biology at Newark High School in Newark, Delaware since 1980, and received the Outstanding Biology Teacher award for Delaware in 1993. She has taught a course in human heredity and development at the University of Delaware, and is a co-author of the NIH/BSCS module "The Brain: Understanding Neurobiology Through the Study of Addiction." Paula commented on appropriateness of content, level, and style of all project components.

Patrick Phillips, Ph.D., is an associate professor of biology and a member of the Center for Ecology and Evolutionary Biology at the University of Oregon. His research focuses on theoretical and empirical studies of evolutionary genetics. He teaches foundations of biology, evolution, population genetics, and experimental design; and is the creator of the evolutionary biology Web site, EvoNet.org. Patrick was lead advisor for the Evolution and Phylogenetics, Genetics of Development, Human Evolution, Neurobiology, and Biodiversity units.

John Postlethwait, Ph.D., is a professor of biology in the Institute of Neuroscience at the University of Oregon. His research interest is in developmental genetics; he and his group have discovered a genome duplication event that occurred before the vast radiation of teleost fish, which account for half of all species of vertebrates. His lab is currently investigating the genetic mechanisms that may help account for that explosion of biodiversity. The author of two non-majors textbooks for college students, John is committed to undergraduate education and has taught introductory biology to mostly non-biology majors since 1964. John provided critical assistance for the Genetics of Development unit and parts of several other units.

About the Contributors, cont'd.

Carol Wheeler is a biology teacher and department chair at Pine Creek High School in Colorado Springs, Colorado. She worked in medical research and was a certified histocompatibility technologist prior to teaching. She received a Christa McAuliffe grant to develop a molecular biology course, and an Intel grant designed to help get students eligible to compete in science fairs at the international level. Carol commented on appropriateness of content, level, and style of all project components.

Online Text Authors

Amy Does, Ph.D., is a microbiology instructor at Portland Community College in Portland, Oregon. In addition to teaching prenursing students, she provides professional development for elementary school teachers who conduct after-school science clubs. She has developed exhibits for a science museum, designed science software for middle school students, and taught college-level biology online. Amy is the author of the Microbial Diversity, Emerging Infectious Diseases, HIV and AIDS, and Genetically Modified Organisms chapters.

Norman A. Johnson, Ph.D., is an adjunct research assistant professor at the University of Massachusetts at Amherst. His research has focused on speciation and several other areas of evolutionary genetics. In addition to the University of Massachusetts, Norman has also taught at the University of Chicago and the University of Texas at Arlington. Norman served as the style editor for all thirteen chapters, and is the author of the Evolution and Phylogenetics, Genetics of Development, Human Evolution, Neurobiology, and Biodiversity chapters.

Teresa Thiel, Ph.D., is a professor of biology at the University of Missouri-St. Louis. Her main interests are molecular biology, microbiology, and bioinformatics. She directs a program for high school teachers and students called "Science in the Real World: Microbes in Action" that includes a Web site of the same name. She teaches microbiology and microbial genetics to undergraduate and graduate students, and offers summer workshops in microbiology for teachers. Teresa is the author of the Genomics, Proteins and Proteomics, Cell Biology and Cancer, and Biology of Sex and Gender chapters.

Activity Writers

Chris Tachibana, Ph.D., has taught undergraduate biology since 1992 at Salt Lake Community College, Penn State University, and the University of Washington. She is a research scientist at the University of Washington Biochemistry Department and the Carlsberg Research Labs in Denmark. Chris developed two case studies: The Genetics of Resistance to HIV and Designing an Anti-Cancer Drug. She also authored the learning activities for the Genomics, Proteins and Proteomics, Emerging Infectious Diseases, HIV and AIDS, Cell Biology and Cancer, Biology of Sex and Gender, and Genetically Modified Organisms units. In addition, she produced the learning activity course guides for all 13 units, and gave the learning activities for all units a common voice.

Andrea (Andi) White, Ph.D., is a postdoctoral research associate at the University of California, Berkeley. As a graduate student at the University of New Hampshire, she was a teaching assistant for marine ecology, honors biology, economic botany, and a lab coordinator for plant biology. Her current research interests focus on algal stress physiology and biochemistry, and the generation of environmentally friendly, alternative fuel sources from green algae. Andi developed two case studies: Evolution of Tungara Frog Mating Calls and Plant Genetic Modification. She also authored learning activities for the Evolution and Phylogenetics, Microbial Diversity, Genetics of Development, Human Evolution, Neurobiology, and Biodiversity units.

Norman A. Johnson, Ph.D., (see biography under online author) also contributed to the learning activities for the Evolution and Phylogenetics, Microbial Diversity, Genetics of Development, Human Evolution, Neurobiology, and Biodiversity units.

About the Contributors, cont'd.

Research Staff

Rediscovering Biology would not be possible without the hard work of the research and production staff at Oregon Public Broadcasting. The research staff provided critical support for video producers, authors, and activity developers.

Cindy Lefton has a bachelor's degree in zoology and a master's degree in mass communication with an emphasis on science writing and editing. She has served as the editor of a medical news magazine, and has edited several medical textbooks and journal articles. Her interests in science and nature have lead to volunteer service as an education coordinator for a wildlife rehabilitation facility, a zoo guide, and a science fair coordinator.

Liza Nicoll earned a bachelor's degree in biology and a bachelor's degree in health science at the University of Massachusetts at Amherst in the spring of 2001. Since completing work on *Rediscovering Biology* she has continued to work in television production, researching for a world history educational series.

Stephani Sutherland earned a doctorate in neuroscience from the Vollum Institute at Oregon Health & Science University, where she coordinated an outreach program in public junior high and high schools called Kids Interested in Discovering Science (KIDS). Since leaving the research laboratory in 2001, she has worked as a science news reporter for the *Los Angeles Times* and traveled around the world. She now works for the *Journal of Neuroscience* and writes freelance science news for various journals. Stephani was also a co-author for the Neurobiology chapter of the online text.

Unit 1

Genomics

Description

Having determined the complete DNA nucleotide sequence of several organisms, including humans, attention shifts to identifying genes within those sequences and determining their function. Tools, including BLAST searches and microarray experiments, coupled with computers that can handle the tremendous amount of data generated, allow researchers to examine thousands of genes at a time, and gain insight into how organisms normally function and how diseases might be treated.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Before and After (20 minutes + 30 minutes of video)

A list of terms to consider and discuss before and after viewing the video.

Activity 2: Making a Microarray (40 minutes)

A diagram to fill in to predict the results of a microarray. The microarray measures changes in gene expression in yeast growing aerobically and anaerobically.

Activity 3: “CSI, Crime Scene Investigation” (45 minutes)

DNA fingerprints are used to come up with possible solutions to a mystery.

Activity 4: Quick Discussion (15 minutes)

A discussion on the application of genomic information to predicting and diagnosing medical conditions.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Before and After requires:

- One copy of the List of Terms and Topics per two people (master copy provided)
- Tips and Suggested Answers

Activity 2: Making a Microarray requires:

- One copy of the Microarray Grid Diagram per two people (master copy provided)
- One copy of the Key to the Genes on the Microarray per two people (master copy provided)
- Transparency of the Microarray Grid Diagram (or a sketch of the grid on a blackboard)
- Transparency of Aerobic and Anaerobic Pathways in Yeast (master copy provided)
- Transparency of Figure 5 from the Genomics online text chapter (master copy provided)
- One copy of the Genomics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Optional: red, green, and yellow pens *or* red, green, and yellow sticky dots (one set per two people)
- Tips and Suggested Answers

Activity 3: “CSI, Crime Scene Investigation” requires:

- One copy of the Mystery Story per person (master copy provided)
- One Figure of DNA Fingerprints From the Inhabitants per person (master copy provided)
- One set of the Figures of DNA Fingerprint Evidence (master copy provided) **Special Instructions:** After making a copy, cut along the dotted lines into individual samples.
- Transparency of the Map of the Room (master copy provided)
- Tips and Suggested Answers

Activity 4: Quick Discussion requires:

- One copy of the Discussion Questions per two people
- Tips and Suggested Answers

Facilitator: Make sure that the room has these supplies:

- | | |
|----------------------------------|-------------------------------------------|
| • pens or pencils and paper | • VCR and TV |
| • overhead projector and markers | • black/white board with chalk or markers |

Session Activities and Video

Activity 1a: Before and After—Pre-Video Discussion (10 minutes)

- Arrange into pairs.
- Read the Setup and have each pair take a List of Terms and Topics.
- Spend 10 minutes discussing and writing thoughts and questions about the terms.
- Emphasize that these are “off the top of your head” answers that will be updated after watching the video.

Video (30 minutes)

- Watch the Genomics video.

Activity 1b: Before and After—Post-Video Discussion (10 minutes)

- Go over the list of terms again as a group. For each term come up with a brief definition or description.
- Example definitions are in the Tips and Suggested Answers.
- Variation: For each term, ask for volunteers to say what they wrote down before the video and what they wrote down afterwards, and if they still have questions about the topic. Ask if anyone has anything to add.
- Variation: Have each person write one question they still have about the topic. As a class, go over the questions and see if the entire group can come up with an answer.

Activity 2: Making a Microarray (40 minutes)

- Arrange into pairs and read the Setup.
- Have each pair take one Microarray Grid Diagram and one Key to the Genes on the Microarray, and a set of colored pens/pencils or dots (if used).
- Put the Aerobic and Anaerobic Pathways in Yeast diagram on the overhead projector.
- Spend 20–25 minutes reading the background and then coloring or marking each dot on the microarray grid as red, green, or yellow.
- Have the Genomics online text chapter available as a reference for microarrays.
- Hints for getting started, if necessary:
 - One way to begin is to decide if expression for each gene would be higher, lower, or the same in anaerobic vs. aerobic conditions; then go through and decide what color its spot would be in the microarray.
 - For genes that are not involved in anaerobic or aerobic growth, decide if their expression would change or not in anaerobic vs. aerobic conditions and then color them accordingly.

(continued, next page)

Session Activities and Video, cont'd.

(Activity 2, continued)

- When everyone is finished, put a transparency of the grid on the overhead and go over it as a group, discussing any spots for which there is no consensus.
- Look in the Tips and Suggested Answers section for an explanation of each spot.
- As a group, discuss the Post-Activity Discussion question.

Activity 3: “CSI, Crime Scene Investigation” (45 minutes)

- Read the Setup.
- Have each person take a copy of the Mystery Story and a Figure of DNA Fingerprints From the Inhabitants.
- Pass around the individual Figures of DNA Fingerprint Evidence until every person has at least one and they are all distributed.
- Put the Map of the Room on the overhead projector.
- Spend 10 minutes individually determining who contributed the pieces of evidence and where the evidence was found in the room.
- As a group, go over the map of the room, marking where the inhabitants were, according to the evidence that was collected.
- Discuss scenarios that explain the evidence.
 - Hint: DNA fingerprints can be used for more than just matching evidence samples with suspects and victims.
- See the Tips and Suggested Answers for possible explanations.

Activity 4: Quick Discussion (15 minutes)

- Arrange into pairs and read the Setup.
- Have each pair take a copy of the Discussion Questions.
- Spend 10–15 minutes discussing the questions, with an overall group discussion if time permits.
- See the Tips and Suggested Answers.

Summary (5 minutes)

- If time permits, as a group or in pairs define the major ideas or “take home” lessons of this unit and its applications.

Unit 2

Proteins and Proteomics

Description

It is the proteins made by a cell that determine what that cell does. The complement of proteins in a cell varies not only from organism to organism, but also within an organism depending upon the type of tissue, the age of the organism, and the environment. Understanding protein structure and knowing how proteins interact with one another is crucial to understanding the mechanisms of normal cell function.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Word Series (20 minutes + 30 minutes of video)

Terms used in the field of proteomics to think about before, and define after, watching the video.

Activity 2: Form and Function (30 minutes)

Pairs examine figures that show different ways of representing proteins and look for specific protein structure features.

Activity 3: 2D (15 minutes)

Shows 2D gel data and explains how it can detect protein modification in response to cellular changes.

Choose either Activity 4 or Activity 5:

Activity 4: Tool Box (45 minutes)

Defines protein investigation techniques and gives examples of how they are applied.

Activity 5: Two-Hybrid (45 minutes)

Uses diagrams that represent the components of a two-hybrid experiment to show how this technique detects interactions between proteins. Data from a large-scale two-hybrid experiment are examined.

(continued, next page)

(Menu of Unit Activities, continued)

Choose either Activity 6 or Activity 7:

Activity 6: Quick Discussion on Proteomic Profiling
(10 minutes)

Discussion on the use of proteomics in medicine.

Activity 7: Quick Discussion on *Deinococcus*
(10 minutes)

Discussion on the use of proteomics to decipher a stress-resistant bacterium.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Word Series requires:

- One copy of the Word List per person (master copy provided)
- One copy of the Proteins and Proteomics online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

Activity 2: Form and Function requires:

- Transparency of Protein Structure Terms and Discussion Questions (master copy provided)
- One set of Protein Structure Diagrams (master copy provided; to make a set, make one copy and cut along dotted lines)

Activity 3: 2D requires:

- Transparencies of 2D Gels, each on a separate transparency (master copies provided)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Before the Session, cont'd.

Choose either Activity 4 or Activity 5:

Activity 4: Tool Box requires:

- One copy of the Tool Set for every person (master copy provided)
- Transparency of Tasks and Applications (master copy provided), or can be written on board
- One copy of the Proteins and Proteomics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Activity 5: Two-Hybrid requires:

- One set of the Yeast Two-Hybrid Diagram Parts per four people (master copy provided; to make a set, cut on the dotted lines after copying)
- One copy of the Yeast Two-Hybrid Diagram Instructions per four people (master copy provided)
- Transparency of Prey Data (master copy provided)
- One copy of the Discussion Questions per person (master copy provided)
- One copy of the Proteins and Proteomics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Choose either Activity 6 or Activity 7:

Activity 6: Quick Discussion on Proteomic Profiling requires:

- One copy of the Discussion Questions per person (master copy provided)

Activity 7: Quick Discussion on *Deinococcus* requires:

- Transparency of the Discussion Questions (master copy provided)
- Tips and Suggested Answers

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- VCR and TV
- overhead projector and markers
- black/white board with chalk or markers

Session Activities and Video

Activity 1a: Word Series—Pre-Video Discussion (10 minutes)

- Read the Setup and arrange into pairs.
- Have each person take a copy of the Word List.
- Spend 10 minutes discussing the words. Pairs should work at their own pace, but don't use more than 10 minutes. The emphasis is on starting to think about the unit topic.

Video (30 minutes)

- Watch the Proteins and Proteomics video.

Activity 1b: Word Series—Post-Video Discussion (10 minutes)

- Spend a few minutes as a group discussing the significance of each term to the field of proteomics. Have one person look up terms that are still unclear in the Proteins and Proteomics online text.

Activity 2: Form and Function (30 minutes)

- Read the Setup and arrange into pairs.
- Have each pair take one of the Protein Structure Diagrams. Extra diagrams can be put into a separate pile.
- Put the transparency of Protein Structure Terms and Discussion Questions on the projector.
- Spend about 2 minutes working in pairs on each diagram, finding any of the structures listed on the transparency. When one pair is finished with a diagram, they should swap with another pair or take a new diagram from the pile of extras.
- When all pairs have seen all diagrams, discuss the questions as a group.
- Variation: Instead of working in pairs, make transparencies of the diagrams and put them on the overhead projector. Go through the diagrams and discuss the questions as a group.

Activity 3: 2D (15 minutes)

- Read the Setup.
- Put the first transparency (0 minutes) on the projector.
- Examine the data for a few seconds, then lay the second transparency (3 minutes) on the projector, lining up the corners so it is easy to see which spots moved and which did not.
- Repeat with the transparencies of the subsequent timepoints.
- Discuss the questions and mark the gel orientation on the transparencies. (See the Tips and Suggested Answers section for the correct gel orientation.)

Session Activities and Video, cont'd.

If you chose Activity 4: Tool Box (45 minutes)

- Read the Setup.
- Have each person take one copy of the Tool Set.
- As a group, come up with a brief description of each item in the Tool Set, focusing on the method of the technique rather than its function. Consult the Proteins and Proteomics online text if necessary.
- Put the transparency of Tasks and Applications on the overhead projector. Look at only one at a time.
- For each task, allow 1–2 minutes for everyone to choose a tool from their set and discuss their choice with their neighbor. Consult the Proteins and Proteomics online text if necessary.
- Then, as a group, spend 2–3 minutes discussing the various tools that could be used for that task before moving to the next task. Confirm decisions by looking in the Tips and Suggested Answers section.

If you chose Activity 5: Two-Hybrid (45 minutes)

- Read the Setup and arrange in teams of four.
- Have each team take one set of the Yeast Two-Hybrid Diagram Parts and the Yeast Two-Hybrid Diagram Instructions.
- Allow 20 minutes for Part 1, then go over the expected outcomes for each situation as a group.
- Allow 10 minutes for Part 2, then show the Prey Data transparency with the lists of prey proteins.
- As a group, discuss the questions. See the Tips and Suggested Answers section for answers to some questions.

If you chose Activity 6: Quick Discussion on Proteomic Profiling (10 minutes)

- Read the Setup and have each person take a copy of the Discussion Questions.
- Discuss the questions as a group.

If you chose Activity 7: Quick Discussion on *Deinococcus* (10 minutes)

- Read the Setup and put the transparency of the Discussion Questions on the overhead projector.
- Discuss the questions as a group. (See the Tips and Suggested Answers section for potential answers.)

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Notes

Unit 3

Evolution and Phylogenetics

Description

Molecular tools, including the ability to compare large DNA sequences from many different organisms, are refining our knowledge of evolutionary histories. Many relationships that were based on fossil evidence are being confirmed and some new relationships are being discovered. Some evolutionary tools and techniques are also useful in forensic applications.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Classifying Life (35 minutes + 30 minutes of video)

A hands-on exercise that compares the Linne, Whittaker, and Woese classification systems by classifying six different organisms.

Activity 2: Construction of a Phylogenetic Tree (60 minutes)

Morphological and molecular characteristics are used to make phylogenetic trees of swordtail fish.

Activity 3: HIV and the Dentist (25 minutes)

A case study on the use of comparative evolution to trace the source of an infectious agent.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Classifying Life requires:

- One set of the Organisms To Be Classified (master copy provided, cut along dotted lines after copying)
- One copy of the Evolution and Phylogenetics online text chapter per team (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Activity 2: Construction of a Phylogenetic Tree requires:

- One copy of the Instructions and Physical Information per person (master copy provided)
- One copy of the Genetic (rDNA) Information and Discussion Questions per person (master copy provided)
- One copy of the Evolution and Phylogenetics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Activity 3: HIV and the Dentist requires:

- One copy of the Comparative Genomics Data and Questions per person (master copy provided)
- Tips and Suggested Answers

Facilitator: Make sure that the room has these supplies:

- | | |
|----------------------------------|-------------------------------------------|
| • pens or pencils and paper | • VCR and TV |
| • overhead projector and markers | • black/white board with chalk or markers |

Session Activities and Video

Activity 1a: Classifying Life—Pre-Video Activity (30 minutes)

- Read the Setup.
- Divide the group into six teams.
- Each team should take one organism to classify and one copy of the Evolution and Phylogenetics chapter.
- Spend five minutes working in teams. Each team will determine the classification of its organism in the three different systems.
- Go through the organisms as a group. Each team should spend a few minutes stating the classification of its organism in the three different systems, with a brief explanation of each classification. Answers are in Tips and Suggested Answers.

Video (30 minutes)

- Watch the Evolution and Phylogenetics video.

Activity 1b: Classifying Life—Post-Video Discussion (5 minutes)

- Discuss the Discussion Questions as a group.

Activity 2: Construction of a Phylogenetic Tree (60 minutes)

- Read the Setup.
- Divide the group into four teams.
- Each person should take one copy of the Instructions and Physical Information, and one copy of the Genetic (rDNA) Information and Discussion Questions.
- Each team should take one copy of the Evolution and Phylogenetics chapter.
- Spend 10–15 minutes working in teams to construct a phylogenetic tree based on the physical information.
- Spend 10–15 minutes working in teams to construct a phylogenetic tree based on the genetic information, and comparing the two trees.
- As a group, compare trees made by the different teams and discuss the Discussion Questions. Answers are in Tips and Suggested Answers.

Session Activities and Video, cont'd.

Activity 3: HIV and the Dentist (25 minutes)

- Read the Setup.
- Arrange into pairs and have each person take a copy of the Comparative Genomics Data and Questions.
- Spend 10 minutes working in pairs on the Questions.
- Spend 15 minutes discussing the Applications and Ramifications as a group.
- Answers are in Tips and Suggested Answers.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 4

Microbial Diversity

Description

Microbes are adapted to live in strange and varied habits, where they use inorganic as well as organic materials for energy. Phylogenetic comparisons of DNA sequences of genes that produce ribosomes indicate that the single large group of prokaryotes comprises two very different groups: the Bacteria and the Archaea. Understanding how microbes communicate with each other and interact with their environment (as in the production of biofilms), can help us to control disease, limit microbial damage, and harness the skills of microorganisms to rehabilitate damaged ecosystems.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Indescribable (30 minutes)

Confronting the issues involved in collecting and cultivating microorganisms from extreme environments, using the bacteria that will be featured in the video as examples.

Activity 2: PCR Demonstration (60 minutes)

A hands-on demonstration of how polymerase chain reaction amplifies a specifically targeted section of DNA, with discussion questions and a reading about how this is used to investigate microbial diversity.

Activity 3: Biofilm Stars (15 minutes)

A discussion on the impact of microbial biofilms on everyday life and on medical treatments.

Activity 4: The Fall of Biosphere 2 (15 minutes)

Readings and discussion on how miscalculations about microbial metabolism affected the Biosphere experiment.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Indescribable requires:

- One copy of the List of Organisms and Instructions per person (master copy provided)
- One transparency of the Table of Terms from the Microbial Diversity online text chapter (master copy provided)
- Tips and Suggested Answers

Activity 2: PCR Demonstration requires:

- One set of PCR materials per four people. Each set contains:
 - two cardboard strips, 12 inches long and 2 inches wide, with a light-colored surface that can be written on
 - 10 cardboard strips, 2 inches long and 2 inches wide, with a light-colored surface
 - eight cardboard strips, 10 inches long and 2 inches wide, with a light-colored surface
 - 40 rubber bands that can fit around the cardboard strips
 - one roll of tape
- One black marker per person
- One copy of the Instructions per person (master copy provided)
- One copy of the Discussion Questions and Microbial Diversity Chapter Excerpt per person (master copy provided)

Activity 3: Biofilm Stars requires:

- One copy of the Microbial Diversity online text chapter per three people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Activity 4: The Fall of Biosphere 2 requires:

- One copy of the Discussion Questions per person
- One transparency of the Table of Terms from the Microbial Diversity online text chapter (master copy provided; see Activity 1)

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- VCR and TV
- overhead projector and markers
- black/white board with chalk or markers

Session Activities and Video

Activity 1: Indescribable (30 minutes)

- Read the Setup and arrange in pairs.
- Have each person take a copy of the List of Organisms and Instructions.
- Put the Table of Terms transparency on the overhead.
- Spend five minutes working in pairs on Exercise 1, using the terms in the table to describe the organisms described in the list.
- As a group, go through the list quickly, and compare answers to those found in Tips and Suggested Answers.
- Spend 10–15 minutes working in pairs on Exercise 2.

Video (30 minutes)

- Watch the Microbial Diversity video.

Activity 2: PCR Demonstration (60 minutes)

- Read the Setup and arrange into teams of four.
- Have each team take a set of PCR materials.
- Have each person take a copy of the Instructions and a copy of the Discussion Questions and Microbial Diversity Chapter Excerpt.
- Spend a few minutes in teams reading and reviewing PCR from the instructions.
- Working in teams, make the target and primer sequences, and go through the PCR demonstration according to the instructions.
- In teams, answer the Discussion Questions and read the Chapter Excerpt.

Facilitator: After all teams have finished, remind everyone that they can view the PCR animation at <http://www.learner.org/channel/courses/biology>.

Activity 3: Biofilm Stars (15 minutes)

- Read the Setup and arrange into teams of three.
- Have each person take a copy of the Discussion Questions. Have each team take a copy of the Microbial Diversity chapter.
- Spend 10–15 minutes working in teams of three on the questions.
- As a group, quickly compare lists and answers with each other, and with the answers in Tips and Suggested Answers.

Session Activities and Video, cont'd.

Activity 4: The Fall of Biosphere 2 (15 minutes)

- Put the Table of Terms transparency on the overhead.
- Read the Setup and have each person take a copy of the Discussion Questions.
- Discuss the questions as a group.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 5

Emerging Infectious Diseases

Description

New diseases arise and old diseases, such as malaria and influenza, return with renewed vigor. Among the causes of emerging infectious diseases are increased travel by humans; misuse of antibiotics; and habitat changes, including those brought on by global climate change, which expand the ranges of some disease vectors.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Emerging Diseases: Causes and Effects (15 minutes + 30 minutes of video)

A discussion about what causes a disease to be emerging or re-emerging, and what diseases fall into these categories.

Activity 2: Lifecycles of the Infectious and Famous (30 minutes)

Groups make diagrams of the infection cycles of four diseases using pre-copied diagram pieces. The diagrams are used to discuss strategies to treat and contain the diseases.

Activity 3: Koch's Postulates (30 minutes)

Application of the criteria used to establish that an infectious agent is the cause of a disease, using SCV (SARS-associated coronavirus) and SARS as an example.

Activity 4: Shifting Antigens (30 minutes)

The ability of influenza to evade the immune system is analyzed by piecing together clues about the components of the virus and the vaccine against the virus.

(continued, next page)

(Menu of Unit Activities, continued)

Choose either Activity 5 or Activity 6:

Activity 5: Wrapping It Up (15 minutes)

A discussion that includes a review of the lists of diseases and causes that were generated in Activity 1.

Activity 6: A Picture's Worth a Thousand Words (15 minutes)

A game in which participants get their partners to say the names of emerging diseases by drawing clues, such as the type of infectious agent or the means of transmission.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Emerging Diseases: Causes and Effects requires:

- One copy of the Instructions per person (master copy provided)
- Tips and Suggested Answers

Activity 2: Lifecycles of the Infectious and Famous requires:

- One set of Replication Cycle Steps and Arrows per two people (master copy provided; to make a set, cut out the arrows to separate them, cut on the dotted lines of the lifecycle steps and scramble them)
- One set of Discussion Questions per person (master copy provided)
- One copy of the Emerging Infectious Diseases online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Activity 3: Koch's Postulates requires:

- One copy of the Instructions and Discussion Questions per person (master copy provided)
- One transparency of Koch's and Rivers's Postulates (master copy provided)
- One transparency of Experimental Results for SARS and SCV (master copy provided)
- Tips and Suggested Answers

Activity 4: Shifting Antigens requires:

- One set of Clues to distribute to the group (master copy provided; after copying, cut to separate the clues)
- One transparency of the List of Questions to start the discussion of how influenza pandemics occur (master copy provided)

Before the Session, cont'd.

Choose either Activity 5 or Activity 6:

Activity 5: Wrapping It Up requires:

- The lists made by the group in Activity 1 (List 1: Diseases that might be considered emerging or re-emerging infectious diseases; List 2: Factors that affect the emergence or re-emergence of a disease)
- One transparency of the Factors That Affect the Emergence of Disease (master copy provided)
- One copy of the Discussion Questions per person (master copy provided)

Activity 6: A Picture's Worth a Thousand Words requires:

- Clock with second hand *or* stopwatch
- One set of the List of Items to Draw (master copy provided; to make a set, cut on the dotted lines after copying)

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- overhead projector and markers
- VCR and TV
- black/white board with chalk or markers

Session Activities and Video

Activity 1a: Emerging Diseases: Causes and Effects— Pre-Video Discussion (10 minutes)

- Read the Setup.
- Spend 1–2 minutes as a group making a list of diseases that might be emerging or re-emerging.

Facilitator: Emphasize that this is a brainstorming session. Write down all ideas.

- Spend about 3 minutes as a group reviewing the diseases in the list, eliminating any that all agree are not really emerging or re-emerging diseases and putting question marks next to ones that are in dispute.
- Spend 1–2 minutes making a list of factors or conditions that contribute to the emergence and re-emergence of diseases.

Facilitator: This is another brainstorming session. Write down all ideas.

- Spend about 3 minutes discussing how the list of diseases and factors match.
- Consult the Tips and Suggested Answers section to see if there are other answers your group did not think of, or if your group thought of additional possibilities.

Facilitator: If you chose Activity 5: Wrapping It Up, *keep a copy of the group's lists of diseases and factors.* Activity 5 uses these lists, so don't throw them away!

Video (30 minutes)

- Watch the Emerging Infectious Diseases video.

Activity 1b: Emerging Diseases: Causes and Effects— Post-Video Discussion (5 minutes)

- Spend a few minutes comparing the information just discussed in the video with the class lists of diseases and factors generated in Activity 1.

Facilitator: If you chose Activity 5: Wrapping It Up, a brief review is all that is needed, because Activity 5 is a longer discussion on this topic.

Activity 2: Lifecycles of the Infectious and Famous (30 minutes)

- Read the Setup.
- Arrange into pairs.

(continued, next page)

Session Activities and Video, cont'd.

(Activity 2, continued)

- Have each pair take one set of the Replication Cycle Steps and about 20 arrows, and a copy of the Emerging Infectious Diseases online text.
- Have each person take a copy of the Discussion Questions.
- Spend 10 minutes working in pairs arranging the replication steps.
- Compare the diagrams as a group. See the Tips and Suggested Answers section for the diagram answers.
- Discuss the Discussion Questions as a group, referring to the diagrams made from the scrambled replication cycle steps. Answers are in the Tips and Suggested Answers section.
- Variation: Divide into four groups. Each group is assigned one of the diseases and gives a brief, informal presentation that answers the discussion questions for that disease.

Activity 3: Koch's Postulates (30 minutes)

- Read the Setup and have each person take a copy of the Instructions and Discussion Questions.
- Spend 5 minutes working individually, writing down criteria for assigning a disease to a virus.
- Working as a group, compile from the individual lists a collective list of the criteria for assigning a disease to a virus.
- View the transparency of Koch's and Rivers's Postulates and compare it to the group list.
- View the transparency of Experimental Results for SARS and SCV that fulfilled Koch's and Rivers's postulates, and assign each result to a postulate.
- Go over the Discussion Questions as a group. Potential answers are in the Tips and Suggested Answers section.

Activity 4: Shifting Antigens (30 minutes)

- Read the Setup.
- Pass around the clues, each person taking one at a time until they are all distributed.
- One at a time, view the questions on the List of Questions transparency.
- After reading each question, if anyone has a clue that relates to the question, have that person read their clue. After all relevant clues have been read, come up with a group answer to the question.

If you chose Activity 5: Wrapping It Up (15 minutes)

- Read the Setup and have each person take a copy of the Discussion Questions.
- Look again at the list of diseases and factors from Activity 1.
- Discuss the provided questions while viewing the transparency of Factors That Affect the Emergence of Disease.

Session Activities and Video, cont'd.

If you chose Activity 6: A Picture's Worth a Thousand Words (15 minutes)

- Read the Setup.
- Arrange into pairs, with each pair taking pens or pencils and several pieces of paper.
- Have each team choose the person who will draw first and the person who will guess first.

Facilitator: Choose one of the items from the List of Items To Draw. Show the word to the person in each team who will be drawing. When all teams are ready, say "Go" and start the timer.

- When the first (non-drawing) member of a team names the item, all teams stop drawing. If no team names the item after 3 minutes, stop all teams and reveal the item.
- Have the teams switch drawers and guessers and play again.
- Variation: To emphasize the factual, rather than the fun aspect, add rules such as:
 - drawing must include means of transmission
 - drawing must include a picture that indicates if the cause is viral, bacterial, or eukaryotic.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or "take home" lessons of this unit and its applications.

Unit 6

HIV and AIDS

Description

Understanding how some people are able to resist HIV or how others are able to harbor the virus without developing AIDS has given us insights into the way this virus infects cells of the immune system and may lead to new kinds of treatments for AIDS or even to an HIV vaccine. But even if new treatments or vaccines are developed, this disease will continue to have a huge influence on human societies, especially in some African countries.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Choose either Activity 1 or Activity 2:

Activity 1: Multiple Choice (15 minutes + 30 minutes of video)

Ten quick questions about HIV to go over before and after the video.

Activity 2: According to WHO? (15 minutes + 30 minutes of video)

An alternate warm-up activity that is more open-ended. It focuses on the top 10 worldwide health risks.

Choose either Activity 3 or Activity 4:

Activity 3: The Mighty Immune System (45 minutes)

An overview of the non-specific and specific immune systems, achieved by making diagrams and tables of the individual components and cells, then considering how they interact to fight disease.

Activity 4: Miracle Drugs? (45 minutes)

Presentations to the group on anti-HIV drugs, guided by the online text, video interviews, a suggested outline, and provided transparencies.

Activity 5: DNA Vaccines (10 minutes)

Discussion questions that compare DNA vaccines to traditional vaccines.

(continued, next page)

(Menu of Unit Activities, continued)

Choose either Activity 6 or Activity 7:

Activity 6: Lesson Plans (20 minutes)

A discussion on how HIV and AIDS are presented to high school students now, and what new information might be incorporated into biology classes.

Activity 7: Public Opinion, Public Policy (20 minutes)

A discussion on how HIV infection is perceived and how this perception affects public policy on treatment and prevention.

Before the Session

Facilitator: Copy and assemble the required activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Choose either Activity 1 or Activity 2:

Activity 1: Multiple Choice requires:

- One copy of the Multiple Choice Questions per person (master copy provided)
- Transparency of the Multiple Choice Answers (master copy provided)

Activity 2: According to WHO? requires:

- Transparency of the list of Top 10 Risks for Health from The World Health Report 2002 (master copy provided)

Before the Session, cont'd.

Choose either Activity 3 or Activity 4:

Activity 3: The Mighty Immune System requires:

- One copy of the Activity Instructions per person (master copy provided)
- One copy of the HIV and AIDS online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of Dr. Jay Levy's interview transcript per two people (to approximate marker 11:20:55; available online at <http://www.learner.org/channel/courses/biology>)

Activity 4: Miracle Drugs? requires:

- Video available for people to review
- One copy of the HIV and AIDS online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the interview transcript with Dr. Jay Levy per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the interview transcript with Dr. David Weiner per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the Guide to the Presentations plus Discussion Questions per person (master copy provided)
- Tips and Suggested Answers
- Transparency of the HIV infection cycle (master copy provided)
- Transparencies of the HIV drugs and the molecules they resemble to use for presentations (optional, master copies provided)

Activity 5: DNA Vaccines requires:

- One copy of the Discussion Questions per person (master copy provided)

Choose either Activity 6 or Activity 7:

Activity 6: Lesson Plans requires:

- Transparency of the Activity Instructions and Discussion Questions

Activity 7: Public Policy, Public Opinion requires:

- One copy of the Discussion Questions per person (master copy provided)

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- overhead projector and markers
- VCR and TV
- black/white board with chalk or markers

Session Activities and Video

Facilitator: Follow the instructions for the activities you have chosen.

Activity 1a or 2a: Pre-Video Discussion:

If you chose Activity 1: Multiple Choice (10 minutes)

- Read the Setup and have each person take a copy of the Multiple Choice Questions.
- Let each person go through the questions at their own pace, but don't use more than 5–10 minutes. Stress that answers might vary, especially for statistics, and this is just to get people thinking.
- Variation: Watch the video first and then go through the questions briefly as a group.

If you chose Activity 2: According to WHO? (10 minutes)

- Read the Setup.
- Spend a few minutes making a group list of the Top 10 Risks for Health.
- View the transparency of the Top 10 Risks for Health from the World Health Report and discuss the discussion questions.

Video (30 minutes)

- Watch the HIV and AIDS video.

Activity 1b or 2b: Post-Video Discussion:

If you chose Activity 1: Multiple Choice (5 minutes)

- Put the Multiple Choice Answers on the overhead projector.
- As a group, go through the Multiple Choice Questions while looking at the answers from the video. See if anyone in the group has heard or read information that is different and if so, what the explanation for the discrepancy might be.

If you chose Activity 2: According to WHO? (5 minutes)

- Go over the discussion questions again and see if there are any new ideas for reducing HIV infection risk after viewing the video.

Session Activities and Video, cont'd.

If you chose Activity 3: The Mighty Immune System (45 minutes)

- Arrange into pairs.
- Read the Setup and have each person take a copy of the Activity Instructions.
- Distribute the HIV and AIDS online text chapter and Dr. Jay Levy's interview transcripts so that everyone has a copy of one or the other.
- Spend 5–10 minutes reading/skimming the parts of the text or transcript that are relevant to the specific and non-specific immune systems.
- Swap texts, so people who read the online text now have the interview transcript and vice-versa. Spend another 5–10 minutes reading/skimming the new document.
- Working in pairs, spend 5 minutes making the Non-Specific/Innate vs. Specific defense list according to the activity directions. Refer to the online text and interview transcript whenever necessary.
- Spend 5 minutes making the Specific Immune System list.
- Spend 5 minutes making the Big Picture diagram.
- Choose diseases from the supplemental discussion question list and go through the roles of the non-specific and specific defenses in fighting the disease. Refer to the Big Picture diagrams.
- Variation: Make the Non-specific/Innate vs. Specific and Specific Immune System lists in pairs, then do the Big Picture diagram and the supplemental discussion questions as a group.

If you chose Activity 4: Miracle Drugs? (45 minutes)

- Arrange into pairs.
- Read the Setup and have each person take a Guide to the Presentations plus Discussion Questions.
- Hand out the HIV and AIDS online text chapter and transcripts of Dr. Jay Levy's and Dr. David Weiner's interviews so that each pair has a copy of all three documents.
- Spend 5–10 minutes becoming familiar with the text and transcripts that are relevant to the immune system and the HIV replication cycle.
- From the list of drugs or treatments in the Guide to the Presentations, have each pair choose one or more drugs or treatments to present.
- Spend 15 minutes preparing short descriptions of the assigned drug or treatment.
- As a group, spend about 5 minutes discussing each drug or treatment, led by the pair in charge of investigating the drug. Refer to the transparencies of the HIV infection cycle and HIV drugs.
- As time permits, discuss the Discussion Questions.

Session Activities and Video, cont'd.

Activity 5: DNA Vaccines (10 minutes)

- Read the Setup and have each person take a copy of the Discussion Questions.
- Discuss the questions in pairs or as a group.

If you chose Activity 6: Lesson Plans (20 minutes)

- Arrange into pairs.
- Read the Setup and put the transparency of the Activity Instructions and Discussion Questions on the overhead projector.
- In pairs, make the two lists of five facts about HIV and AIDS.
- As a group, go through the lists generated by the pairs and discuss the Discussion Questions.

Facilitator: Be sure the discussion remains focused on pedagogical issues and does not become a forum for classroom anecdotes!

If you chose Activity 7: Public Opinion, Public Policy (20 minutes)

- If the discussion will be done in pairs, arrange these.
- Read the Setup and have each person take a copy of the Discussion Questions.
- In pairs or as a group, discuss the questions.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 7

Genetics of Development

Description

Organisms as different as flies, fish, and humans share a similar set of genes, known as a genetic toolkit, which guides development in multicellular organisms. Understanding these developmental pathways and processes not only tells us more about how multicellular organisms evolved, it also could help researchers who are attempting to make adult stem cells form new tissues.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Fact or Fiction? (15 minutes + 30 minutes of video)

Ten statements about development are evaluated for the five that are amazing-yet-true.

Activity 2: Mommie Dearest (45 minutes)

Readings and genetic problems that highlight the effects of mutations in genes that direct development.

Activity 3: Small Cells, Big Controversies (60 minutes)

Readings and discussions on the applications of embryonic stem cells and the controversies about generating them.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Fact or Fiction? requires:

- One transparency of the *Drosophila* Head Micrographs
- One copy of the Statements per person (master copy provided)
- One copy of the Genetics of Development online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Activity 2: Mommie Dearest requires:

- One copy of the Readings, Worksheet, and Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Activity 3: Small Cells, Big Controversies requires:

- One copy of the Discussion Questions per person (master copy provided)
- One copy of the Interview Transcript Excerpts per person (master copy provided)

Facilitator: Make sure that the room has these supplies:

- | | |
|----------------------------------|-------------------------------------------|
| • pens or pencils and paper | • VCR and TV |
| • overhead projector and markers | • black/white board with chalk or markers |

Session Activities and Video

Activity 1a: Fact or Fiction?—Pre-Video Exercise (10 minutes)

- Put the transparency of the *Drosophila* Head Micrographs on the overhead and read the Setup.
 - Have each person take a copy of the Statements.
 - Spend 5–10 minutes on the statements, deciding which five are true.
- Facilitator:** Emphasize that participants should try to find the five statements that are entirely true. The point of this exercise is not to get all the right answers, but to think about some amazing or amusing facts of developmental research.

Video (30 minutes)

- Watch the Genetics of Development video.

Activity 1b: Fact or Fiction?—Post-Video Discussion (5 minutes)

- As a group, review the statements, deciding which are true. Compare answers to those found in Tips and Suggested Answers.

Activity 2: Mommie Dearest (45 minutes)

- Read the Setup and arrange into pairs.
- Have each person take a copy of the Readings, Worksheet, and Discussion Questions.
- Spend 5–10 minutes on the reading.
- Spend 10–15 minutes working in pairs on the worksheet problems.
- As a group, go over the answers from the pairs, comparing them to those found in Tips and Suggested Answers.
- As a group or in pairs, talk about the Discussion Questions. Compare challenge question answers to the explanation given in Tips and Suggested Answers.

Session Activities and Video, cont'd.

Activity 3: Small Cells, Big Controversies (60 minutes)

- Read the Setup and arrange into teams of three or four.
- Have each person take a copy of the Discussion Questions and a copy of the Interview Transcript Excerpts.
- Spend five minutes reading Excerpt 1.
- As a group, discuss the corresponding discussion questions.
- Repeat for Excerpts 2 and 3, and their corresponding questions.
- As a group, discuss any questions that were especially controversial in the smaller team discussions.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 8

Cell Biology and Cancer

Description

Cancers result when genes required for normal cell function are mutated and the resulting cells undergo other changes, ultimately leading to uncontrolled growth. Two classes of these genes are proto-oncogenes and tumor suppressor genes. Knowing how these genes work in healthy individuals allows for the possibility of designing drugs that interfere with specific kinds of cancers.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: No Cut-and-Dried Answers (15 minutes + 30 minutes of video)

Ten statements about cancer to consider and discuss before and after the video.

Activity 2: The Price of Proto-Oncogenes (15 minutes)

A quick discussion about the function of proto-oncogenes to be done in the middle of viewing the video.

Choose either Activity 3 or Activity 4:

Activity 3: Family History (60 minutes)

Identification of an allele that contributes to breast cancer risk through pedigree and statistical analysis of families with a history of breast cancer.

Activity 4: Dilemmas of Cell Biology (60 minutes)

Three packets of exercises and discussions on p53 and aging; telomerase, cancer, and aging; and genetics of breast and colon cancer.

Activity 5: The Big Picture (30 minutes)

Discussion questions on the personal and societal impact of cancer and cancer prevention.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: No Cut-and-Dried Answers requires:

- One copy of the Yes/No, But... statements per person (master copy provided)
- Tips and Suggested Answers

Activity 2: The Price of Proto-Oncogenes requires:

- One transparency of the Discussion Questions

Choose either Activity 3 or Activity 4:

Activity 3: Family History requires:

- One copy of the Background Questions for each person (master copy provided)
- One copy of the Pedigrees and Data sheet for each person (master copy provided)
- One copy of the LOD Score Information for each person (master copy provided)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Activity 4: Dilemmas of Cell Biology requires:

- One copy of each of the three different Topic Packets (master copies provided)
- Three copies of the Cell Biology and Cancer unit online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

Activity 5: The Big Picture requires:

- One copy of the Discussion Questions per person (master copy provided)

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- overhead projector and markers
- VCR and TV
- black/white board with chalk or markers

Session Activities and Video

Activity 1a: No Cut-and-Dried Answers—Pre-Video Discussion (10 minutes)

- Read the Setup and have each person take a copy of the Yes/No, But... statements.
- Let each person go through the statements at their own pace, finishing in about 5–10 minutes.

Facilitator: Stress that participants can either agree or disagree, and should write down qualifying statements if they can't decide.

- Variation: Go through each statement as a group divided into two sides. Have people from one side of the room say a few words that support the statement as true. Then, the people on the other side of the room respond by saying a few words that suggest the statement is false.

Video, Part 1 (6 minutes)

- Watch the first part of the Cell Biology and Cancer video.

Facilitator: Start timing the Cell Biology and Cancer video at the Annenberg/CPB logo and watch the first 5:54 minutes. Stop the video just after Dr. Robert Weinberg says, "Without the proto-oncogenes, embryos wouldn't be able to develop, adult tissues would not be able to be maintained. However, the price of carrying these proto-oncogenes in our genomes is occasionally they become damaged and mutated and convert into oncogenes, and thus become converted into agents for causing cancer."

Activity 2: The Price of Proto-Oncogenes (15 minutes)

- Read the Setup.
- As a group, come up with a list of possible normal cellular functions that might be performed by proto-oncogenes.
- View the transparency of the Discussion Questions and talk about them as a group.

Video, Part 2 (24 minutes)

- Finish watching the Cell Biology and Cancer video.

Activity 1b: No Cut-and-Dried Answers—Post-Video Discussion (5 minutes)

- Spend a few minutes looking through the list of statements for the activity and see if anyone's opinions changed, or if there is new information to add to the qualifying statements.
- Compare the group's statements with those in the Tips and Suggested Answers section.

Session Activities and Video, cont'd.

If you chose Activity 3: Family History (60 minutes)

- Read the Setup and arrange into pairs.
- Have each person take a copy of the Background Questions, one copy of Pedigrees and Data, one copy of the LOD Score Information, and one copy of the Discussion Questions.
- Spend 5–10 minutes working in pairs on the background questions.
- Spend 5 minutes going over possible answers as a group, comparing answers with those in the Tips and Suggested Answers section.
- Spend about 30 minutes going through the Pedigrees and LOD Score Information.
- As a group, spend 10 minutes discussing the questions, comparing group answers to those in the Tips and Suggested Answers section.

If you chose Activity 4: Dilemmas of Cell Biology (60 minutes)

- Read the Setup and divide into three teams.
- Have each team take one of the packets.
- Spend 15–20 minutes working in teams on the packet.
- Have the teams switch packets and spend 15–20 minutes working on the new packet.
- Have the teams switch packets again and work on the final packet for 15–20 minutes.

Activity 5: The Big Picture (30 minutes)

- Read the Setup and have each person take a copy of the Discussion Questions.
- Discuss the questions in pairs or as a group.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 9

Human Evolution

Description

Modern humans and chimpanzees have both diverged from the common ancestor they shared four or five million years ago, and *Homo sapiens* are now the only living representative of what was once a multibranched bush of hominid species. One of several legacies that is revealed by studying genetics of modern humans is a remarkably small amount of genetic variation within our species; this results from one or more population bottlenecks at sometime in the past.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: What's the Relationship? (15 minutes + 30 minutes of video)

A discussion of the terms and concepts used in human evolution that is a preview to watching the video.

Activity 2: Molecular Clock (60 minutes)

A molecular clock is constructed using sequences that represent humans, baboons, and chimps. The clock is then used to calculate divergence times.

Activity 3: Genealogies (15 minutes)

Construction of genealogies to trace inheritance of mitochondrial DNA and Y chromosomes.

Activity 4: Icelandic Perspectives (25 minutes)

Small groups debate the issues surrounding the use of genealogical information for commercial and research purposes.

Activity 5: Roots (15 minutes)

A group discussion on companies and non-profit organizations that use DNA testing to determine ancestry.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: What's the Relationship? requires:

- One copy of the List of Terms per person (master copy provided)
- One copy of the Human Evolution online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

Activity 2: Molecular Clock requires:

- One piece of graph paper per person
- One copy of the Worksheet of Instructions and Sequences per person (master copy provided)
- Transparency of Molecular Clock (master copy provided)
- Tips and Suggested Answers
- One copy of the Human Evolution online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

Activity 3: Genealogies requires:

- Transparency of the Example Genealogy and Questions (master copy provided)
- Tips and Suggested Answers

Activity 4: Icelandic Perspectives requires:

- One copy of the Discussion Questions per person (master copy provided)

Activity 5: Roots requires:

- One copy of the Discussion Questions per person (master copy provided)
- Transparency of More Quotes and Perspectives (master copy provided)

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- VCR and TV
- overhead projector and markers
- black/white board with chalk or markers

Session Activities and Video

Activity 1a: What's the Relationship?—Pre-Video Discussion (10 minutes)

- Read the Setup.
- Arrange into pairs and pass out the List of Terms.
- Spend 10 minutes discussing what the terms might mean and how they relate to each other.

Facilitator: Stress that these are not necessarily terms everyone will know. The point of the exercise is to make educated guesses about the terms and to begin thinking about the topics in this unit.

Video (30 minutes)

- Watch the Human Evolution video.

Activity 1b: What's the Relationship?—Post-Video Discussion (5 minutes)

- As a group, go over the terms and concepts again. Briefly define or explain them. Any that are still uncertain can be looked up in the Human Evolution online text chapter.

Activity 2: Molecular Clock (60 minutes)

- Read the Setup.
- Arrange into pairs and have each person take a piece of graph paper and a Worksheet of Instructions and Sequences.
- Spend 10–15 minutes working on Part 1. As a group, compare answers and discuss any questions or discrepancies.
- Begin working on Part 2. When everyone has finished Exercise A, look at the transparency of the Molecular Clock as a group. Compare the clocks made by the pairs with the example on the transparency.
- When everyone has completed the entire exercise, spend a few minutes as a group discussing the results generated by each pair.
- Answers to Part 2 can be found in the Tips and Suggested Answers.

Activity 3: Genealogies (15 minutes)

- Read the Setup while looking at the transparency of the Example Genealogy.
- Each person will draw their genealogy or work with the example on the transparency.
- After a few minutes to make the genealogies, as a group, discuss the questions. Compare the group's conclusion with the one in the Tips and Suggested Answers.

Session Activities and Video, cont'd.

Activity 4: Icelandic Perspectives (25 minutes)

- Read the Setup and arrange into teams of four. Each team of four will divide into two pairs. One pair will choose to represent a company like Decode and the other pair will represent the community of citizens whose genetic information and genealogy is being researched.
- Each person should take a copy of the Discussion Questions.
- Spend 10 minutes working in pairs, with each pair deciding how the questions would be answered by the group they represent.
- Spend 10 minutes in teams of four debating the questions, with each pair presenting their perspective to the other side.
- As a group, spend a few minutes discussing the overall conclusions of the individual teams.

Activity 5: Roots (15 minutes)

- Read the Setup.
- Have each person take a copy of the Discussion Questions.
- As a group, discuss the questions. After discussing each question, put the transparency of More Quotes and Perspectives on the overhead projector for additional information.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 10

Neurobiology

Description

Neurons are wired together in a complicated network and connect to other neurons via synapses. Day-to-day changes in synapses can lead to a higher rate of response to a given level of neurotransmitter at a particular synapse; these changes are partly responsible for memory and learning. Neurotransmitters, such as dopamine, that function in the reward pathway underlie behaviors essential for survival, and also are responsible for behavioral problems associated with addictive drugs.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Connections (15 minutes + 30 minutes of video)

A discussion around a diagram of neurobiology terms that have conceptual connections.

Activity 2: Penny for Your Thoughts (10 minutes)

Recalling details of an everyday object as a way to stimulate thought on what makes something forgettable or memorable.

Activity 3: Action Potentials (60 minutes)

A demonstration of how ions change the membrane potential of neurons, using small objects to represent ions, and boxes with openings to represent neurons with ion channels.

Activity 4: Sex, Drugs, and Neurobiology (25 minutes)

Readings from the Neurobiology online text on drugs and the nervous system, with discussion questions.

Activity 5: Fountain of Youth (10 minutes)

A discussion on the development of neurons in adults and the role of environmental stimulus in neurogenesis.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Connections requires:

- One copy of the Diagram of Terms per person (master copy provided)

Activity 2: Penny for Your Thoughts requires:

- One set of the Parts of a Penny per two people (master copy provided; to make a set, cut on the dotted lines after copying)
- One penny per two people (as the answer key)

Activity 3: Action Potentials requires:

- One copy of the Instructions per four people (master copy provided)
- One copy of the Neurobiology online text chapter per four people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the Discussion Questions per person (master copy provided)
- One set of 100 small, colored objects per four people. (The objects can be balls, chocolate-covered candies, jellybeans, coins, or gumdrops. Each set should have 30 blue, 49 red, and 21 green objects.)
- One box for every four people. The boxes should be open at the top to allow visualization and manipulation of the colored objects. Make two panels in the sides of each box that can be opened and closed, and are sufficiently large that they permit the addition and removal of the objects. For Exercise 2, have at least three boxes.
- One piece of graph paper per person
- Tips and Suggested Answers

Activity 4: Sex, Drugs, and Neurobiology requires:

- One copy of the Neurobiology Online Text Chapter Excerpts per person (master copy provided)
- One copy of the Discussion Questions per person (master copy provided)

Activity 5: Fountain of Youth requires:

- One copy of the Discussion Questions per person

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- VCR and TV
- overhead projector and markers
- black/white board with chalk or markers

Session Activities and Video

Activity 1a: Connections—Pre-Video Activity (10 minutes)

- Read the Setup.
- Arrange into pairs.
- Have each person take a handout with the Diagram of Terms.
- Spend 10 minutes in pairs, making and explaining as many connections between the terms as possible.

Facilitator: Emphasize that not all the terms will be familiar. This is just a warm-up exercise to begin thinking about neurobiology.

Video, Part 1 (7 minutes)

- Watch the first part of the Neurobiology video.

Facilitator: Start timing the Neurobiology video at the Annenberg/CPB logo and watch for about 7 minutes. Stop the video just after the host says, “One area of study that forges ahead as scientists understand more about how neuronal connections change is the study of learning and memory.”

Activity 2: Penny for Your Thoughts (10 minutes)

- Read the Setup.
- Arrange into pairs.
- Have each pair take a set of the Parts of a Penny.
- Spend 10 minutes in pairs, making the diagram and discussing why the details of everyday objects are—or are not—familiar, and what makes an object or an event memorable.
- Check finished diagrams against a real penny.
- Variation: Do the activity before beginning to watch the video.

Video, Part 2 (23 minutes)

- Finish watching the Neurobiology video.

Activity 1b: Connections—Post-Video Discussion (5 minutes)

- As a group, go over the Connections Diagram of Terms. See if anyone picked up new connections to make after watching the video.

Session Activities and Video, cont'd.

Activity 3: Action Potentials (60 minutes)

- Read the Setup.
- Arrange into teams of four. Within each team, designate one person as scorekeeper, one as gatekeeper, and two as ball handlers.
- Have each team take a copy of the Instructions, the Neurobiology online text, one set of colored objects, and one box.
- Have each person take a copy of the Discussion Questions and a piece of graph paper.
- Spend 20–30 minutes in teams doing Exercise 1 and discussing the Discussion Question.
- Set up the boxes and colored objects to do Exercise 2 as a group.
- Spend 20–30 minutes doing Exercise 2 and discussing the Discussion Questions.

Activity 4: Sex, Drugs, and Neurobiology (25 minutes)

- Read the Setup.
- Arrange into teams of three.
- Have each person take a copy of the Neurobiology online text excerpt and a copy of the Discussion Questions.
- Spend 5–10 minutes reading Section 1 of the text excerpt, then discuss the first set of questions in teams of three.
- Spend 5 minutes reading Section 2 of the text excerpt, then discuss the second set of questions in teams of three.
- Review some of the questions and points that were the most controversial in the small team discussions as a group.

Activity 4: Fountain of Youth (10 minutes)

- Read the Setup.
- Have each person take a copy of the Discussion Questions.
- Discuss the questions as a group.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 11

Biology of Sex and Gender

Description

Scientists are beginning to unravel the evolution of sex. Several genes have been identified that help determine what makes a human embryo develop female or male sexual anatomies. Recent findings have challenged scientific beliefs about the roles of anatomy, environment, and genetics in the determination of gender.

Menu of Unit Activities

Choose either Activity 1 or Activity 2:

Activity 1: 1 in 4000 (15 minutes)

A quick discussion and review of human gender determination and the causes of abnormalities.

Activity 2: Birds Do It, Bees Do It (15 minutes)

A quick discussion on the diverse mechanisms used by different organisms to generate sexes.

Choose either Activity 3 or Activity 4:

Activity 3: What About Meiosis? (15–30 minutes, depending on the experience of the participants)

Paper chromosomes are used to work through situations of X and Y chromosome pairing, segregation, and recombination.

Activity 4: What Are Our Roles? (30 minutes)

Situations for discussion or role-playing that involve an intersex infant or student.

Activity 5: Let's Call the Whole Thing Off (60 minutes)

Readings and simple tests on male-female differences in intellectual and motor skills.

Choose either Activity 6 or Activity 7:

Activity 6: Y? (15 minutes)

Discussion questions on the genetic implications of the XY sex-determination system.

Activity 7: You Be the Judge (15 minutes)

Discussion questions on gender testing for athletic events, and what determines true maleness and femaleness.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Choose either Activity 1 or Activity 2:

Activity 1: 1 in 4000 requires:

- Tips and Suggested Answers

Activity 2: Birds Do It, Bees Do It requires:

- Tips and Suggested Answers

Choose either Activity 3 or Activity 4:

Activity 3: What About Meiosis? requires:

- One set of Paper Chromosomes per two people (master copy provided; to make a set, cut after copying, so that each chromosome is separate)
- One copy of the Instructions and Situations per two people (master copy provided)
- A box of small- to medium-sized paper clips
- A roll of tape
- Tips and Suggested Answers

Activity 4: What Are Our Roles? requires:

- One copy of the Situations and Discussion Topics per person (master copy provided)

Activity 5: Let's Call the Whole Thing Off requires:

- One copy each of the article excerpt, Tests, and Discussion Questions per person (master copy provided)
- A stopwatch, watch with second hand, or timer for each team of four
- A tennis ball or other small ball for each team of four
- A wastebasket or other target for each team of four

Choose either Activity 6 or Activity 7:

Activity 6: Y? requires:

- Optional: Paper Chromosomes (master copy provided in Activity 3)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Activity 7: You Be the Judge requires:

- One copy of the Discussion Questions per person (master copy provided)

Facilitator: Make sure that the room has these supplies:

- pens or pencils and paper
- VCR and TV
- overhead projector and markers
- black/white board with chalk or markers

Session Activities and Video

If you chose Activity 1: 1 in 4000 (15 minutes)

- Read the Setup and arrange into pairs.
- Spend about five minutes working in pairs to discuss gender determination and abnormalities.
Facilitator: If hints would help the pair discussions, give the categories into which you'll be putting the ideas during the group discussion (see Tips and Suggested Answers).
- As a group, discuss and categorize the ideas from the pair discussions. Compare the group's list with the list in Tips and Suggested Answers.

If you chose Activity 2: Birds Do It, Bees Do It (15 minutes)

- Read the Setup and arrange into pairs.
- Spend about five minutes working in pairs to discuss different mechanisms of gender determination.
Facilitator: If hints would help the pair discussions, give the categories into which you'll be putting the ideas during the group discussion (see Tips and Suggested Answers).
- As a group, discuss and categorize the ideas from the pair discussions. Compare the group's list with the list in Tips and Suggested Answers.

Video (30 minutes)

- Watch the Biology of Sex and Gender video.

If you chose Activity 3: What About Meiosis? (15–30 minutes)

- Read the Setup and arrange into pairs.
- Have each pair take one set of Paper Chromosomes and one copy of the Instructions and Situations.
- Have each pair take at least four paper clips and some tape.
- Spend two to five minutes working on each situation in pairs, working out the solution with the paper chromosomes.

Facilitator: If the group is experienced in using objects to represent chromosomes, skip the first few situations and begin with situation 2, 3, or 4. Make up the extra time in the session by doing an additional activity from the choices. For example, do both Activities 1 and 2, or both Activities 6 and 7.

- Compare the group's answers with those in Tips and Suggested Answers.

Session Activities and Video, cont'd.

If you chose Activity 4: What Are Our Roles? (30 minutes)

- Read the Setup and arrange into teams of three or four.
- Have each person take a set of the suggested topics.
- Spend about 20 minutes role-playing or discussing the topics.
- As a large group, discuss the fundamental issues that arose from the smaller discussions and role-playing.
- Variation: Divide the group into a few teams of two or three people, with the rest as the audience. All the teams are given the same situation, such as an intersex child being introduced to a classroom at grade 5. One team at a time does a short role-play about the situation while the rest of the teams are out of the room. After all the teams have done their role-play, discuss as a group what common issues came up and what different issues were addressed.
- Variation: Instead of role-playing, choose a situation to discuss. List all the individuals who would be affected, and explore all their possible responses.

Activity 5: Let's Call the Whole Thing Off (60 minutes)

- Read the Setup and arrange into teams of four. If possible, have two men and two women per team.
 - Have each person take a copy of the *Scientific American* article excerpt.
 - Spend 10 minutes reading the article.
 - Each team should take a stopwatch, and some pens or pencils and paper.
- Facilitator:** Have one person per team be in charge of administering and timing tests. Give this person four copies of the *first page only* of the Tests (Test 1).
- The person in charge of each team should distribute one copy of Test 1 to each person on the team, who will leave it face down until the timer starts.
 - Spend five minutes in teams, doing Test 1 and recording the results.

Facilitator: Give the person in charge of each team four copies of the *second page only* of the Tests (Test 2).

- The person in charge of each team should distribute one copy of Test 2 to each person on the team, who will leave it face down until the timer starts.
- Spend 5–10 minutes in teams, doing Test 2 and recording the results.

Facilitator: Give the person in charge of each team four copies of the *third page only* of the Tests (Test 3).

(continued, next page)

Session Activities and Video, cont'd.

(Activity 5, continued)

- The person in charge of each team should distribute one copy of Test 3 to each person on the team, who will leave it face down until the timer starts.
- Spend 5–10 minutes in teams, doing Test 3 and recording the results.

Facilitator: While teams are doing Test 3, set up the balls and targets for Test 4.

- Spend 5–10 minutes in teams doing Test 4 and recording the results.
- Have each person take a copy of the Discussion Questions and spend 10 minutes as a group discussing them.

If you chose Activity 6: Y? (15 minutes)

- Read the Setup and pass out sets of paper Y chromosomes, if desired.
- Have each person take a copy of the Discussion Questions and talk about them as a group.
- See Tips and Suggested Answers for additional information.

If you chose Activity 7: You Be the Judge (15 minutes)

- Read the Setup and have each person take a copy of the Discussion Questions.
- Talk about the questions as a group.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or 'take home' lessons of this unit and its applications.

Notes

Unit 12

Biodiversity

Description

By combining mathematical models and field experiments, scientists hope to learn how ecosystems function and how environmental changes might affect biodiversity. In one study, a more diverse system was better able to withstand stress such as drought than a less diverse system. With current extinction rates rivaling those of previous episodes of mass extinction, such knowledge may help in efforts to minimize the problems associated with a loss of biodiversity.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Before and After (15 minutes + 30 minutes of video)

Six discussion questions that outline biodiversity issues covered in the video.

Activity 2: Quantifying Biodiversity (45 minutes)

Explanation and exercises in generating and comparing biodiversity indices. Indices are made for a representative community that undergoes habitat fragmentation.

Activity 3: Extinction Risk (25 minutes)

A discussion on factors that increase the risk of extinction. Two examples of species with various factors are provided for context and comparison.

Activity 4: Concept Maps (20 minutes)

Four lists of concepts for making concept maps, with discussion questions.

Activity 5: Wrap-Up Discussion (15 minutes)

Questions that explore ethical issues in preserving biodiversity and practical issues in teaching the concepts of the field.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Before and After requires:

- One copy of the list of Biodiversity Questions per person (master copy provided)

Activity 2: Quantifying Biodiversity requires:

- One copy of the Worksheet for Quantifying Biodiversity with Diversity Indices plus Discussion Questions per person (master copy provided)
- Approximately 125 small, colored objects to represent individuals in an ecosystem. For every two people, have five small items (e.g., strips of paper, toothpicks, matchsticks, colored candies, etc.) in each of five different colors (five red, five green, five white, etc.), for a total of 25 per two people.
- Tips and Suggested Answers

Activity 3: Extinction Risk requires:

- One copy of the Pairs of Species To Compare for Extinction Susceptibility plus Discussion Questions per person (master copy provided)
- One copy of the Biodiversity online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Activity 4: Concept Maps requires:

- One copy of the list of Suggested Concepts To Map per person (master copy provided)
- One copy of the Biodiversity online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

Activity 5: Wrap-Up Discussion requires:

- One copy of the Discussion Questions per person (master copy provided)

Facilitator: Make sure that the room has these supplies:

- | | |
|----------------------------------|-------------------------------------------|
| • pens or pencils and paper | • VCR and TV |
| • overhead projector and markers | • black/white board with chalk or markers |

Session Activities and Video

Activity 1a: Before and After—Pre-Video Discussion (10 minutes)

- Read the Setup.
- Arrange into pairs.
- Have each person take a copy of the Biodiversity Questions to discuss.
- Discuss the list of questions.

Video (30 minutes)

- Watch the Biodiversity video.

Activity 1b: Before and After—Post-Video Discussion (5 minutes)

- Go over the biodiversity issues as a group and see if anyone would change or amend their answers, and if everyone's questions were answered.

Activity 2: Quantifying Biodiversity (45 minutes)

- Read the Setup.
- Arrange into pairs and have each pair take a Worksheet.
- After reading the background, do Exercise 1 in pairs.
- If necessary, the facilitator can check answers with the Tips and Suggested Answers section.
- While the pairs are finishing Exercise 1, the facilitator can distribute the objects that represent individuals of different species.
- As a group, build the ecosystem for Exercise 2 by having each pair contribute their objects.
- Do Exercise 2.
- Discuss the questions as a group.

Session Activities and Video, cont'd.

Activity 3: Extinction Risk (25 minutes)

- Read the Setup.
- Arrange into teams with three or four people per team.
- Have each person take a handout of the Pairs of Species To Compare for Extinction Susceptibility plus Discussion Questions.
- Pass out a copy of the Biodiversity online text to each team.
- Do Activity 3, consulting the text and the Tips and Suggested Answers.

Activity 4: Concept Maps (20 minutes)

- Read the Setup.
- Have each person take a copy of the Suggested Concepts To Map and draw a concept map that includes all of them.
- The facilitator should encourage the addition of concepts or new lists of concepts to map.
- Have copies of the online text available for reference.
- When everyone is finished, arrange groups of three or four to compare concept maps and discuss the questions associated with each.

Activity 5: Wrap-Up Discussion (15 minutes)

- Read the Setup.
- Have each person take a copy of the Discussion Questions.
- Discuss the questions as a group.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Unit 13

Genetically Modified Organisms

Description

While genetic modification of organisms has been going on for millennia, we now have the tools to be able to insert intentionally selected genes from one organism into germ cells of unrelated species. Such genetically transformed organisms are increasingly common in agriculture, industry, and medicine. Their potential benefits and risks are explored.

Menu of Unit Activities

Note: All activities, handouts, solutions, and tips can be found in the Appendix of this guide.

Activity 1: Coming Attractions (15 minutes + 30 minutes of video)

A series of quick questions about genetically modified organisms, whose answers are found in the video.

Activity 2: What's the Difference? (50 minutes)

Brief, team-led discussions on related terms in genetic engineering and cloning.

Activity 3: Troubleshooter (50 minutes)

Pairs read about failed genetic engineering experiments, find the flaws, and think of tests that would determine what went wrong.

Activity 4: Two Thumbs Up? (5 minutes)

Group discussion on how to interpret and apply the unit information.

Before the Session

Facilitator: Copy and assemble the following activity materials. (See the Activities section in the Appendix of this guide for master copies of transparencies and handouts, plus Tips and Suggested Answers.)

Activity 1: Coming Attractions requires:

- Transparency of the Coming Attractions Questions (master copy provided)
- Transparency of the Coming Attractions Answers (master copy provided)

Activity 2: What's the Difference? requires:

- One copy of What's the Difference? Questions per person (master copy provided)
- Transparency of What's the Difference? Questions (master copy provided)
- Tips and Suggested Answers
- One copy of the Genetically Modified Organisms online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

Activity 3: Troubleshooter requires:

- One copy of each of The Genetic Engineering Experiment Cases (master copy provided)
- Tips and Suggested Answers
- One copy of the Genetically Modified Organisms online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

Activity 4: Two Thumbs Up? requires:

- One copy of the Discussion Questions per person (master copy provided)

Facilitator: Make sure that the room has these supplies:

- | | |
|----------------------------------|-------------------------------------------|
| • pens or pencils and paper | • VCR and TV |
| • overhead projector and markers | • black/white board with chalk or markers |

Session Activities and Video

Activity 1a: Coming Attractions—Pre-Video Discussion (10 minutes)

- Read the Setup.
- Put the transparency of the Coming Attractions Questions on the overhead. Answer the questions individually, looking at them one by one and spending 30 seconds to one minute on each.

Facilitator: Stress that correct answers are not expected. This is just to get people thinking about the topics in the video.

- Variation: Instead of each person answering each question, do the activity as a group, with the session facilitator writing down consensus answers or guesses on a blackboard or transparency.

Video (30 minutes)

- Watch the Genetically Modified Organisms video.

Facilitator: If possible, leave the transparency of Coming Attractions Questions up during the video.

Activity 1b: Coming Attractions—Post-Video Discussion (5 minutes)

- After the video, put the transparency of the Coming Attractions Answers on the overhead.
- Go over the questions as a group, with the answers from the video.

Activity 2: What's the Difference? (50 minutes)

- Arrange into pairs.
- Read the Setup.
- Have each person take a handout of the What's the Difference? Questions.
- Have each pair take at least one of the questions to work on, and one copy of the Genetically Modified Organisms online text chapter for reference.
- Spend five to 10 minutes preparing answers.
- Put the transparency of the questions on the overhead projector. As a group, read each question, waiting a few seconds to think of individual answers.
- Spend 3 minutes per question listening to the answer the assigned pair came up with, adding details and discussing similarities between the terms.
- See the Tips and Suggested Answers.

Session Activities and Video, cont'd.

Activity 3: Troubleshooter (50 minutes)

- Arrange the group into a total of five teams, if possible. Otherwise, form pairs.
- Read the Setup.
- Distribute the pieces of paper with the cases so each team has one case.
- Each team will spend 5–10 minutes going through the case, then exchange cases with another team. Repeat until each team has discussed all five cases.
- As a group, discuss each case briefly, including the experiment ideas generated by the teams.
- See the Tips and Suggested Answers.

Activity 4: Two Thumbs Up? (5 minutes)

- Read the Setup.
- Have each person take a copy of the Discussion Questions.
- Discuss the discussion questions in pairs or as a group.

Summary (5 minutes)

- If time permits, as a group or in pairs, define the major ideas or “take home” lessons of this unit and its applications.

Appendix

Activities, Handouts, Solutions, and Tips	71
Unit 1	71
Unit 2	93
Unit 3	129
Unit 4	153
Unit 5	167
Unit 6	197
Unit 7	219
Unit 8	235
Unit 9	249
Unit 10	265
Unit 11	283
Unit 12	309
Unit 13	323
Credits	341

Activity 1: Before and After

Based on video content

20 minutes (10 minutes before and 10 minutes after the video)

Setup

Before watching the video, take a few minutes to consider your impressions of the field of genomics. In pairs, look at the list of some terms and topics that will be covered in the video. Discuss, or write a few brief sentences summarizing what comes to mind when you see the term.

After the video, go over the terms again as a group. For each one, discuss the group's impressions about the term before the video, and how they changed after viewing the video. As a group, try to come up with a brief definition or summary for each term.

Materials

- One copy of the List of Terms and Topics per two people (master copy provided)
- Tips and Suggested Answers

List of Terms and Topics

1. genome
2. Human Genome Project
3. open reading frame (ORF)
4. BLAST (basic local alignment search tool)
5. homology
6. SNPs (single nucleotide polymorphisms, “snips”)
7. DNA fingerprint
8. microarrays
9. cDNA (complementary DNA)
10. *Arabidopsis*
11. knockout organism
12. Type II diabetes

Note: For possible ways to define these terms, see the Tips and Suggested Answers that follow.

List of Terms and Topics, Suggested Definitions

1. genome

The entire collection of DNA found in an organism, including genes and additional sequences. The sequence varies slightly between individuals, but "genome" refers to the collective, common DNA of a species.

2. Human Genome Project

A collaborative effort to determine the sequence of the 3 billion nucleotides found in the entire collection of human DNA.

3. open reading frame (ORF)

A DNA sequence that has the potential to encode a protein. It has a series of amino acid codons without a stop and all in the same "reading frame" that is theoretically long enough to encode a protein.

4. BLAST (basic local alignment search tool)

A computer program that compares and aligns DNA sequences to find similarities. For example, it can look for genes that are common in two different species.

5. homology

Similarity between genes, DNA sequences, proteins, or other components of organisms, which suggests a common ancestral origin.

6. SNPs (single nucleotide polymorphisms, "snips")

Places in the genome where single base differences are found when the DNA from individuals within the same species are compared.

7. DNA fingerprint

Variations in DNA base sequence that are characteristic of an individual.

8. microarrays

Glass chips dotted with tiny amounts of "probe" DNA. They can be used to detect which genes are expressed in an organism under different conditions by detecting the RNA from cells of the organism.

9. cDNA (complementary DNA)

DNA that is made from RNA by "reverse transcription." It can be hybridized to a microarray of genes, to determine which RNAs are made by a cell under certain conditions.

10. *Arabidopsis*

A mustard plant that is used as a "model organism" for genetics.

11. knockout organism

An organism in which a gene has been deleted, or "knocked out" using genetic engineering techniques.

12. Type II diabetes

An inability to regulate blood glucose levels, because of defects in the regulatory system that makes and responds to insulin. This diabetes usually has an adult onset.

Activity 2: Making a Microarray

Based on video and online text content

40 minutes

Setup

Microarrays are small slides or chips of glass onto which microscopic dots of DNA are spotted. Each dot has DNA of a difference sequence. When the microarray of DNA dots is incubated with test DNA that has been fluorescently labeled, the test DNA hybridizes to specific spots, of known sequence, in the microarray. A fluorescence detector reveals where the test DNA has hybridized, so the microarray “probes” the test DNA to find out which sequences, and in what quantity, are in the test DNA.

Often, the test DNA is cDNA generated from the RNA of cells exposed to specific conditions. By comparing the RNA made by cells under the specific conditions to RNA made under standard conditions, we can see how cells respond to a drug or a new environmental condition.













In this exercise, you will receive a diagram with a grid of dots that represents a microarray. You will also have a key to the gene sequences found at each dot on the grid, and an explanation of how the test DNA was made. Work in pairs to predict how each dot in the grid will hybridize to the test DNA. Then predict the appearance of each dot in the grid, and mark each dot according to your predictions. After everyone has marked the microarray, go over it as a group, and discuss any spots that are in dispute. See the Tips and Suggested Answers for explanations.

If necessary, review the microarray technique from the Genomics video or Genomics online text chapter, or look at Figure 5 from the text, before doing this exercise.

Materials

- One copy of the Microarray Grid Diagram per two people (master copy provided)
- One copy of the Key to the Genes on the Microarray per two people (master copy provided)
- Transparency of the Microarray Grid Diagram (or a sketch of the grid on a blackboard)
- Transparency of Aerobic and Anaerobic Pathways in Yeast (master copy provided)
- Transparency of Figure 5 from the Genomics online text chapter (master copy provided)
- One copy of the Genomics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Optional: red, green, and yellow pens or red, green, and yellow sticky dots (one set per two people)
- Tips and Suggested Answers

Microarray Grid Diagram

	1	2	3	4
A				
B				
C				

On the microarray, a gene whose expression is *increased in aerobic growth* will appear **red**. A gene whose expression is *increased in anaerobic growth* will appear **green**. A gene that is *equally expressed in both conditions* will appear **yellow**.

For each microarray spot, show whether you would predict it to appear red, green, or yellow, by coloring it or labeling it R, G, or Y.

Key to the Genes on the Microarray

Background

This microarray will be used to see how the expression (transcription) of several yeast genes changes as the cells change their metabolism. First, a little background. Yeast cells can metabolize anaerobically or aerobically. Anaerobic metabolism (fermentation) uses the pathway of glycolysis to break down glucose to pyruvate. Pyruvate is converted to acetaldehyde, then ethanol, which is how the alcohol in wine or beer is produced. In aerobic metabolism, the glycolysis pathway enzymes are still active. However, pyruvate is not converted to ethanol. Instead, metabolic byproducts are sent to the TCA cycle (also called the citric acid, or Krebs cycle) and the electron transport chain. The TCA cycle and electron transport chain occur in mitochondria, and require oxygen.

How the Test DNA Was Made

The test DNA that is hybridized to the microarray contains two batches of labeled cDNAs made from yeast mRNAs. cDNAs from yeast growing *aerobically* are labeled with a *red* fluorescent dye; cDNAs from yeast growing *anaerobically* are labeled with a *green* dye.

On the microarray, *red* spots will show genes that are highly expressed in *aerobic* growth; *green* spots show genes that are highly expressed in *anaerobic* growth. If cDNA from a gene is equally abundant in both the green and red labeled cDNA batches, both will bind equally to a spot and it will appear *yellow*.

Key to Genes on the Microarray

Spot	Gene	Function of Gene Product
A1	PGK1	enzyme in glycolysis
A2	RPB8	RNA polymerase subunit, enzyme in transcription
A3	PDC1	enzyme that converts pyruvate to acetaldehyde
A4	ADH1	enzyme that converts acetaldehyde to ethanol
B1	DBP2	DNA polymerase subunit, enzyme in DNA replication
B2	COX1	cytochrome oxidase, part of the respiratory electron transport chain
B3	CIT1	enzyme in the TCA (citric acid, Krebs) cycle
B4	RPL10	ribosomal protein for translation in the cytoplasm
C1	MRP1	ribosomal protein for translation in mitochondria
C2	SIP4	activates transcription of genes required for aerobic metabolism
C3	HSP12	protects against oxidative (oxygen-generated) stress
C4	ACT1	actin protein for cell structure

Post-Activity Discussion

Once we know how genes increase or decrease in expression when yeast metabolism changes, how might this information be applied? Consider the fact that yeast is used in baking, brewing, and as a model organism for cell and genetic research. (See the Tips and Suggested Answers section for ideas.)

Source and Answers

The Tips and Suggested Answers has the information about which genes in this activity were expressed in aerobic and anaerobic conditions. The data come from microarrays described in the following article: DeRisi, J.L., Iyer, V.R., Brown, P.O. 1997. "Exploring the metabolic and genetic control of gene expression on a genomic scale." *Science* 278(5338):680–686.

Aerobic and Anaerobic Pathways in Yeast

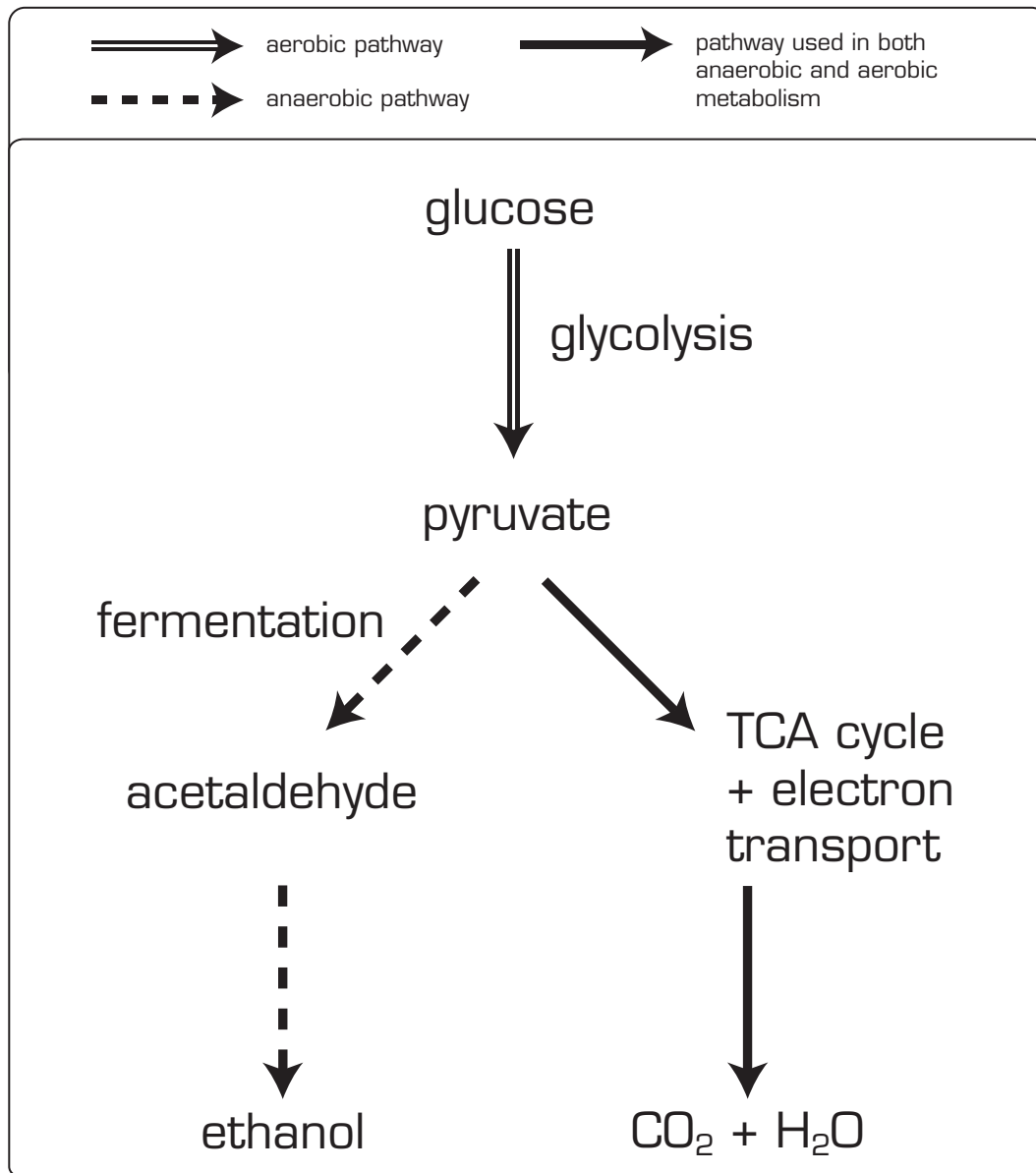


Figure 5 From the Online Text Chapter

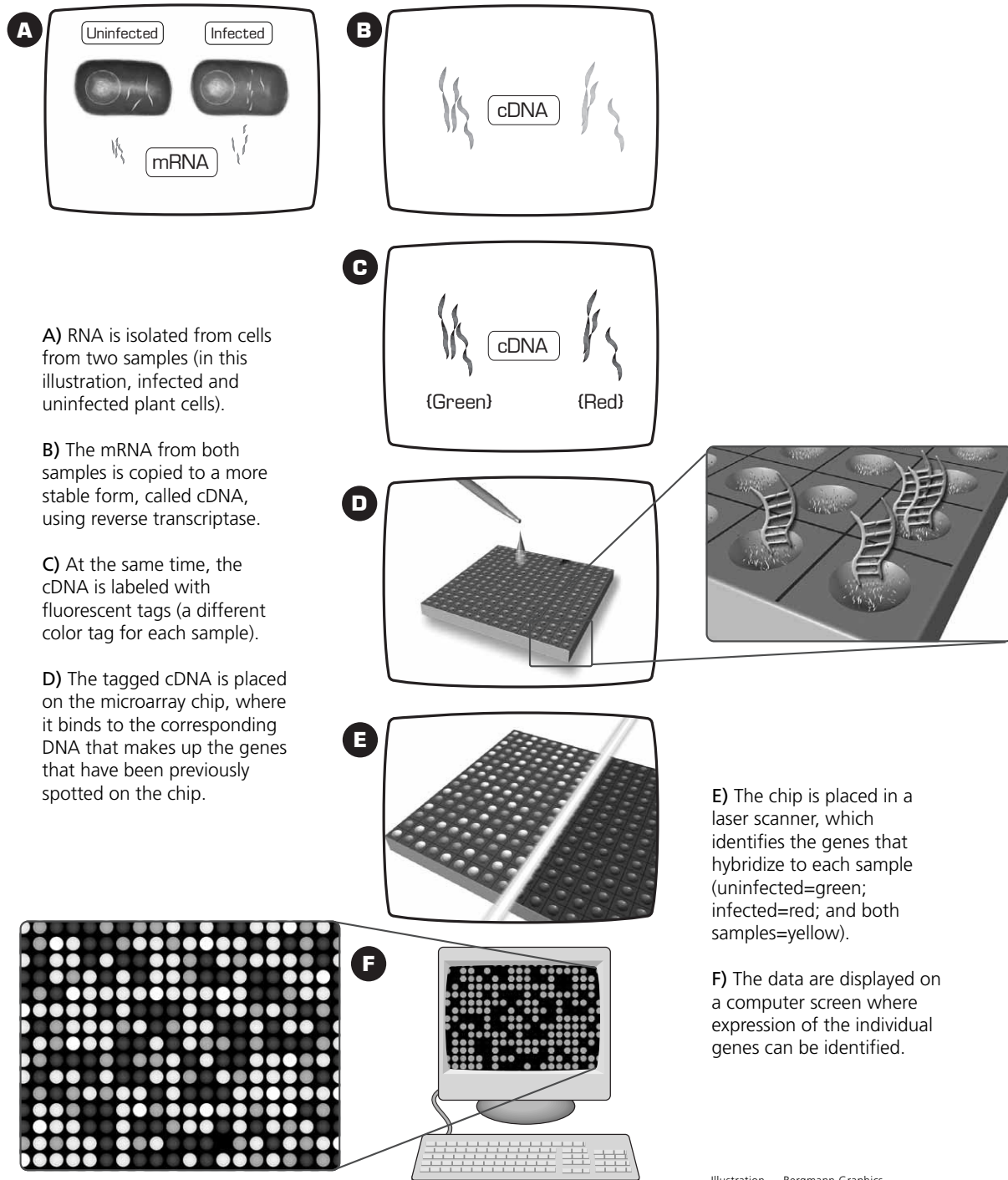


Illustration — Bergmann Graphics

Making a Microarray

from DeRisi, J.L., Iyer, V.R., Brown, P.O. (1997). "Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale." *Science* Oct 24; 278(5338):680-686.

Microarray Answers

In general, we can divide the genes into three categories and use these to predict its expression in conditions of aerobic and anaerobic metabolism:

1. *Yellow spots* are predicted for genes that are expressed equally in aerobic and anaerobic conditions. These encode RNAs and proteins with "housekeeping" functions that occur in any conditions. Housekeeping genes include those for glycolysis, since many are active in both aerobic and anaerobic conditions.
PGK1, RBP8, DBP2, RPL10, ACT1
2. *Green spots* are seen for genes that encode enzymes that function in *anaerobic* growth (fermentation) and are more highly expressed under these conditions.
ADH1, PDC1
3. *Red spots* are seen for genes that encode enzymes that function in *aerobic* metabolism, mitochondrial function (where the TCA cycle and electron transport chain occur) or response to the increased oxidative damage that occurs during aerobic metabolism. The product of the *SIP4* gene is required for the transcriptional activation of other aerobic metabolism genes, so it also increases in aerobic metabolism.
COX1, CIT1, MRP1, SIP4, HSP12

In reality, this type of yeast normally grows anaerobically, so the expression level of several of the housekeeping genes like *RPL10* is higher during anaerobic growth. *ADH1* is expressed equally in aerobic and anaerobic conditions, indicating that the cell controls the protein, which is made constantly, rather than controlling the gene.

Post-Activity Discussion Question Answer

Knowing the gene expression changes that occur when yeast convert from anaerobic to aerobic growth, or v.v., might provide information that could be used to optimize brewing or baking applications. For example, strains of yeast that are specialized for brewing or baking could be subjected to the same microarray analysis, to find out if they are optimized for production of ethanol, or for anaerobic growth. New strains might be selected based on their gene expression "profile" determined by this experiment.

Since yeast cells are used as models for studying basic cellular processes in other organisms, learning how gene expression changes in yeast when metabolic conditions change might provide insights into what happens in the cells of other organisms under similar conditions. Of course, human cells do not ferment glucose to ethanol, but muscle cells ferment glucose to lactic acid under aerobic conditions.

Activity 3: “CSI, Crime Scene Investigation”

Based on video and online text content

45 minutes

Setup

This activity is a “whodunit” that uses DNA fingerprints as the identifying features of individuals. You are the Crime Scene Investigation team, investigating the death of a man in a household full of people.

The sequences used as identifying markers in DNA fingerprinting are STRs (short tandem repeats). These are repeated sequences that are found at a particular locus on a chromosome, but the number of copies of repeats varies in the population. Like the single nucleotide polymorphisms (SNPs) that are described in this unit’s video and text, STRs are inherited just like alleles of genes: one from each parent. Like SNPs, several STRs in a section of a chromosome make up a haplotype.

After reading the mystery story, use (fabricated) STR information to come up with possible solutions for the situation in the story.

Materials

- One copy of the Mystery Story per person (master copy provided)
- One Figure of DNA Fingerprints From the Inhabitants per person (master copy provided)
- One set of the Figures of DNA Fingerprint Evidence, cut into individual samples (master copy provided)
- Transparency of the Map of the Room (master copy provided)
- Tips and Suggested Answers

Mystery Story

A man is found dead of a head wound in the library of a large mansion. Near his head is a heavy, broken vase. The deceased is 20-year-old John, the chauffeur for the family who owned the mansion. John's parents, Jamison and Lilly, are the butler and maid of the mansion. The family who own the mansion are Sir Roderick and Lady Madeline, and their children Roberta (age 18) and Bertram (age 15). Also in the house is Roberta's fiancé Paul (age 21). The family has a cat named Spike and a dog named Spot.

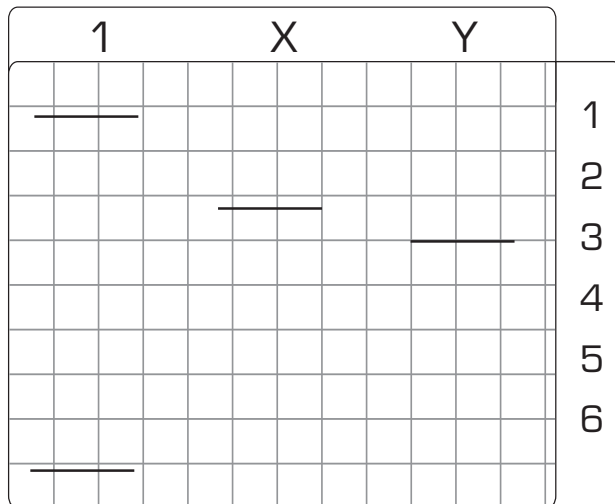
The window of the room was open. It was a dry, windy night and there were no footprints on the hard ground outside the window. A cat hair was found on the vase. Sir Roderick seems to think that a valuable stamp collection that was in the bookcase is missing, although his wife Madeline, daughter Roberta, and son Bertram claim they haven't seen the stamp collection for years, and thought he lost or sold it long ago.

Evidence samples have been taken from various parts of the room. In addition, cell samples were taken from each person in the household. DNA fingerprint analysis was performed on the evidence and the cell samples, and is now available for review by the CSI team. The following chromosomal regions were tested to generate the DNA fingerprints: a region on chromosome 1, a region on the X chromosome and for the males, a region on the Y chromosome. Note that the diagrams of DNA fingerprints supplied here are simplified, to save time. Normally, many more chromosomal loci are used in a DNA fingerprint investigation.

Each person will take at least one piece of DNA Fingerprint Evidence, along with a copy of the Figure of DNA Fingerprints From the Inhabitants. Use the Map of the Room and the Fingerprints From the Inhabitants to figure out who your evidence came from and where it was found. Come up with possible scenarios—accident or murder—that could have caused the death. If you suspect murder, was it an inside job or an intruder? What are the possible motives?

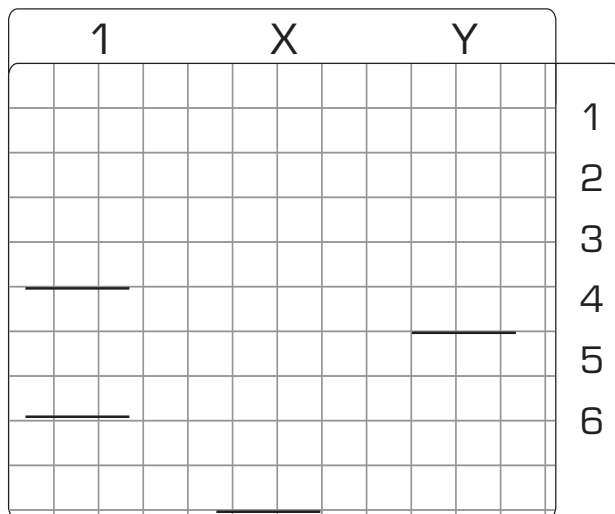
As a group, describe the scenarios you came up with to explain the evidence. See the Tips and Suggested Answers for possible answers.

Figures of DNA Fingerprint Evidence



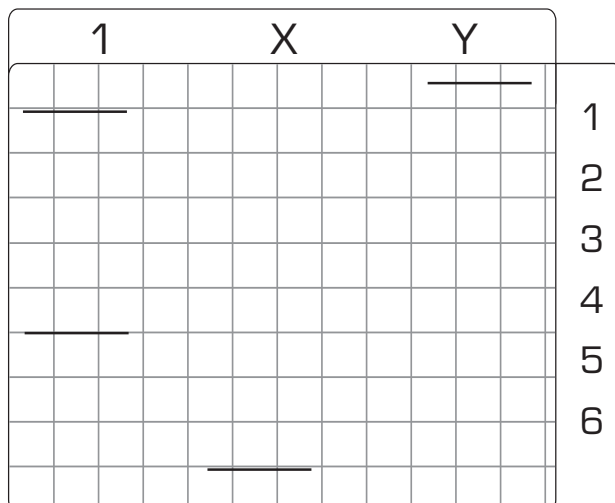
Evidence 1: From hair found on the floor
(see map for location)

(Grid lines and numbers along the side are for reference.)



Evidence 2: From skin cells found on the desk
(see map for location)

(Grid lines and numbers along the side are for reference.)



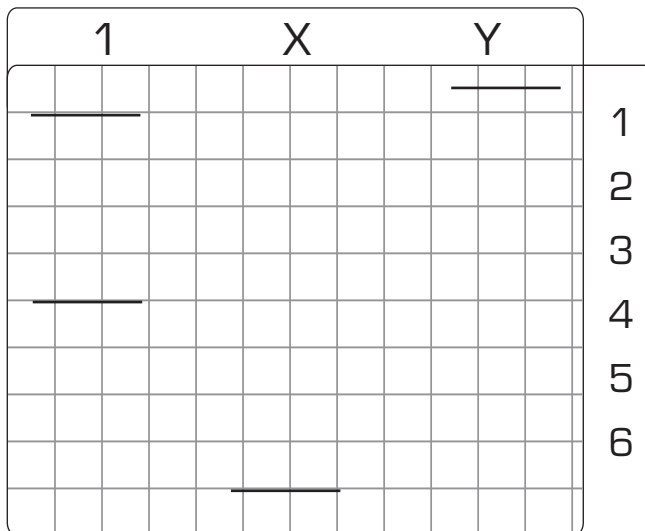
Evidence 3: From hair found on the floor
(see map for location)

(Grid lines and numbers along the side are for reference.)

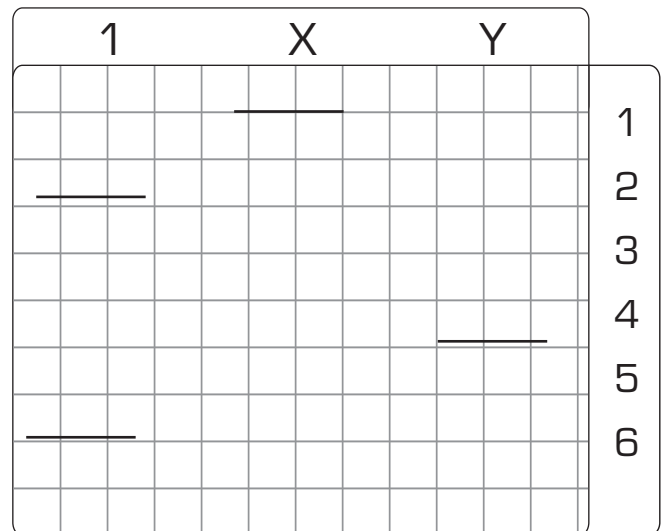
Figures of DNA Fingerprints From the Inhabitants

(Grid lines and numbers along the side are for reference.)

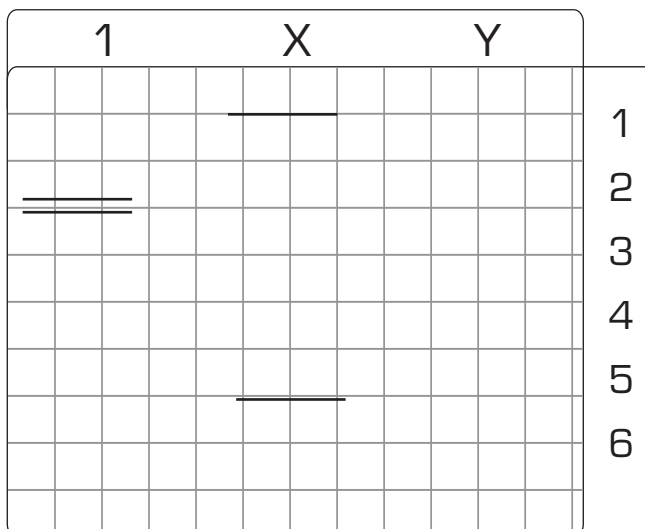
Butler (Jamison)



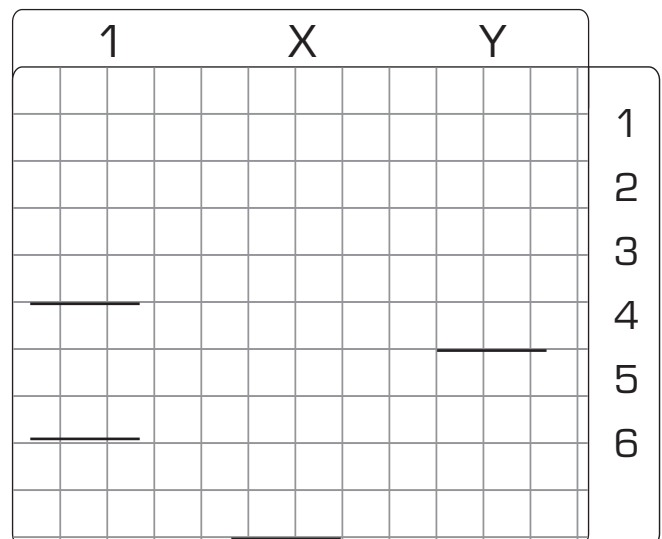
Chauffeur (the deceased, John)



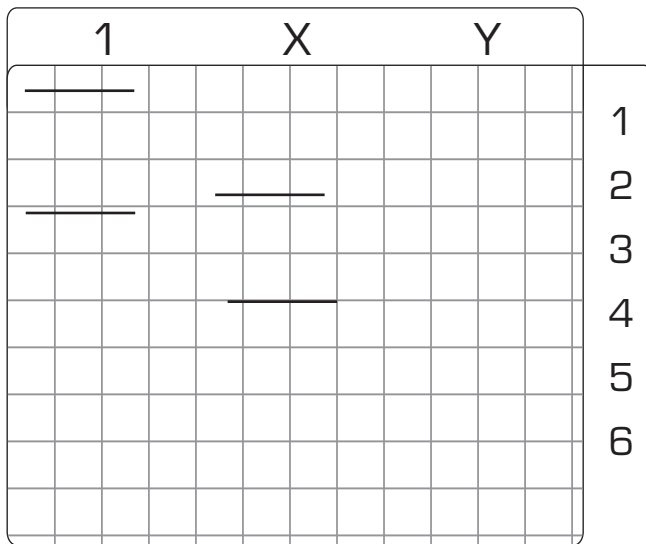
Maid (Lilly)



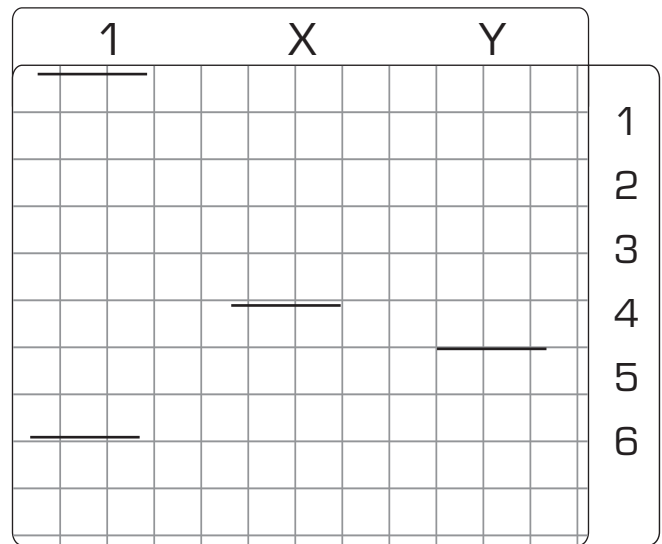
Owner of the mansion (Sir Roderick)



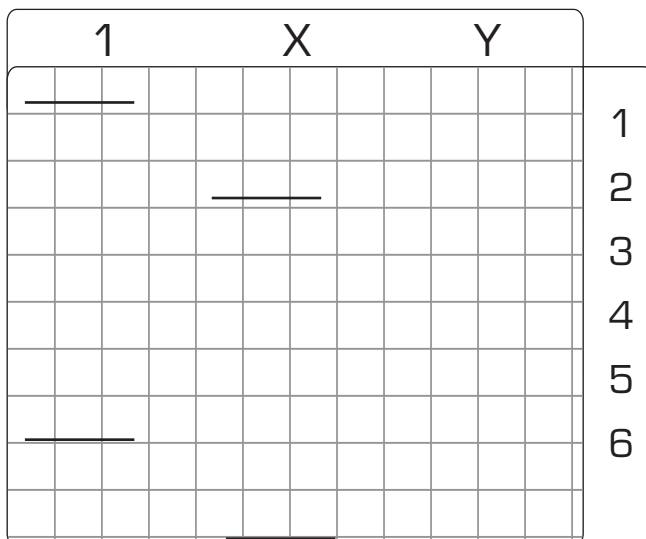
Owner of the mansion (Lady Madeline)



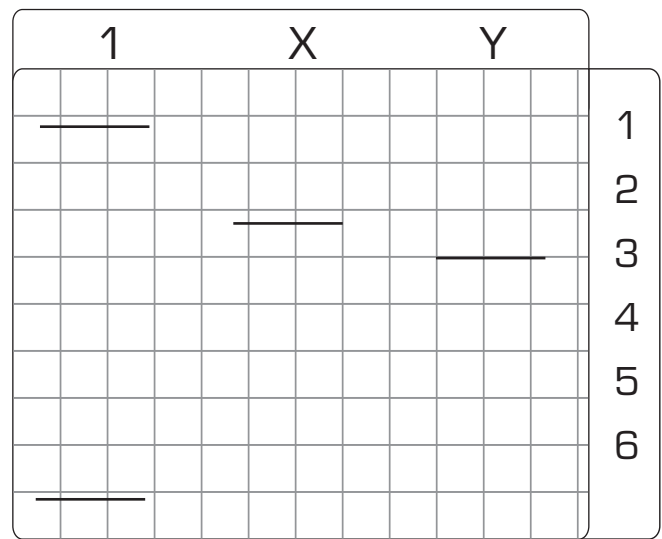
Son of Roderick and Madeline (Bertram)



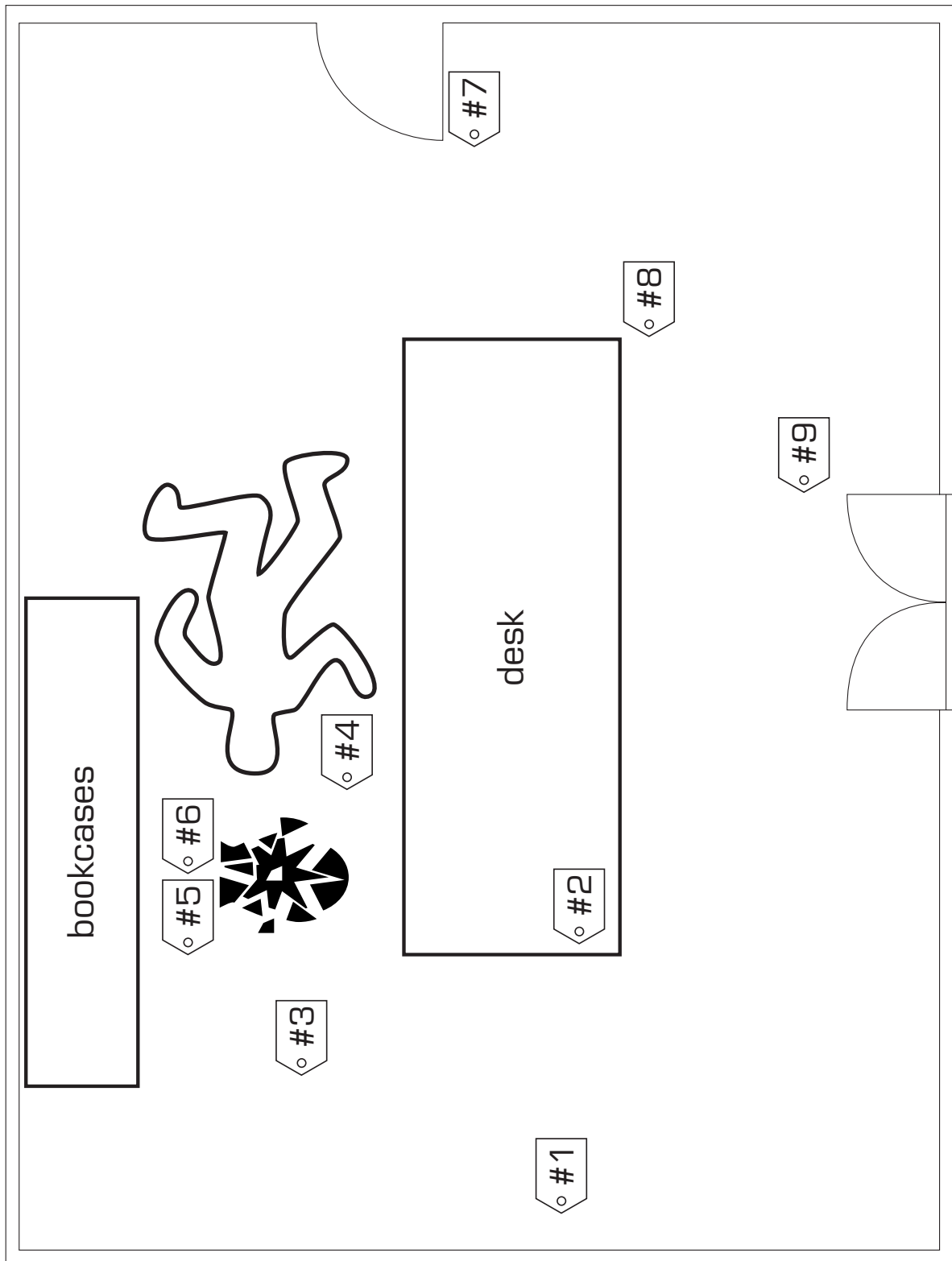
Daughter of Roderick and Madeline (Roberta)



Fiancé of Roberta (Paul)



Map of the Room



CSI, Crime Scene Investigation Possible Answers

1. The butler did it. His motivation? Paternity issues. Each person inherits one copy of each gene, SNP and STR from each parent. For autosomes (non-sex chromosomes like chromosome 1), everyone inherits 2, one from each parent. For the sex chromosomes and the genes and DNA fingerprint markers on them, males inherit an X from their mother and a Y from their father. Notice that the deceased, John, is supposed to be the child of the butler Jamison and the maid Lilly, but his DNA fingerprints suggest his father is Sir Roderick.
2. Sir Roderick did it, for the same reasons as the butler.
3. Lady Madeline did it. The partially damaged DNA fingerprint has some markers in common with her DNA fingerprint, although only for one of the loci tested here. Perhaps she recently discovered that her husband was the father of the chauffeur, and wanted to protect the inheritance of her own children.
4. Bertram did it, perhaps for the same reasons as Lady Madeline. His blood is on the floor near the window. Perhaps he cut himself on the broken vase and tried to escape out the window before the others came?
5. The maid did it. Lilly's skin cells are on the vase, although as the maid, she would be expected to pick it up and clean it from time to time.
6. The cat did it. The cat's hair is on the vase. It may have been up on the bookcases, perhaps chased by the dog, and dislodged the vase just as the victim walked below.
7. The wind did it. The window was open and it was a windy night. Perhaps the vase was in an unsteady position and blew over onto the victim.
8. An outsider did it, coming in and escaping through the open window, possibly stealing the stamp collection. (The partial DNA fingerprint might belong to the intruder.)

Activity 4: Quick Discussion

Based on video and online text content

15 minutes

Setup

Medicine is just one of the fields that is being revolutionized by the application of genomics techniques. Often, however, diagnostic applications precede applications that can cure or treat a condition. In pairs, spend a few minutes discussing each of the following questions.

Materials

- One copy of the Discussion Questions per two people
- Tips and Suggested Answers

Discussion Questions

1. Imagine it is possible to do a complete genomic analysis of an individual human. The analysis can determine which variants (alleles) of genes the person has, and the level at which the person's genes are expressed. Speculate about the kind of information a genomic analysis might reveal. What could one's genes, or their level of expression, predict about one's medical conditions, personality, or other personal characteristics? (See the Tips and Suggested Answers for possible answers.)
2. If individual genomic analysis tests were available, and they determined your risk of developing 100 diseases including Type II diabetes, different types of cancer, Alzheimer's, and susceptibility to viral and bacterial infections, would you have the test done? Why or why not?
3. If that type of data were available, who should have access to it? You and your physician? Your family members? Should insurance companies, the World Health Organization, the NIH, or the Center for Disease Control be able to access an anonymous, aggregate of the data, for statistical analysis?
4. What if genomics showed that a certain set of SNPs showed that some persons had an 80% chance of becoming alcoholic. Should we pass a law that would prevent them from buying alcohol?
5. Iceland automatically includes everyone's medical records in a database, unless a person requests to be left out. This database has found genes associated with several genetic disorders. Should we use a similar approach in this country?

Quick Discussion Answers

1. Imagine it is possible to do a complete genomic analysis of an individual human. The analysis can determine which variants (alleles) of genes the person has, and the level at which the person's genes are expressed. Speculate about the kind of information a genomic analysis might reveal. What could one's genes, or their level of expression, predict about one's medical conditions, personality, or other personal characteristics?

Possibilities include

- Information about a person's genes for insulin or the insulin receptor, or levels at which they are expressed, which might reveal information about susceptibility to diabetes.
- Information about a person's immune system genes or the level at which they are expressed might reveal information about their susceptibility to viral or bacterial infection, allergies, or risk of cancer.
- Knowing the level of expression of genes for enzymes that produce or release neurotransmitters or genes for neurotransmitter receptors might provide information about a person's mental health or personality.

Notes

Activity 1: Word Series

Based on video and online text content

20 minutes (10 minutes before and 10 minutes after the video)

Setup

Before viewing the Proteins and Proteomics video, think about some of the terms and concepts used in this field. With a partner, brainstorm about the relationship between the words in these pairs or series. After the video, take a few minutes to discuss the terms as a group. See if they are used the way you thought, or if their meanings in the field of proteomics are different from what you expected. If there are terms that are difficult to describe, have one person check the Proteins and Proteomics online text chapter for more information.

Materials

- One copy of the Word List per person (master copy provided)
- One copy of the Proteins and Proteomics online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

Word List

What is the relationship between the terms in each pair or group?

- a. gene: genome
- b. protein: proteome
- c. genomics: proteomics
- d. primary structure: secondary structure: tertiary structure: quaternary structure
- e. active site (or catalytic domain): ligand (or substrate)
- f. kinase: phosphatase
- g. intron: exon: splicing: alternative splicing
- h. alpha-helix: beta sheet
- i. mass spectroscopy: proteomic profiling (fingerprinting)
- j. glycosylation: phosphorylation
- k. SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis): 2D gel
- l. protein microarray: DNA microarray
- m. protein structure: rational drug design

Activity 2: Form and Function

Based on video and online text content

30 minutes

Setup

Proteins are more than just chains of amino acids: Folding into a specific native structure is required for function. One part of proteomics is understanding how structure relates to function. This exercise will look at different methods for representing protein structure and how each method emphasizes different features of a protein.

In pairs, take one of the Protein Structure Diagrams. Spend a minute defining the protein structure terms listed on the transparency, then point out any that you can see on your diagram. When you're finished, switch diagrams with another pair until the entire group has seen all the diagrams. Then discuss the questions as a group.

Materials

- Transparency of Protein Structure Terms and Discussion Questions (master copy provided)
- One set of Protein Structure Diagrams (master copy provided; to make a set, make one copy and cut along dotted lines)

Protein Structure Terms and Discussion Questions

primary structure

tertiary structure

active site/ligand binding site

beta sheet (or beta pleated sheet)

secondary structure

quaternary structure

alpha-helix

turn-between beta sheets

random coiled region

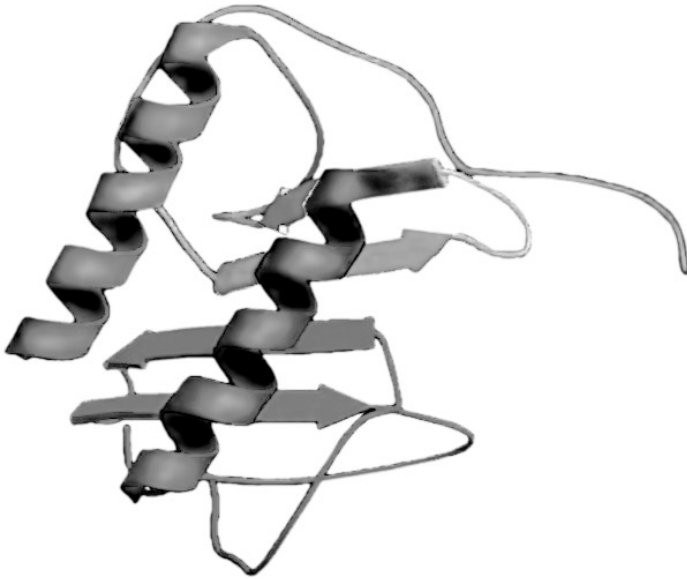
Discussion Questions

1. Which of the diagrams is best suited for showing secondary, tertiary, and quaternary structure?
2. How could looking at a protein's structure allow one to make an educated guess about the protein's function?

Protein Structure Diagrams

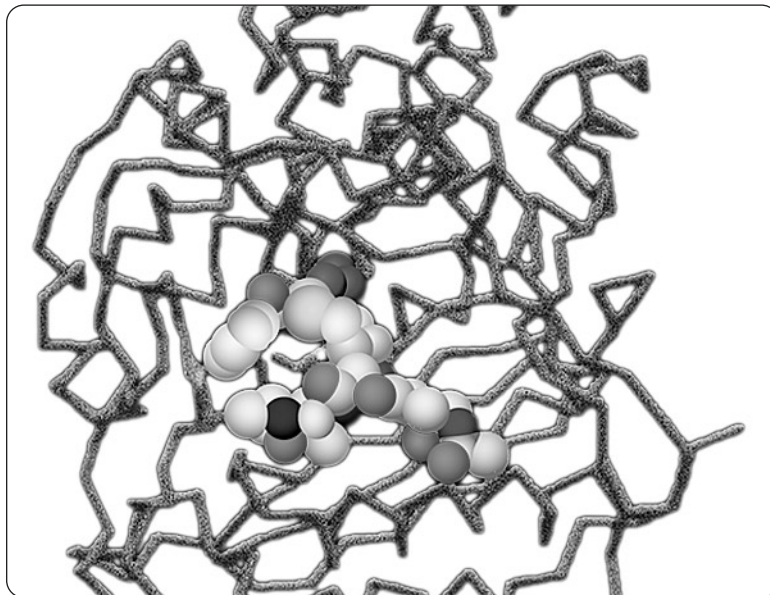
(Cut on the dotted lines after copying.)

1. interleukin 8, a small signaling protein



Source: online text

-
2. penicillin-binding protein with the drug cephalosporin in the middle



Source: online text

3. Abl kinase with the drug Gleevec in the middle



Source: Naqar, B. et al. 2002. *Cancer Res.* 62:4236–43, Figure 3A

4. LDL receptor adaptor with PTB (phosphotyrosine binding domain) and amino acid changes caused by several inherited mutations (e.g., P202H means proline number 202 is changed to histidine).



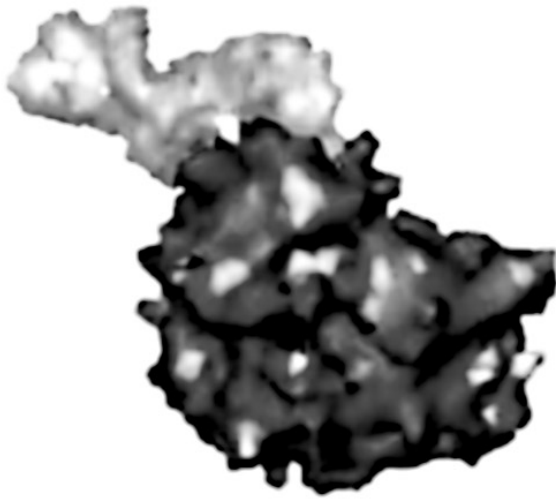
Source: Garcia et al. 2001. *Science* 292:1394–98, Figure 2C

5. CCR5 cytokine receptor, DNA, and amino acid sequence

[illegible]

Source: Raport et al. 1996. *J. Biol. Chem.* 271(29):17161–66, Figure 1

6. ribosome



Source: Stark H., et al. 1997. *Nature* 389:403–6, Figure 3B

Activity 3: 2D

Based on video and online text content

15 minutes

Setup

Two-dimensional gels can be used to make a proteomic profile of the abundance and modification of the proteins of a cell. Comparing the proteomic profiles of cells under different conditions can identify proteins that change in response to stimuli.

In this exercise, you will see a series of 2D gels. They show the proteins of a cultured immune system cell line, just before the cells are stimulated by binding of a ligand to a cell surface receptor and at several time points afterward.

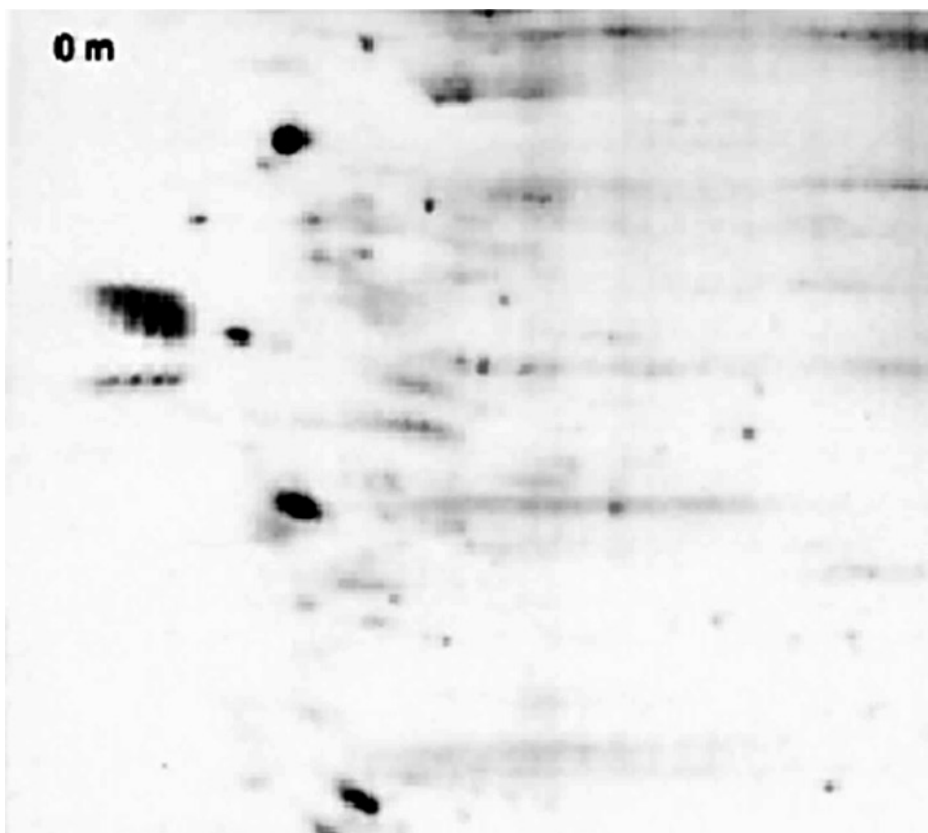
First, view the transparency of the 0 minute 2D gel. Then overlay the transparency of the next time point, which is 3 minutes after the ligand is added, directly onto the 0 minute transparency. Then overlay the next time point and the next, comparing changes to the proteomic profile each time. As a group, discuss the supplemental discussion questions.

Materials

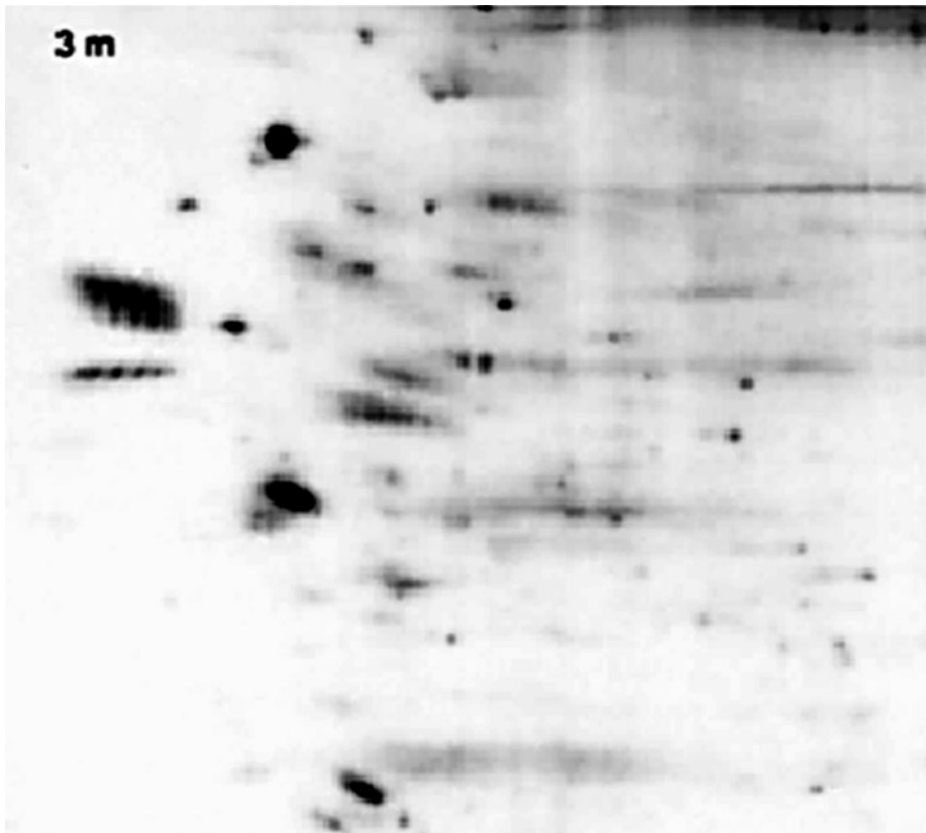
- Transparencies of 2D Gels, each on a separate transparency (master copies provided)
- Pens to write on transparency
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

2D Gels

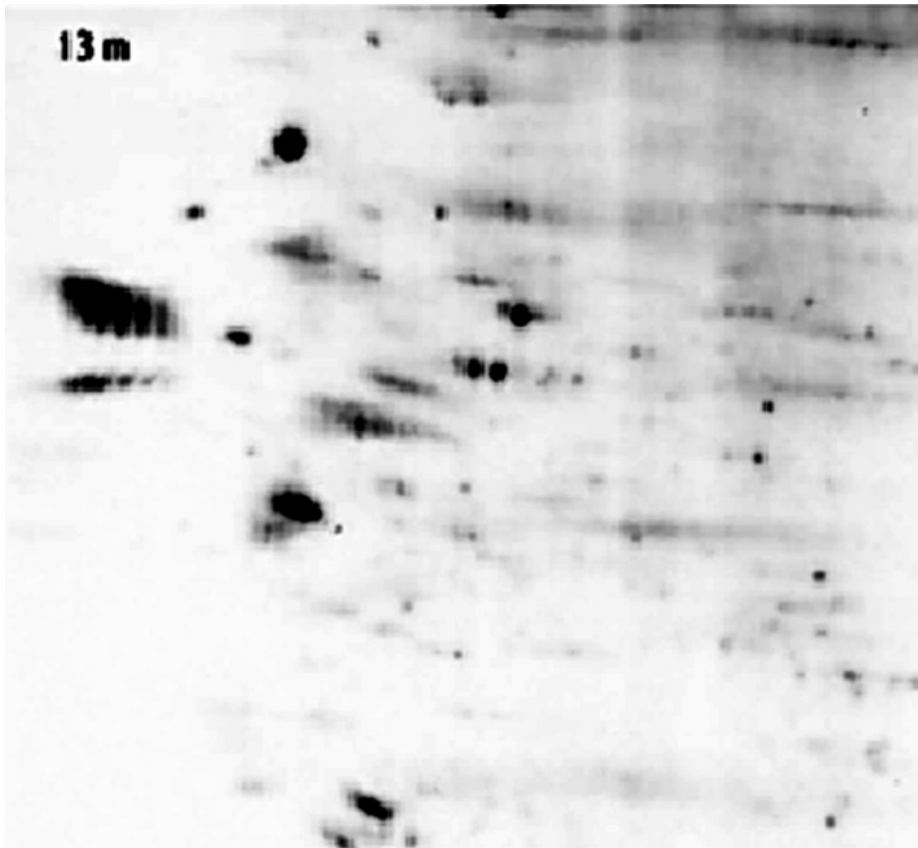
(Copy each onto a separate transparency.)



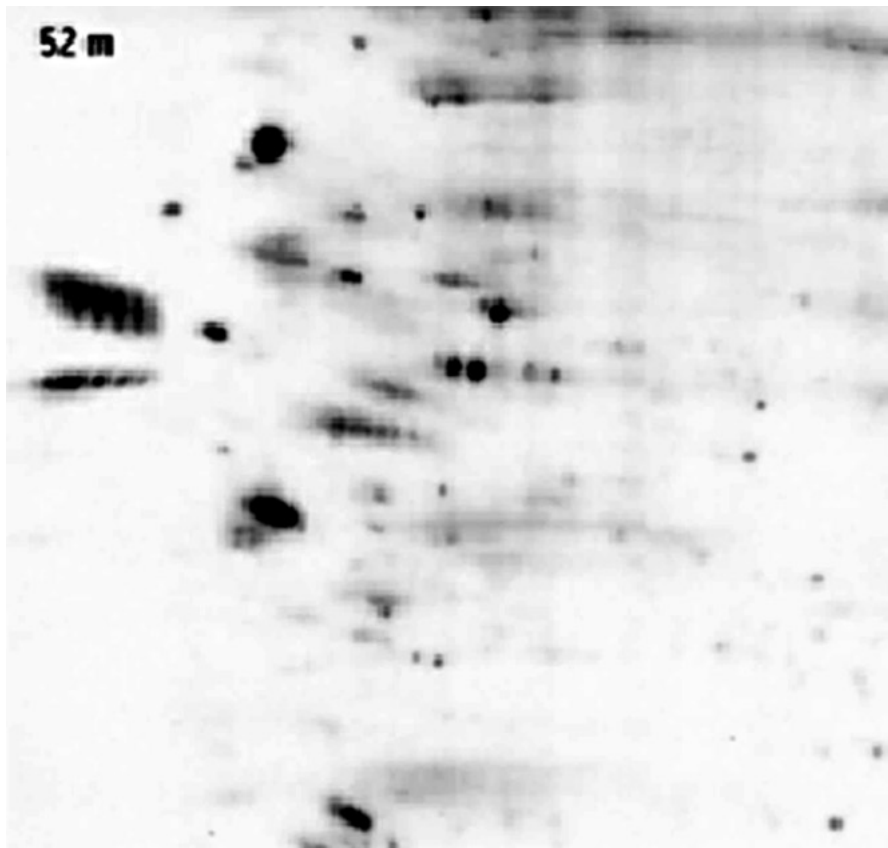
Source: <http://www-lecb.ncifcrf.gov/phosphoDB/>



Source: <http://www-lecb.ncifcrf.gov/phosphoDB/>



Source: <http://www-lecb.ncifcrf.gov/phosphoDB/>



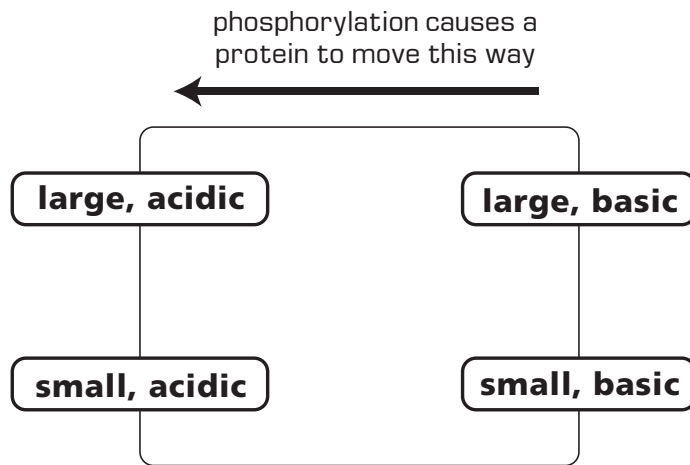
Source: <http://www-lecb.ncifcrf.gov/phosphoDB/>

Discussion Questions

1. What proteins change in abundance?
2. What proteins change in modification?
3. The first dimension was separated by isoelectric focusing point, or pI; this is the pH at which the protein has a net neutral charge. For example, this gel might be oriented so that the low-pH end of the isoelectric focusing gradient is to the left, and the high-pH end is to the right. The second dimension is separated vertically by size, with larger proteins at the top. With this information, label the corners of the gel, "large, acidic," "large, basic," "small, acidic," and "small, basic." The correct orientation is in the Tips and Suggested Answers.

Acidic (negatively charged) proteins have a low pI because they must migrate to a position of low pH (high H⁺ concentration) in the isoelectric focusing gel before their acidic groups are neutralized by protonation. Basic (positively charged) proteins migrate to a high-pH position in the isoelectric focusing gel, because they must reach a high pH before their protonated groups lose the H⁺ that causes them to be charged.

With this information, how would the position of a protein change in the 2D gels if it acquired a negatively charged group, like a phosphate?
4. Choose one of the proteins that changes position during the time course of the experiment. What kinds of modifications do you think are happening at each time point, based on how they change the size and charge of the protein?

Discussion Question #3 Answer

Activity 4: Tool Box

Based on video and online text content

45 minutes

Setup

Proteomics is a broad field, covering a range of studies about proteins, including their interactions, structures, and functions. Accordingly, there is a diverse collection of techniques that are used in this field. Sometimes, there is more than one tool that can be used for a given task, similar to the way a real tool box might contain a flathead and a Phillips screwdriver: One may be more generally useful than the other and both have specific uses, but usually one is best suited for a particular task.

In this exercise, you'll be given a set of proteomics "tools." Start out as a group, giving a brief definition of each tool. Don't go into detail about how it is used, because that is what this exercise explores. Instead describe the technique in a few sentences.

Then consider the task or applications on the transparency. For each task, look through your tool set and pull out the one(s) you would use. Decide individually, and then compare your selection with your neighbor's. Consult the Proteins and Proteomics online text chapter if necessary. As a group, discuss what tool(s) each person or pair chose and why one might be better suited to the task than others.

Materials

- One copy of the Tool Set for every person (master copy provided)
- Transparency of Tasks and Applications (master copy provided), or can be written on board
- One copy of the Proteins and Proteomics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Tool Set

(Cut on dotted lines.)

X-ray crystallography

twp-hybrid analysis

protein microarry

2D gel

mass spectrometry

NMR
(nuclear magnetic resonance)

PAGE
(polyacrylamide gel eletrophoresis)

Tasks and Applications

See the Tips and Suggested Answers.

1. Making a model of the structure of a protein.
2. Determining what proteins are in a multi-subunit complex, such as a polymerase, transcription factor complex, or membrane ion transporter.
3. Finding out which proteins become more abundant when a cell is subjected to oxidative stress.
4. Finding out how a particular protein's modification changes after the cell is stimulated with a growth factor.
5. Making a proteomic profile of a blood sample to use as a diagnostic test for cancer.
6. Seeing how the structure of a protein receptor changes when it binds its ligand.
7. Finding out if two proteins interact *in vivo*.
8. Determining the precise distance between the side groups of two amino acids of a protein when it is folded into its native structure.

Tasks and Applications Answers

1. Making a model of the structure of a protein.
X-ray crystallography or NMR; NMR is more suitable for quick analysis of small, soluble proteins. X-ray crystallography would be used for more a detailed analysis of a protein structure.
2. Determining the proteins in a multi-subunit complex, like a polymerase, transcription factor complex, or membrane ion transporter.
Mass spectrometry, two-hybrid analysis, protein microarray, 2D gel, or SDS-PAGE combined with mass spectrometry. Mass spectrometry could be used if the protein complex was purified but the subunits are unknown. In this case, amino acid sequences of unknown proteins would be identified by mass spectrometry. These sequences would then be compared to a protein or DNA sequence database of the organism from which the multi-subunit complex was purified. 2D gel or SDS-PAGE could be used first to identify the number of protein subunits and estimate their sizes. Protein subunits could be purified from the 2D or SDS-PAGE gel for analysis by mass spectrometry.
Two-hybrid analysis could be used if genes for candidates in the complex were already cloned, and could be engineered into bait and prey fusion proteins. Another approach would be to use a microarray that had antibodies against candidate proteins. The proteins to be identified would be denatured, and the identified by the antibodies they bound to in the microarray
3. Finding out which proteins become more abundant when a cell is subjected to oxidative stress.
Protein microarray or 2D gel, possibly mass spectroscopy. The proteome (collection of proteins expressed under certain conditions) from stressed and unstressed cells could be compared by comparing their abundance with a protein microarray, or by seeing which proteins produce a darker spot on a 2D gel of total extracted proteins. Mass spectroscopy could be done on subsets of partially purified protein extracts.
4. Finding out how a particular protein's modification changes after the cell is stimulated with a growth factor.
SDS-PAGE or 2D gel. Either a "1-dimensional" SDS-PAGE gel or a 2D gel could determine size and charge changes that accompany phosphorylation, proteolytic processing, or other modifications. Theoretically, X-ray crystallography, mass spectrometry or other techniques could as well, but the gel techniques are probably easier.
5. Making a proteomic profile of a blood sample to use as a diagnostic test for cancer.
Mass spectrometry or protein microarrays could show the appearance, disappearance or change in abundance of an indicator protein. This might also be possible with 2D gels, but they are not as sensitive.
6. Seeing how the structure of a protein receptor changes when it binds its ligand.
X-ray crystallography of the protein receptor co-crystallized with its ligand, and crystallized and analyzed in the absence of its ligand.

7. Finding out if two proteins interact *in vivo*.

Two-hybrid (possibly mass spectrometry, 2D gel or SDS-PAGE, protein microarray). If the genes for the proteins are cloned, two-hybrid bait and prey fusion proteins can be made and tested for *in vivo* interaction.

Co-purification is another possibility. One of the proteins could be purified from the cell with a specific antibody against the protein. Then mass spectrometry, SDS-PAGE, or 2D gel electrophoresis could be used to see if the other protein “co-purified” with it, suggesting *in vivo* association. If one protein is present on a protein microarray, one could test if the other protein bound to it, but this would be an *in vitro* or *in silico* interaction.

8. Determining the precise distance between the side groups of two amino acids of a protein when it is folded into its native structure.

NMR or X-ray crystallography. Assuming the protein is in its native structure when crystallized, X-ray crystallography would give the most precise distance measurement.

Activity 5: Two-Hybrid

Based on video and online text content

45 minutes

Setup

The yeast two-hybrid system can be used to find protein-protein interactions between any proteins, not just those found in yeast. Part 1 of this exercise makes a diagram showing the mechanics of this technique. Part 2 analyzes data from a large-scale, high-throughput project that used this technique.

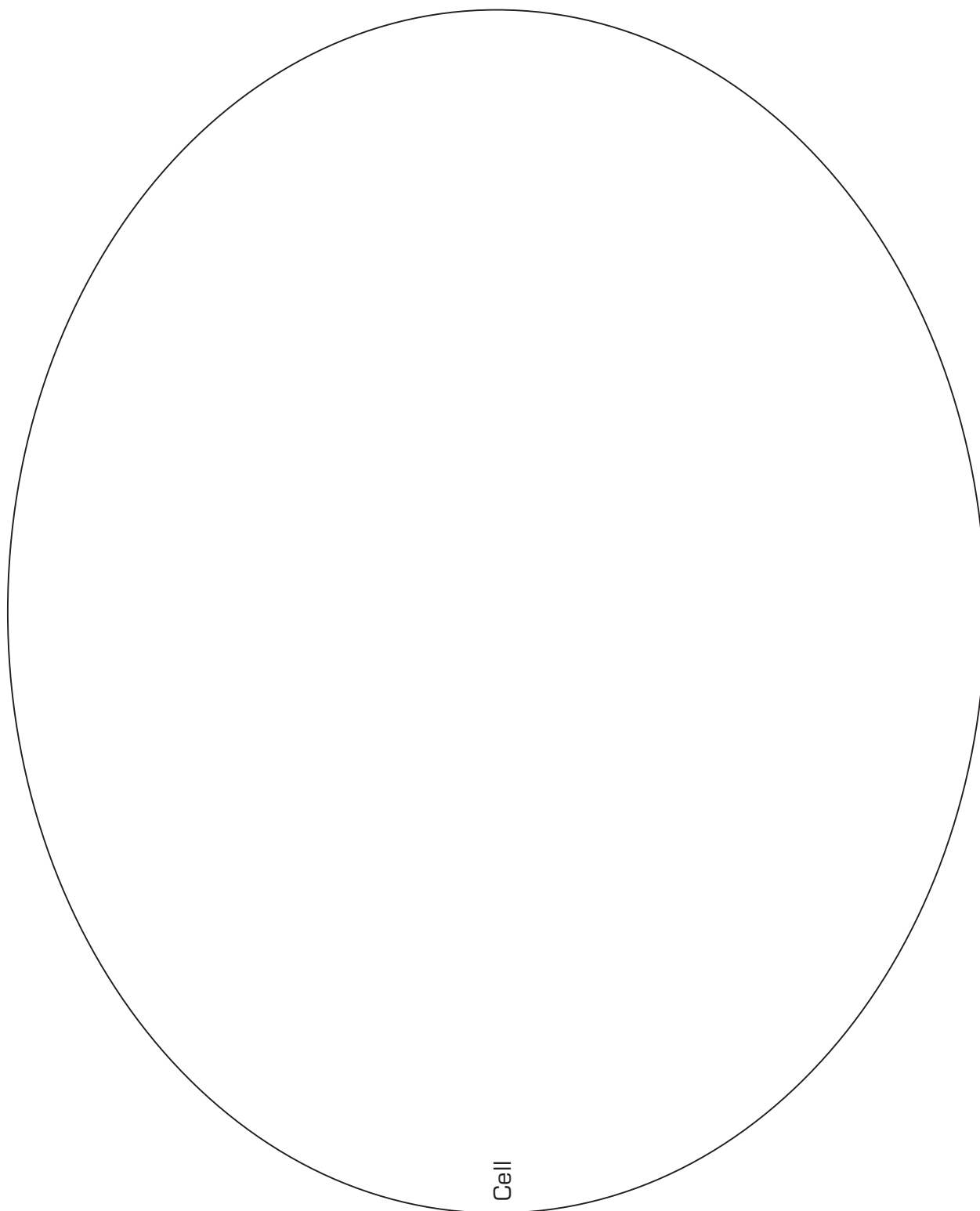
Work in teams of four. Each team will take one set of the Yeast Two-Hybrid Diagram Parts and Instructions. Take a few minutes to put the diagram parts together with the “positive control” parts and then with the “negative control” parts, according to the instructions.

For Part 2, think about one of the “bait” protein examples and list the proteins you would expect to interact with it. List as many ideas as you can come up with. Then look at the data on the transparency, which shows the two-hybrid interactions that were found for the example proteins. Finally, discuss the Discussion Questions as a group.

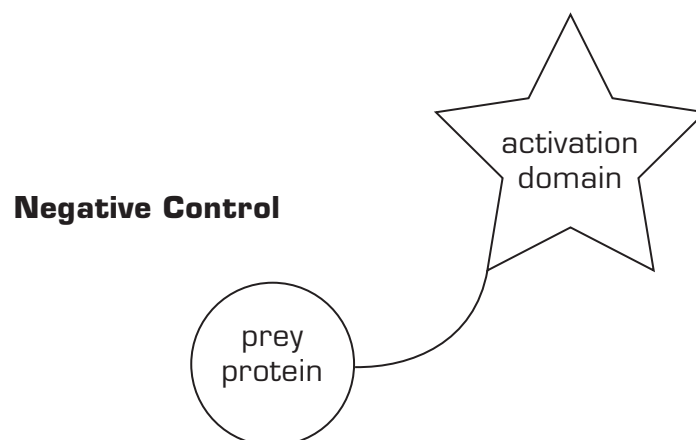
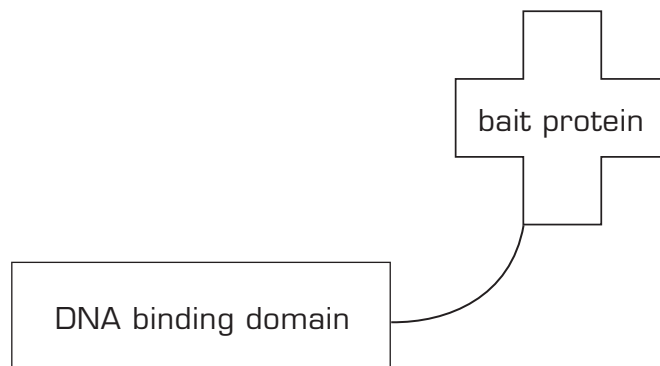
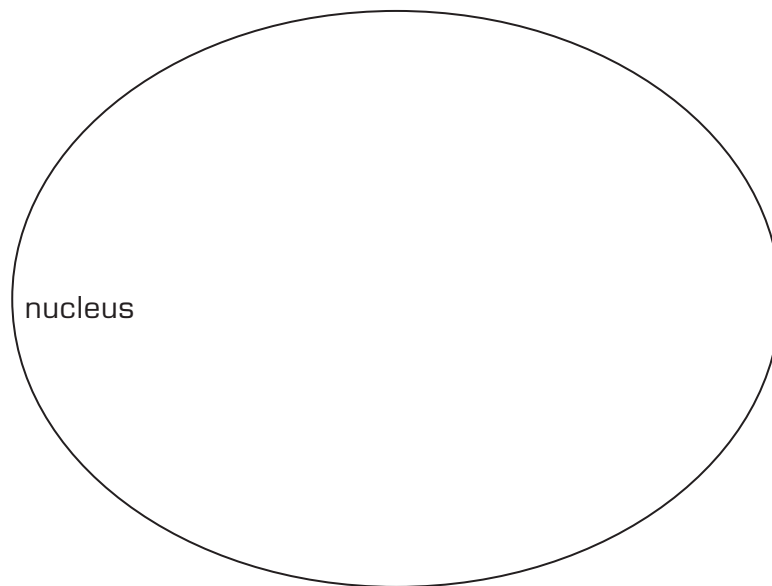
Materials

- One set of the Yeast Two-Hybrid Diagram Parts per four people (master copy provided; to make a set, cut on the dotted lines after copying)
- One copy of the Yeast Two-Hybrid Diagram Instructions per four people (master copy provided)
- Transparency of Prey Data (master copy provided)
- One copy of the Discussion Questions per person (master copy provided)
- One copy of the Proteins and Proteomics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

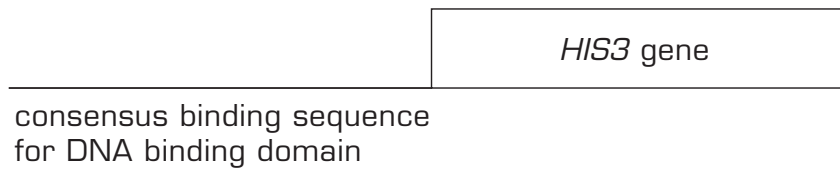
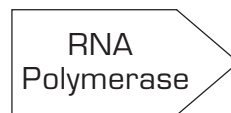
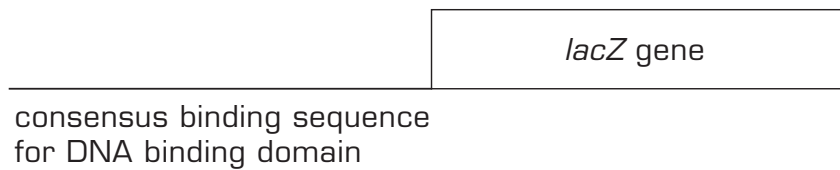
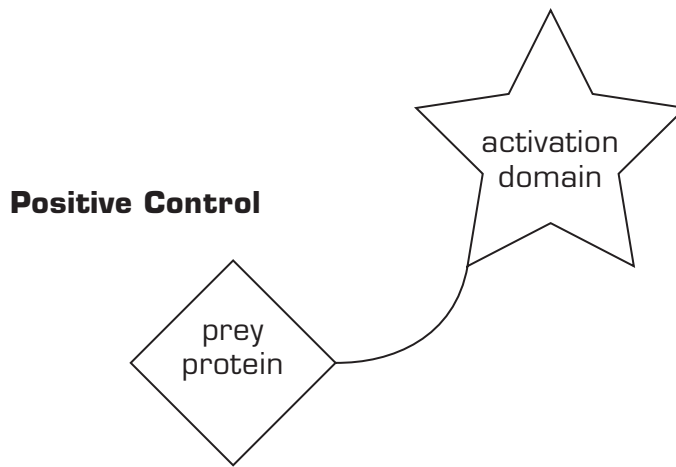
Yeast Two-Hybrid Diagram Parts



(Cut on dotted lines.)



(Cut on dotted lines.)



Yeast Two-Hybrid Diagram Instructions

Part 1:

1. First, check that you have all the parts for the yeast two-hybrid system. The components are:

- yeast cell(s)

A eukaryote that normally synthesizes its own histidine amino acid if it has all the histidine catabolic enzymes.

- a bait protein

The amino-terminal half is a domain that binds to a specific DNA sequence; the carboxyl-terminal half is the protein whose interactions are being investigated.

Yeast cells are genetically engineered to produce this “fusion protein” from a single gene. The DNA binding domain is invariant, used for all two-hybrid experiments. The other half acts as the bait to catch interacting proteins. The bait fusion protein has a nuclear localization signal.

- prey proteins

The amino-terminal half is a protein that might interact with the bait; the carboxyl-terminal half is a transcription activation domain.

Again, yeast cells are genetically engineered to produce this fusion protein from a single gene. The transcription activation domain is invariant, used for all two-hybrid experiments. When brought near a gene, it activates its transcription. The other half of the protein is being tested for interaction with the bait protein. All prey fusion proteins have a nuclear localization signal.

- reporter genes

The *lacZ* gene is from bacteria. It encodes the enzyme β -galactosidase (β -gal), which breaks the glycosidic bond in lactose. Commercially available substrates can indicate the presence of the enzyme by converting from colorless to colored substances when acted on by β -gal.

The *HIS3* gene encodes a histidine biosynthesis enzyme.

Both genes are engineered to have the DNA sequence bound by the DNA-binding domain of the bait protein close to the transcription start of the gene.

- RNA polymerase

This transcribing enzyme is recruited to a gene by transcription activators like the one that is part of the prey proteins.

2. Putting it together:

- a. Start with the Positive Control situation, in which the bait and prey interact. In this case, the bait might be something like alpha tubulin, which is one subunit of the microtubule cytoskeletal protein complex. Put the cell together so all components that should be in the nucleus are in the nucleus. (For this first example, use only the Positive Control prey.)
- b. To start, place the DNA-binding domain part of the bait protein wherever its recognized sequence is found.
- c. If the positive control prey is something like beta tubulin, which is the other subunit of the microtubule cytoskeletal protein complex, we would predict that alpha and beta tubulin would interact, fitting together like adjoining pieces of a jigsaw puzzle.

What will happen at the *HIS3* and *lacZ* genes when both bait and prey are in the nucleus? Will this cell grow on a medium that lacks histidine? If we test with the β -gal substrates, will we detect the enzyme?

- d. Now remove the positive control prey and make the negative control situation. Again put the DNA-binding domain part of the bait protein where its recognized sequence is found.
 - e. If the negative control prey is something like a TCA-cycle enzyme that is normally found in the mitochondrial matrix, and does not interact with the alpha tubulin prey protein, what will happen at the *HIS3* and *lacZ* genes when both bait and prey are in the nucleus? Assuming a reasonably high, steady level of transcription is required to make enough *HIS3* enzyme and β -galactosidase to detect, will this cell grow on a medium that lacks histidine? If we test with the β -gal substrates, will we detect the enzyme?
3. We have now demonstrated how the two-hybrid system works with proteins that do interact (alpha and beta tubulin) and proteins that do not interact (alpha tubulin and a TCA cycle enzyme). Interaction is detected by growth on medium without histidine (because the cells make the *HIS3* enzyme and can produce their own amino acid), and presence of the β -gal enzyme. Lack of interaction is seen when cells that contain both bait and a potential prey do not grow on medium without histidine and do not produce β -gal.

To use this system to investigate interactions between proteins, proteomicists make bait proteins whose interactions are unknown or untested. To find interacting proteins, a bait protein is put into cells and tested against collections of prey to see which ones interact.

Part 2:

4. This system has been used in a large-scale, high-throughput screen of all the yeast open reading frames. Every possible yeast protein was made as a bait and tested against every possible yeast protein made as a prey.

Choose one or more of the following bait examples. Predict the prey proteins that a two-hybrid screen would uncover.

- a. One of the bait proteins was the cytoskeletal protein actin, which is found in animal muscles. Yeast don't have muscles, but they have actin, as do plants. With what prey proteins do you think actin would interact?
 - b. Another bait protein was histone H2A, which is one of the histones found in nucleosomes. With what prey proteins do you think H2A would interact?
 - c. Another bait protein was RAS—which is a protein in the signal transduction pathway between the cell surface—where growth hormone and other signals are received, and the cytoplasm and nucleus, where responses are generated. (See the Cell Biology and Cancer unit for more information.) With what type of prey proteins do you think RAS would interact?
5. Once you have made a list of predicted prey proteins, check it against the data on the transparency; this lists the proteins that interacted with this bait in a two-hybrid experiment.

Prey Data

Actin as bait interacted with these proteins as prey:

- Myo4: a microfilament motor protein
- Fus1: a cell fusion protein
- Glk1: glucokinase
- Aip2: lactate dehydrogenase
- Rpp2B: ribosome component
- Sac6: crosslinks actin filaments
- Oye2: NADPH dehydrogenase
- Bud6: cytoskeletal regulatory protein
- Aip1: actin microfilament severing protein
- Srv2: cellular response to *RAS* signaling, cytoskeletal organization
- Bni1: cytoskeletal regulatory protein

Histone H2A as bait interacted with this protein as prey:

- Nap1 (nucleosome assembly factor)

RAS as bait interacted with these proteins as prey:

- Cdc25: regulatory protein for adenylate cyclase, an enzyme that generates second messengers in signal transduction
- Sdc25: a homolog (similar protein) to Cdc25

Discussion Questions

See the Tips and Suggested Answers.

1. Are the interacting proteins for the bait examples the ones you expected?
 - a. Are there interactions that you expected that didn't appear in the data? If so, how do you explain the lack of interaction?
 - b. Are there any unexpected interactions? If so, what might explain them?
2. What can we learn from discovering new protein interactions or confirming known interactions?

Sources: Uetz, P. et al. *Nature* February 10, 2000.; <http://portal.curagen.com>

Discussion Question Answers

1. Are the interacting proteins for the bait examples the ones you expected?
 - a. Are there interactions that you expected that didn't appear in the data? If so, how do you explain the lack of interaction?
 - b. Are there any unexpected interactions? If so, what might explain them?

A lack of expected interaction could mean that the proteins don't really interact in the cell. However, sometimes known interactions—like between one histone and its partners in the nucleosome—do not show up. Among the many reasons are that the protein structure might change when it is made as a fusion protein with another protein. Structural changes could also mean the “prey” fusion protein is unstable or does not efficiently enter the nucleus. Transient interactions, like between RAS and receptors or kinases, might not be stable enough to be detected in this experiment.

Unexpected interactions could point to a previously unknown function for either the bait or the prey proteins. They could also mean that one or the other protein, when made as a fusion, creates a domain that resembles the interaction domain of another protein; or that one or the other protein is “sticky” and non-specifically binds to many proteins in this type of experiment.

2. What can we learn from discovering new protein interactions or confirming known interactions?

Discovering new protein interactions can illuminate the functions of uncharacterized proteins and determine the regulation of known proteins. This technique has also been elaborated to look at protein-protein interactions that occur only under certain conditions, like the presence of a growth hormone or certain nutrients.

Activity 6: Quick Discussion on Proteomic Profiling

Based on video content

10 minutes

Setup

One of the potential uses of proteomics is to make a profile of a specific organ's proteins (for example, the blood proteins). If data are available for the general population on how this profile typically changes in the presence of a specific disease, proteomic profiling could be used as a fast, non-invasive, diagnostic method. However, as usual with humans and medicine, the reality could be a little more complex. Take a few minutes to discuss these questions about the implications of proteomic profiling.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. Pre-symptomatic screening for cancer and other diseases can be a powerful tool for early detection and treatment. The drawbacks are that not everyone will fit the same pattern of protein expression. Expert interpretation will be crucial because false negatives give an unwarranted sense of security, while false positives may lead to unnecessary treatment or invasive additional testing.

If you had the opportunity to get a full blood proteomic profile that would tell you if you have indicators for several types of cancer, would you do it? If you would, how much faith would you put in the information? If not, why not?

2. Both genetic and proteomic testing might reveal information about a person's current and also potential medical status. For example, either type of profile might indicate a higher than average risk for type-2 diabetes or Alzheimer's.
 - a. Which do you think would be a more accurate indicator of disease risk: a genetic or a proteomic profile? Why?
 - b. For either genetic or proteomic profiling, who should have access to this kind of information? Is there a difference in the privacy issues for information about the gene alleles that you inherited and the proteins produced by your cells?

Activity 7: Quick Discussion on *Deinococcus*

Based on video and online text content

10 minutes

Setup

One of the uses of proteomics is to identify proteins that give a particular type of cell its unique characteristics. In 2002 for example, researchers from Pacific Northwest National Lab, Louisiana State University, and Uniformed Services University of the Health Sciences published over 60 percent of the proteome from *Deinococcus radiodurans* (1,900 proteins). This was one the first, large-scale proteomics projects to publish results. Take a few minutes to consider the ramifications and applications of this information.

Materials

- Transparency of the Discussion Questions (master copy provided)
- Tips and Suggested Answers

Discussion Questions

See the Tips and Suggested Answers.

1. What is *Deinococcus radiodurans*? (See the Genomics unit for more information about this organism.) If you've never heard of it, do you think it is a plant, an animal, a bacterium...?
Does the name tell you anything about its characteristics?
2. *Deinococcus radiodurans* is a high-radiation and stress-resistant bacterium. (It can survive 3,000 times the dose of radiation that is lethal for humans). We already know the genome (entire DNA sequence) for this organism. Why would we be interested in knowing the proteome of this organism? What more could this tell us?
3. What uses and applications can you think of for this bacterium?

Discussion Question #3 Answer

3. The proteome under different stress conditions can be compared so that the proteins that give the bacterium its stress resistance characteristics can be determined.

Some applications being investigated for *Deinococcus radiodurans* include bioremediation of pollution sites that are severely contaminated with chemicals or radiation, and use in space travel for producing medicines and recycling waste.

Notes

Activity 1: No Cut-and-Dried Answers

Based on video and online text content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

Before viewing the video on Cell Biology and Cancer, look at the ten statements on the Yes/No, But... handout. Take a few minutes to read each, and write “Yes” if you think the statement is true or “No” if you think it is false. The statements are rather cut-and-dried, but biology—especially cancer biology—rarely is. Therefore, for some of the statements you might want to answer “Yes, but...” or “No, but...” In those cases, write a phrase or two about what makes you want to qualify your answer. After viewing the video and reading the text, look through your list again to see if any of your ideas about cancer were changed and if any were reinforced.

Materials

- One copy of the Yes/No, But... statements per person (master copy provided)
- Tips and Suggested Answers

Yes/No, But...

Write either “yes” or “no” next to each question. You may want to qualify your answer with a phrase or two about why this cut-and-dried statement really can’t be answered with a simple yes or no. See the Tips and Suggested Answers for possible points of view.

1. Cancer “runs in families”; it is inherited from one’s parents.
2. Cancer is a genetic disease.
3. “Cancer” is really hundreds of different diseases.
4. Over half of cancers in this country are preventable.
5. Scientists no longer believe that viruses cause cancer.
6. Oncogenes are cancer-causing genes.
7. The function of “tumor suppressor genes” is to suppress tumors.
8. Telomeres (the ends of eukaryotic chromosomes), get shorter each time the chromosome is replicated and the cell divides.
9. Prostate cancer can be diagnosed with a simple blood test for prostate-specific antigen (PSA).
10. Twenty-five percent of people in the U.S. will develop cancer in their lifetime.

No Cut-and-Dried Answers: Possible Answers and Qualifying Statements

1. Cancer “runs in families”; it is inherited from one’s parents.
A person can inherit a *predisposition* or an *increased risk* for certain types of cancer by inheriting “mutant” alleles of genes that are involved in cell division.
2. Cancer is a genetic disease.
Cancers result from mutations in genes, so they are genetic diseases. However, environmental factors like exposure to mutagens and carcinogens may cause the mutations that lead to cancer.
3. “Cancer” is really hundreds of different diseases.
All cancers, fundamentally, are cells undergoing uncontrolled cell division. However, there are many mutations and combinations of mutations that can lead to this outcome.
4. Over half of cancers in this country are preventable.
Preventing most of the cancers in the U.S. would require stopping smoking, cleaning up environmental hazards, and changing our diet—an ideal goal that might not be entirely practical. Also, spontaneous mutations caused by errors in DNA replication and other random events mean that some cancers are inevitable.
5. Scientists no longer believe that viruses cause cancer.
Viruses are no longer thought to be the sole cause of cancer, but some viral infections are associated with cancers—for example Hepatitis B and liver cancer, or papilloma virus and cervical cancer—because they can cause mutations that contribute to cancer.
6. Oncogenes are cancer-causing genes.
An oncogene is a mutated form of a gene that normally functions in growth and development; the mutated gene can contribute to the development of a cancer cell. The unmutated version is called c-oncogene, cellular oncogene, or proto-oncogene, although the term “oncogene” is sometimes used for both the mutated and unmutated form.
7. The function of “tumor suppressor genes” is to suppress tumors.
Tumor suppressor genes did not evolve to suppress cancers, but to play a part in the normal regulation of cell division. However, when their normal function is lost through mutation, this contributes to cancer.
8. Telomeres (the ends of eukaryotic chromosomes), get shorter each time the chromosome is replicated and the cell divides.
Telomeres get shorter with each cell division in normal adult human cells. This is not true in all cells; for example, in germ line cells, many plant cells, and some cancer cells, the telomeres do not get shorter.
9. Prostate cancer can be diagnosed with a simple blood test for prostate-specific antigen (PSA).
PSA is normally present in the blood of adult males, so just its presence, even at a somewhat high level, does not definitively indicate prostate cancer. Not all prostate cancers cause elevated levels of PSA. However, a high level of PSA is an indication that further testing should be done.
10. Twenty-five percent of people in the U.S. will develop cancer in their lifetime.
The lifetime risk for women in the U.S. is 1 in 3; for men it is 1 in 2. However, these are generalized numbers for the entire population.

Activity 2: The Price of Proto-Oncogenes

Based on video content

15 minutes (during the video)

Setup

Stop the video after about five minutes, just after Dr. Robert Weinberg says, “Without the proto-oncogenes, embryos wouldn’t be able to develop, adult tissues would not be able to be maintained. However, the price of carrying these proto-oncogenes in our genomes is occasionally they become damaged and mutated and convert into oncogenes and thus become converted into agents for causing cancer.”

Take a few minutes to think of what cellular functions normal proto-oncogenes might have. Remember, these are genes that have a role in normal cell division for growth, development, and cell replacement. Make a list of some functions they might perform, then discuss the discussion questions. Restart the video and see how some proto-oncogenes normally function.

Materials

- One transparency of the Discussion Questions

Discussion Questions

1. One of the proto-oncogenes that is frequently found to be mutated in a cancer encodes the protein Ras. It is found just on the inside of the cell's plasma membrane and is activated when signaling molecules, like growth factors, bind receptors. What might Ras be doing?
2. Some studies have found an association between long-term hormone replacement therapy for menopause symptoms and an increased risk for certain types of cancer. What might be the connection between hormone therapy and an increased risk of cancer?
3. One treatment for some types of cancer includes drugs that block estrogen receptors and antiandrogens, which block testosterone production. Speculate on how this therapy might slow or prevent the growth of cancerous cells.

Activity 3: Family History

Based on video and online text content

60 minutes

Setup

In 1990, the first gene associated with early-onset inherited breast cancer was mapped to chromosome 17. The technique for mapping a gene to a chromosome is to follow simultaneously the inheritance of the trait caused by the gene, and the inheritance of easily followed sequences called markers on all the chromosomes. Genes and markers that are on different chromosomes or far apart on the same chromosome will segregate randomly. Genes and markers that are close to each other on a chromosome tend to be inherited together. Only occasionally will crossing-over occur between them. Because the chromosomal location of the marker is known, the tendency of a trait and a marker to inherit together maps the approximate location of the gene on a particular chromosome. The frequency of the occasional crossing-over events give the distance between the marker and the gene.

The technique is a great deal of work, but it is a classic pedigree and statistical analysis. In this exercise, you'll see some of the pedigrees used to locate the BRCA1 gene and you will go over some of the theory behind the technique. Working in pairs, start by discussing the background questions. Then get copies of the Pedigrees and Data sheet and LOD Score Information sheet, and work through the exercises. As a group, talk about the discussion questions at the end.

Materials

- One copy of the Background Questions for each person (master copy provided)
- One copy of the Pedigrees and Data sheet for each person (master copy provided)
- One copy of the LOD Score Information for each person (master copy provided)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Background Questions

Read these questions and discuss them before starting on the Pedigrees and Data sheet. Answers are in the Tips and Suggested Answers.


1. Breast cancer can arise from both inherited and spontaneous mutations. The alleles that are responsible for inherited breast cancer are incompletely penetrant, which means that people who inherit the allele do not always show the phenotype. How does this cause problems for studying inherited cases of breast cancer? What criteria would you use to find families to use in a study of inherited breast cancers?
2. How would you confirm cases of breast cancer, even if the person in the family was deceased? How would you confirm that someone did NOT have breast cancer?
3. What would you use for chromosome markers to follow along with the inherited trait?
4. Up to 25 percent of pedigrees are incorrect because parentage is not as expected. What would you do to correct for this?


Pedigrees and Data


After reading and discussing the background questions, work through these pedigrees by answering the following questions. The pedigrees are modified from ones done for the study that identified the chromosomal region with BRCA1.

1. Do the pedigrees suggest autosomal or sex-linked inheritance? Dominant or recessive?
2. Read the LOD Score Information sheet. If a hypothesis about linkage between a gene and a marker has a high LOD score (for example, 4) what does it mean? If a hypothesis for linkage has a low LOD score (for example, -2) what does it mean?
3. For each family, look at the pedigree and the LOD scores and answer these questions:
Is there evidence of linkage between marker D17S74 and breast cancer? If not, give an explanation in a few sentences to your partner and see if s/he agrees, disagrees, or has any clarifications to add. If there is evidence of linkage, what allele of the marker, in this family, is linked to the disease allele of the gene? Why is it not the same in all families?

Legend for Pedigrees

 Circle, Female

 Square, Male

 Open, Unaffected

 Filled In, Affected

 Deceased

If living and unaffected—age at last interview for study is given.

If affected—age at diagnosis with breast cancer is given.

If deceased—age at death is given.

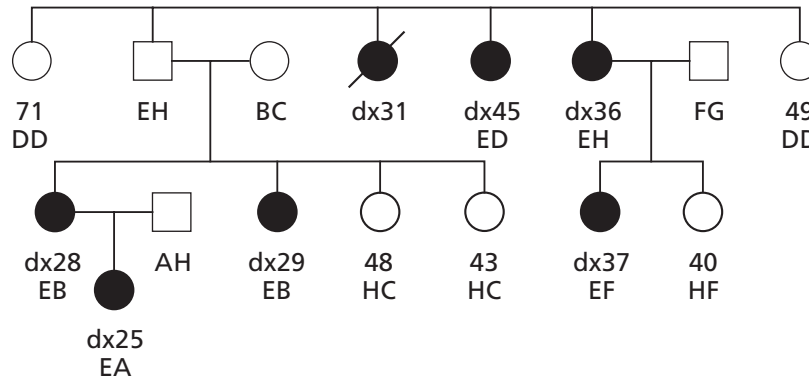
Two letters—represent allele of the D17S74 marker, which was found to be the most closely linked to the breast cancer susceptibility gene.

One letter—given when one allele is known, and the other is unknown.

Family 1

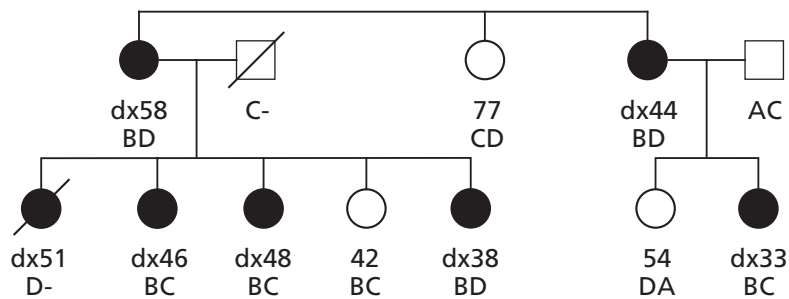
mean age of onset: 33

LOD score for linkage of breast cancer to D17S74 (recombination fraction 0.001) = +2.36

**Family 7**

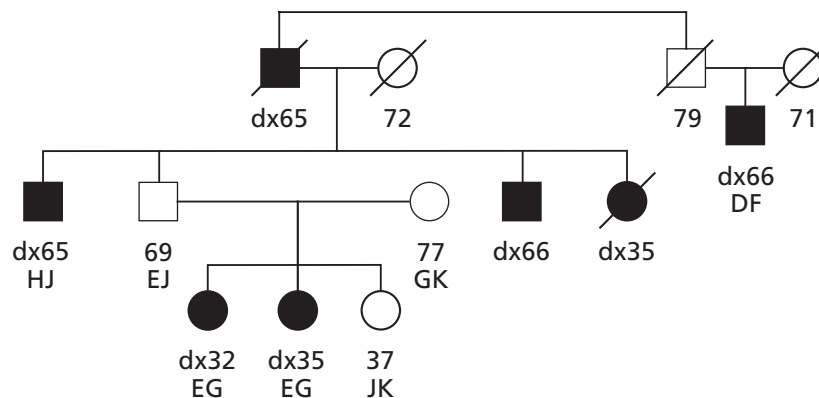
mean age of onset: 44

LOD score for linkage of breast cancer to D17S74 (recombination fraction 0.001) = +0.7

**Family 16**

mean age of onset: 52

LOD score for linkage of breast cancer to D17S74 (recombination fraction 0.001) = -2.71

Sources: Hall et al. 1990. *Science* 250:1684

LOD Score Information

LOD stands for “log of the odds of linkage.”

In this case, “linkage” means the gene for the trait of inherited tendency for breast cancer is located close on the same chromosome to a genetic marker—for example, a small, variable sequence that is easily followed. Traditionally, linkage is measured in units of recombination frequency, with one map unit equaling one percent recombination. In this case, we’re following inheritance of risk for breast cancer and inheritance of a marker—for example a restriction fragment polymorphism or a single nucleotide polymorphism (SNP) (see the Genomics unit). If 1 individual out of a 100 observed showed evidence that crossing over changed the combination of marker and breast cancer gene on a chromosome, that would be a one percent recombination frequency, or one map unit.

To standardize the evaluation of evidence for linkage, the LOD is calculated. “Log of the odds” is the \log_{10} of a ratio, calculated for a number of different possible values of X, as shown below:

LOD =

$\log_{10} \frac{\text{probability that the observed pattern of inheritance occurred by chance}}{\text{probability that it occurred because the gene and the marker are linked X map units apart}}$

A series of LOD scores shows the probability of linkage at several certain distances. Because it is a \log_{10} value, a LOD score of 3 means odds are 1000 to 1 in favor of linkage. LOD 4 means the odds are 10,000 to 1 in favor of linkage. LOD scores of 3 to 4 are generally accepted as indicating linkage.

Discussion Questions

Possible answers are in the Tips and Suggested Answers.

1. What are some explanations for pedigrees included in this study that showed negative LOD scores?
2. In the region identified by the linkage analysis were several candidate genes. Among them were a gene with homology to human epidermal growth factor receptor, a gene involved in steroid hormone synthesis, and a protein that binds the possibly anticarcinogenic retinoic acid. How would mutations in these genes contribute to cancer?
3. From the marker allele inherited, some of the women in the study should have inherited a breast cancer susceptibility allele, yet did not have breast cancer. What are some explanations?
4. Breast cancer is not the most common cancer in women (lung cancer is the most common). Only 5–10 percent of breast cancers are inherited, and of these, only 15–20 percent are attributed to mutations in BRCA1 or BRCA2. Do you think it is worth the effort to find genes implicated in inherited breast cancer, or is it better to spend the research dollars to investigate the 90–95 percent of breast cancers that are not inherited?

Family History

Background Question Answers

1. The criteria used by the authors of this study were families with extended history and many cases of breast cancer, younger age at diagnosis, frequent bilateral breast cancer (both breasts), and higher incidence of breast cancer in men in the family.
2. The authors of this study obtained pathology records, medical records, or death certificates for any individuals who had had surgery. For individuals who did not have breast cancer, the authors relied on self-reporting for living family members, and death certificates or reports from relatives for deceased family members.
3. The authors of this study used VNTRs (variable number tandem repeats), which are repeated sequences whose number varies widely in the population; and RFLPs (restriction fragment length polymorphisms), which are sequence variations that can be detected with a restriction enzyme.
4. The authors of this study followed inheritance of 183 different markers to confirm parentage.

Discussion Questions, Possible Answers

1. Explanation of LOD scores
Note that for families 7 and 16, with low and negative LOD scores, the age of onset is relatively late. The gene identified in this study is responsible for early-onset familial breast cancer. The explanations given by the researchers for low and negative LOD scores in some families include a mutation in a different gene (for example, the BRCA2 gene that was discovered later); or a high incidence of spontaneous cases of breast cancer just by chance.
2. How might mutations contribute to cancer?
Actually, BRCA1 turned out to be a completely new gene. None of the candidates first identified in the region were responsible for inherited breast cancer.
3. Explanations between inheritance of marker but not breast cancer
Linkage between particular markers and gene alleles can change if crossing-over occurs between them. Inheritance of the breast cancer alleles just increases risk, it does not always result in cancer.

Activity 4: Dilemmas of Cell Biology

Based on video and online text content

60 minutes

Setup

The topic of cancer is woven tightly into other topics of biology—like cell division, cell death, aging, and medical ethics. This exercise comes with three packets of information, each addressing a different issue in which cancer research has an impact on another area of biology. Divide into three teams. Each team should take one packet of information, go through the exercises, and discuss the questions. After 15–20 minutes, pass your packet to the next team and take one from another team. Continue until all teams have gone through all packets.

Materials

- One copy of each of the three different Topic Packets (master copies provided)
- Three copies of the Cell Biology and Cancer unit online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

Topic Packet 1: p53

Part1: As a team, discuss these points.

1. What is the function of p53?
(Here's a suggestion for how to approach this part: As a team, make a list of what you know about p53, with one person writing down all the ideas. After a minute or so, organize your list into a description of p53's function.)
2. If you didn't cover this idea in question 1, consider how a loss-of-function mutation in p53 would affect cell division. Would this mutation be dominant or recessive? That is, would a mutation in one copy of the p53 gene be sufficient to see the phenotype or would two mutant p53 alleles be necessary?
3. Is p53 classified as a tumor suppressor or an oncogene?
4. The human p53 gene has been cloned and could, theoretically, be used in gene therapy. Come up with a strategy for introducing unmutated alleles of p53 into cells that have p53 mutations. What might be some of the dangers, drawbacks, or limitations of your strategy? What might be some of the advantages of your strategy over other cancer therapy methods?

If your team is unfamiliar with gene therapy, read the following hints:

- a. Most current gene therapy techniques introduce a gene in an inactivated virus that can infect a cell but cannot produce more viruses.
 - b. Some gene therapies are designed to integrate the introduced gene permanently into the chromosome, but the gene inserts into a random location. We cannot direct where in the genome the gene will be inserted, so it may disrupt or alter the expression of another gene.
 - c. The introduced gene is not always expressed at precisely the same level as the normal gene. The cell with the introduced gene might express a little more or a little less of the protein than normal.
 - d. Successful use of this gene therapy technique requires a virus that can be engineered to infect and not replicate. The virus must be able to access and infect the targeted cells. In addition, if the therapy is done by removing cells and infecting them in vitro, the corrected cells must be reintroduced into the patient.
 - e. New gene therapy techniques may use small interfering RNAs (siRNAs) that *prevent* expression of a gene by causing its mRNA to be degraded.
5. Do you think there would be any consequences to introducing *too much* p53? If so, what would they be?

Part 2: After discussing points 1–5, read the following summary of work done by Dr. Lawrence A. Donehower of Baylor University and answer the following questions as a team.

In 1992, researchers in the Donehower lab made “p53 knockout” mice that had their p53 genes knocked out, so they completely lacked functional p53 protein. The mice died of cancer at an early age.

In 2002, they tried to make more mutant p53 mice, but these were not knockout mice. Instead, a p53 gene was introduced that still produced protein, but had a small change in the amino acid sequence. The mice they generated did not get cancer, but developed a different phenotype. They looked “old.” They also had osteoporosis, shriveled organs, and a shorter lifespan.

6. What do you think happened? Explain the relationship between mutant p53 and the phenotypes it caused.

After discussing point 6, read the following.

Dr. Donehower’s lab investigated the mice that became “old” quickly and, as you might have deduced, they were producing *extra* amounts of normal p53.

One of p53’s functions is inducing apoptosis (programmed cell death). In a young organism, organs that require a continuous supply of new cells, like skin, blood, and intestinal lining, produce many new cells. In fact, they can produce more than are needed, by division of stem cells.

7. Why might extra p53 cause a young mouse to age prematurely?
8. UV light causes DNA damage that activates p53. What is a possible connection between UV light and skin that looks “old”?
9. People with Li-Fraumeni syndrome have mutations in the p53 gene that increase their risk of cancer by approximately 100-fold. At this point in our knowledge of p53 and our abilities in gene therapy, would it be possible, practical, and ethical to use gene therapy to correct Li-Fraumeni syndrome? If you would use gene therapy, would you use the technique that introduces an unmutated version of the gene, or a technique like siRNA that prevents expression of a gene?

Sources: *New York Times*. 2002. Cancer fighter exacts a price: cellular aging. Jan 8.;
Science. 2003. Aging and genome maintenance: lessons from the mouse? 299[5611]:1333–34.
February 28.;
<http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispim?151623>

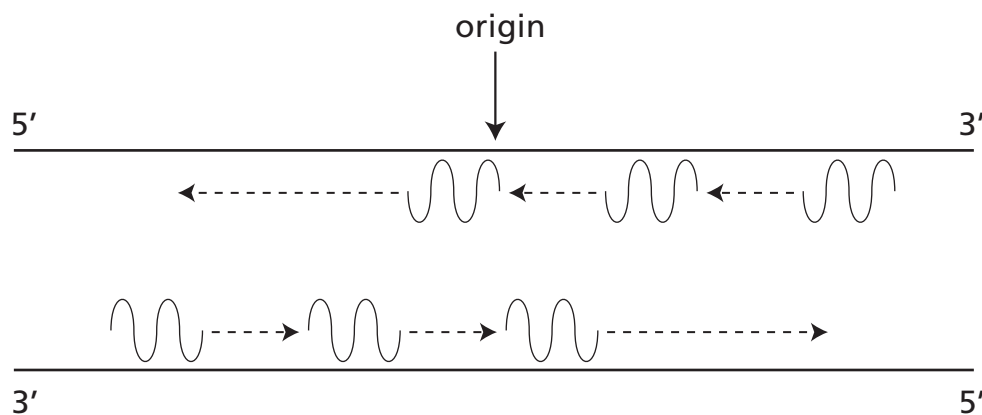
Topic Packet 2: Telomerase

As a team, discuss these points.

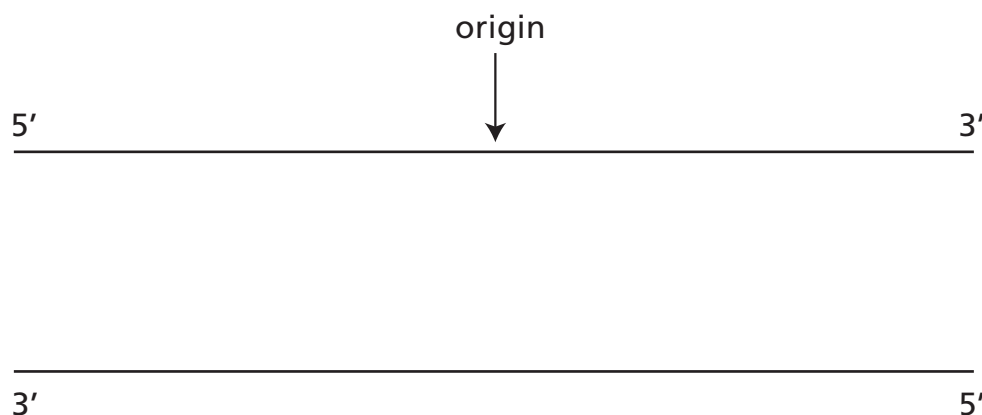
1. Start by describing the telomeres of chromosomes and their functions as completely as possible. Review the video and Cell Biology and Cancer online text chapter if necessary. What happens to a chromosome end that lacks telomeric sequences? What are the consequences for the cell?
2. Complete this drawing of the replication of a linear chromosome by:
 - a. Marking 5' and 3' ends on Part 1, and then filling in rest of the newly replicated strands and Okazaki fragments.
 - b. Showing how the DNA will look as the RNA primers are removed on Part 2.
 - c. Showing what the DNA polymerase that removes and replaces the RNA primers will use as a primer and what it will use as a template on Part 2.

Figure legend: Primers made by RNA primase are shown as wavy lines. The newly made strands are dotted lines. Their synthesis started from replication origins in the middle where the "origin" arrow indicates.

Part 1:



Part 2:



3. Using the drawing in 2b as a guide, describe the chromosomal replication problem that is solved by the enzyme telomerase. (Side issue: Do bacterial cells need telomerase? What about viruses?)
4. At which chromosome ends would telomerase be found?
5. In what types of human cells is telomerase active? (You can review Dr. Elizabeth Blackburn's interview in the video, or information in the online text.)
6. For a long time, it was thought that all DNA polymerizing enzymes required a template—a single strand with a sequence complementary to the strand the DNA polymerase enzyme would make. Look at the drawing above. How does telomerase work without a template strand?

If your group is unfamiliar with the details of telomerase, read the following hint:

Telomerase is not a single protein enzyme, but a complex. One of the genes that encodes a telomerase subunit does not encode a protein. When the gene for this subunit is transcribed, the result is not an mRNA, but an RNA that functions on its own.

7. Knockout mice can be made that are completely lacking the telomere subunit you discussed in point 6, because the gene for the RNA has been “knocked out.” What do you think is the phenotype of these mice?

After discussing point 7, read the following:

Mice that are *mtr*^{-/-} are lacking the essential RNA component of telomerase. This component acts as the template for addition of telomeric repeated sequences to the 5' ends of newly replicated DNA, which would otherwise be shortened by the removal of the RNA primer used to start its synthesis. Without the RNA component, telomerase does not work.

The first generation of *mtr*^{-/-} mice are fine; but after three or four generations of breeding mice with this genotype to each other, the offspring show premature gray hair, loss of hair, impaired healing of wounds, cancer, and shortened lifespan.

8. When the cloned sheep Dolly was born, everyone was curious about how long the telomeres of her cell's chromosomes would be. Why? (Hint: She was cloned from a somatic cell of an adult sheep that was already several years old.)
9. Activation of telomerase in cells that lack it has been suggested as a mechanism for delaying aging or extending lifespan. What is your opinion about this idea? Would it work, in principle? What would be the expected negative consequences?

Source: de Lange and Jacks. 1999. *Cell* 98:273.

Topic Packet 3: BRCA and APC

As a team, discuss these points.

1. Start by dividing your team into two sides. Each side will discuss either case a or case b. After a few minutes, each side explains to the other side the situation and the conclusions it reached.
 - a. You have a close friend whose paternal grandmother, father, and brother all had colon cancer by age 35. Your friend is worried about his risk for colon cancer and is asking for your advice.
 - Is there a test he can take that will evaluate his risk?
 - What is the test? How often does it have to be administered? Does it require a colon sample, a blood sample, samples from his relatives...?
 - Is there more than one test? If so, what does each measure?
 - b. You have a close friend whose grandmother, mother, sister, and aunt (on the maternal side) all had breast cancer by age 40. None of them were genetically tested, but your friend is worried about her risk for breast cancer and is asking for your advice.
 - Is there a test she can take to evaluate her risk?
 - What is the test? How often does it have to be administered? Does it require a sample of breast tissue, a blood sample, samples from her relatives...?
 - Is there more than one test? If so, what does each measure?
2. The colon cancer side reads c below. The breast cancer side reads d below. After a few minutes, each side explains the information to the other side.
 - c. Colorectal cancer is the second leading cause of cancer deaths in the U.S. Hereditary colon cancer comes in two forms: FAP (familial adenomatous polyposis) and HNPCC (hereditary nonpolyposis colorectal cancer). Genetic tests are available for the common mutations in these genes. Here is some information about each type of hereditary colon cancer.

FAP

 - less than one percent of colorectal cancers
 - the inherited mutation is in the APC (adenomatous polyposis coli) tumor suppressor gene
 - causes hundreds of polyps to form in the colon as early as age 10 and almost always by age 35
 - failure to remove the polyps almost always leads to cancer
 - 67 percent of FAP patients who see a doctor about symptoms (diarrhea, rectal bleeding) have cancer
 - if a person tests positive for the mutant APC gene, the recommended course is annual colonoscopy

HNPCC

 - the inherited mutation is in one of at least five different genes that function in DNA mismatch repair
 - causes colon cancer at an age of onset of about 45
 - lifetime risk of cancer can be as high as 85 percent
 - if a person tests positive for a mutant allele annual, colonoscopy starting at age 20–25 and annually after age 35

Some tests are physical or visual—like digital rectal exams, colonoscopies, and x-rays. Feces can be tested for minute amounts of blood. For those with a family history, blood marker tests and genetic tests may be recommended.

Does this information change or add to the advice you would have given to your friend? How would you summarize this information, and where would you advise your friend to go for more information?

- d. Approximately 5–10 percent of breast cancer cases are hereditary, which means that a contributing factor is the inheritance of a mutant gene. The major causes of hereditary breast cancer are mutations in either BRCA1 or BRCA2. In general, a woman's lifetime risk for breast cancer is about 12 percent, but inheriting a mutation in one of these genes increases lifetime breast cancer risk to 40–80 percent.

Some mutations are more common in certain populations. A woman in a family with a history of breast cancer could be tested for one of these known mutations. However, if the mutant allele is present, it indicates only an increased risk for breast cancer, because not everyone with a mutant allele will develop breast cancer. On the other hand, if no mutant alleles can be detected, it might be because the family has a different mutation than the ones that are normally tested for. Both the BRCA1 and BRCA2 genes are large, so sequencing the entire genes to look for mutations is not commonly done.

Does this change or add to the advice you would have given to your friend?

3. Would a loss-of-function mutation in APC be dominant or recessive? That is, would a mutation in one copy of an APC gene be sufficient to see the phenotype of increased cancer risk, or would two mutant alleles be necessary? Answer the same question for HNPCC, genes and for BRCA1 and BRCA2.
4. Are the APC, HNPCC, BRCA1, and BRCA2 genes classified as tumor suppressors or as oncogenes? Explain what these classifications are and how genes are put into one category or the other. What was Dr. Mary-Claire King's objection to the "tumor suppressor" categorization? (You can view her interview for the *Rediscovering Biology* project on the video, or read the transcript at <http://www.learner.org/channel/courses/biology/>.)
5. One possibility for people who inherit an allele that increases their risk for a certain type of cancer, like colon or breast cancer, is prophylactic surgery. What do you think this means for colon cancer? What does it mean for breast cancer? Another possibility is prophylactic chemotherapy. What does this mean?

After discussing the question, read the following:

Prophylactic colectomy (removal of colon before cancer develops) is recommended for people with the APC gene only if they have multiple polyps. It is not recommended for people with a family history of HNPCC, or people with the APC gene who do not develop multiple polyps.

Research in 1999 found that prophylactic mastectomy was associated with a 90 percent reduction in incidence in women with a family history of breast cancer. Research in 2001 showed that tamoxifen treatment had little or no effect on women with BRCA1 mutations, but women with BRCA2 mutations had significant benefit.

6. A study in the *Journal of the American Medical Association* found that only 43 percent of family members predisposed to colon cancer because of an HNPCC mutation were likely to get a genetic test to see if they carried the mutant allele. Does this figure seem high or low to you? What factors affect a person's decision to be tested for a gene allele like an APC or HNPCC allele that indicates a high risk for colon cancer, or the BRCA1 or BRCA2 alleles that indicate a high risk of breast or ovarian cancer?
7. In 1994, *Science News* published four real-life ethical dilemmas that genetic counselors had encountered and asked readers for their opinions on the solution. One of the situations was a 30-year-old woman who had been diagnosed with familial adenomatous polyposis (FAP). Doctors and genetic counselors suggested that she inform her siblings and have her children tested. She refused.
Should the genetic counselors inform the family members of their risk, against the woman's wishes?

After discussing the question, read the following:

Although the readers who sent in their opinions split 50–50 on whether or not to inform the family, a panel of four genetic counselors and medical ethicists all agreed that the family should be informed, regardless of the woman's stated intentions.

Sources: Rosen et al. 2003. BRCA1 gene in breast cancer. *J. Cell Physiol.* 196[1]:19–41.
<http://searchosp1.nci.nih.gov/whealth/whr0001/breast.htm>;
<http://www.docguide.com/dg.nsf/PrintPrint/2DC8DA5F0525AD7A852567670068BA5D>;
<http://www.screening-for-colon-cancer.com/index.php3>;
http://www.sciencenews.org/sn_edpik/ms_2.htm

Activity 5: The Big Picture

Based on video and online text content

30 minutes

Setup

The overall goal of cancer research has, of course, been to cure cancer. Although Nixon declared war on cancer in 1971, we haven't found a cure. However, we have learned a great deal about the causes of cancer and how to treat it effectively, as well as fundamental principles about cell division, mutation, and gene regulation. As a final wrap-up to this unit, discuss the following questions about the big picture of cancer and cancer research.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. Billions of dollars have been spent on cancer research in the U.S. Just to give you an idea, the National Cancer Institute's 2003 budget was around \$4.6 billion. Consider what we have learned about the basic workings of the cell and the function of human genes. Weigh the state of cancer prevention and therapy today against the amount of money we have spent.
 - a. In your opinion, has it been worth it?
 - b. How much more money and time will it take until cancer is as life-threatening as, say, a bacterial infection that can be treated with antibiotics?
 - c. What do you see as the future of cancer research and cancer therapy? What new therapies have you heard about or could you imagine being developed?

After discussing question 1c, read the following and answer the questions:

RNA interference, or RNAi, is a mechanism that effectively silences genes completely and with great efficiency. It is found in nearly all organisms—from petunias to worms to mammals. RNAi is initiated by the introduction of double-stranded RNA of a sequence that is designed to target specific genes. The double-stranded RNA triggers a cascade of events, leading to the stimulation of the enzymes Dicer and RISC. These enzymes destroy specific mRNAs, preventing expression of a gene by eliminating any RNA produced from it. So far, interfering RNAs have been able to shut off expression of specific genes in plants, worms, fruit flies, and mammalian cells.

RNAi has been suggested as a possible therapy for many diseases, including cancer. How might it be used to treat cancer? What information would be needed before RNAi therapy for a specific cancer could be started? What difficulties do you foresee for the development of this treatment?

- d. What is your opinion on the amount of attention and research funds that are spent on cancer, compared to other diseases that we are all concerned about? Compare cancer programs to, for example, emerging or re-emerging infectious diseases such as AIDS and tuberculosis; illnesses of Western cultures, like adult onset-diabetes and obesity; mental illness; or any others you can think of. Where would you allocate funds, if it were up to you?

2. Breast cancer occurs less frequently in African American women than white women (100 cases/100,000 vs. 114/100,000). However, the five-year survival rate for African American women is 69 percent, while for white women it is 84.4 percent.

In 1996, the colorectal cancer death rate was 16.4 per 100,000 for white Americans and 22.5 per 100,000 for black Americans.

The death rate for all cancers combined is about 30 percent higher for blacks than for whites.

- a. List factors that might contribute to these disparities.
 - b. Of the possible contributing factors you have thought of, how many are biological and how many are societal?
 - c. What do you think can be done to correct racial disparity in survival and treatment of any diseases, not just cancer?
3. In your biology classes, have you used cancer as a framework for lessons on, for example, cell division or cell signaling? If so, how have you used cancer examples? Will you change your lessons as a result of what you have learned from this unit? If not, will you be incorporating cancer examples in your lessons?
4. As teachers, we have an opportunity to pass on information about cancer causes and prevention to our students. What do you think is the single most important piece of information you can provide to your students? How can it be presented so it has a lasting impact?
5. As teachers, we process a great deal of information about cancer and carcinogens, from advice presented to the general public, to more specialized information on the biology of cancer. As the result of your reading, have you made (or plan to make) any lifestyle changes, with the goal of reducing your own risk for cancer?

Sources: http://cis.nci.nih.gov/fact/1_1.htm;
<http://www.nature.com/horizon/rna/background/interference.html>

Notes

Activity 1: Indescribable

Based on video and online text content

30 minutes

Setup

"...there's this huge diversity out there that has never been described. More than 90 percent of the diversity we see in the environment we've never described." So says Dr. Anna Louise Reysenbach, whose job as a microbial ecologist is to collect and investigate microorganisms. This exercise will explore the challenges of finding and characterizing diverse microbes.

Working in pairs, use the terms explained in the Microbial Diversity online text chapter to describe the metabolism and growth conditions of several microbes that will be highlighted in the video. Then, consider how a researcher who wanted to study them would use this information to cultivate them in a lab.

Materials

- One copy of the List of Organisms and Instructions per person (master copy provided)
- One transparency of the Table of Terms from the Microbial Diversity online text chapter (master copy provided)
- Tips and Suggested Answers

List of Organisms and Instructions

See Tips and Suggested Answers for answers to some questions.

Exercise 1:

1. First, familiarize yourself with the terms that describe the metabolism and growth conditions of microorganisms. Using the terms on the overhead, describe each organism listed below, based on the information that is provided about it.
 - a. *Thermocrinus ruber* grows in hot springs. It oxidizes inorganic molecules for energy and fixes carbon dioxide to generate complex organic molecules.
 - b. *Pseudomonas aeruginosa* is a soil bacterium that is an opportunistic human pathogen; for example, it can live in the mucus-covered lung tissue of a cystic fibrosis patient.
 - c. *Acidithiobacillus ferrooxidans* uses acidic conditions to oxidize iron and sulfur.
2. Now consider how you would cultivate each of these microorganisms in the lab. List the general categories of ingredients you would use if you had to make culture medium on which to grow each these organisms. What approximate conditions of temperature, pH and O₂ levels would you provide?
3. Look at the culture medium recipes you generated in question 2. Would any of the organisms on the list grow in a medium other than its own?

Exercise 2:

1. One of the challenges for microbial ecologists like Dr. Anna Louise Reysenbach is cultivating novel microorganisms. Specifically, she says "...just try to second guess what these organisms like to eat from the environment! Consequently, we've only been able to actually grow less than perhaps 1 percent of all the organisms you see under the microscope."

For each of the following environments, come up with ideas for medium and growth conditions you might use when trying isolate previously uncharacterized bacteria from these areas.

- a. cold ocean waters
 - b. an area with acidic pollution
 - c. Utah's Great Salt Lake
 - d. a sewage treatment facility
 - e. soil from your backyard
2. Researchers are often faced with the predicament of having to identify bacteria that are unculturable in the lab. What are some techniques one could use to survey or sample the microbial diversity without having to cultivate the microorganisms from an environment? How could novel gene sequences or gene products from unnamed species be identified or characterized?
 3. Microorganisms that grow in hot springs or that oxidize unusual minerals are not pathogenic to humans, and they are difficult to cultivate. What reasons can you come up with for studying them? What practical applications can you think of for these microbes?

Table of Terms

Energy Sources	
Phototroph	Light
Chemotroph	Chemicals
Chemoorganotroph	Organic Compounds
Chemolithotroph	Inorganic Compounds
Carbon Sources	
Autotroph	Carbon Dioxide
Heterotroph	Organic Compounds
Growth Conditions	
Aerobe	uses oxygen as an electron acceptor
Anaerobe	uses a non-oxygen electron acceptor
Extremophile	tolerates extremes of pH, temperature, or salinity
Thermophile	grows optimally above 45°C

Exercise 1: Answers

1.
 - a. *Thermocrinus ruber*: chemotroph, chemolithotroph, autotroph (or chemolithoautotroph), extremophile; specifically, a thermophile
 - b. *Pseudomonas aeruginosa*: chemoorganotroph, heterotroph
 - c. *Acidithiobacillus ferrooxidans*: chemolithotroph, extremophile; specifically, an acidophile
2. For all organisms, it might be necessary to experiment with different O₂ levels, pH, or temperatures.
 - a. *Thermocrinus ruber*: water, oxidizable inorganic compounds (containing sulfur and iron, for example), high temperature, and CO₂
 - b. *Pseudomonas aeruginosa*: water, organic molecules (like sugars and amino acids), neutral pH, room temperature up to human body temperature, O₂
 - c. *Acidithiobacillus ferrooxidans*: water, compounds containing iron and sulfur in reduced forms, low pH, room temperature or cooler

Activity 2: PCR Demonstration

Based on video and online text content

60 minutes

Setup

In the first activity, you speculated on how one might survey the microbial diversity in an area, even if the resident microorganisms can't be cultivated in the lab. The video mentioned using PCR (polymerase chain reaction) as a way to detect microbial DNA in a sample. In this exercise, you will see how PCR can take a minute quantity of DNA—theoretically from a single cell—and generate enough DNA for identification and evolutionary analysis of the microbes in the sample.

PCR is simply in vitro DNA replication. The process is fundamentally the same as in cells: separation of DNA strands, priming from a short primer sequence, and semi-conservative replication by DNA polymerase. Work in teams of four to demonstrate the technique of PCR. If you are interested in seeing an animation of PCR, go to <http://www.learner.org/channel/courses/biology>.

Materials

- One set of PCR materials per four people. Each set contains:
 - two cardboard strips, 12 inches long and 2 inches wide, with a light-colored surface that can be written on
 - 10 cardboard strips, 2 inches long and 2 inches wide, with a light-colored surface
 - eight cardboard strips, 10 inches long and 2 inches wide, with a light-colored surface
 - 40 rubber bands that can fit around the cardboard strips
 - one roll of tape
- One black marker per person
- One copy of the Instructions per person (master copy provided)
- One copy of the Discussion Questions and Microbial Diversity Chapter Excerpt per person (master copy provided)

Instructions

1. First, read about the process of PCR in this excerpt from the Microbial Diversity chapter:
To replicate DNA in vitro, PCR takes advantage of a special property of the molecule: the hydrogen bonds. These bonds, which bind the complementary strands of DNA together in a double helix, are broken at elevated temperatures (about 95°C). Each single-stranded piece of DNA (**ssDNA**) is then built upon to form a new, double-stranded molecule (**dsDNA**). To initiate this, short “primers”—specific ssDNA fragments called **oligonucleotides**—must anneal to complementary regions on the single-stranded DNA. Deoxynucleotides (A,T,G and C) and DNA polymerase are added and, in a process called primer extension, the complementary copy of the ssDNA fragment is built. The result is two double-stranded DNA molecules identical to the original. Repeating these steps thirty times can result in a 10⁹-fold amplification of the original molecule.

Careful thermal cycling is required for PCR to proceed. For the primers to anneal to the ssDNA fragments, the temperature is reduced to about 55°C. However, at this temperature the original complementary ssDNA fragments will begin to re-anneal with each other. A high concentration of primers, and the tendency of the shorter primer strands to anneal more readily, ensures primer binding. The temperature is then raised again to about 72°C for primer extension. Underscoring the importance of microbes, the thermophilic bacteria *Thermus aquaticus* is the major source of the heat-tolerant DNA polymerase, which catalyzes primer extension and facilitates PCR.

In order to amplify a particular gene, specific primers, unique to that gene, are used. Two oligonucleotide primers (oligos) are constructed to flank a region of interest. One oligo will be complementary to a region on one strand of DNA, and the other oligo will be complementary to a region downstream on the homologous strand.

2. Next, have one person on the team make the target DNA sequence and the other three make primers:
 - a. Target DNA: The two 12-inch cardboard strips represent the target DNA sequence. On each strip, write 48 nucleotides (one letter per quarter-inch). Note that they are complementary strands, so line them up as well as you can. The sequences are:
top strand (cardboard strip 1)
5' TGATGCCGTA/AGTATTCGAT/AGCTTCTGAT/TGATTACCGT/AGCTCCGG 3'
bottom strand (cardboard strip 2)
3' ACTACGGCAT/TCATAAGCTA/TCGAAGACTA/ACTAATGGCA/TCGAGGCC 5'

Line up the strips so the sequences are complementary and anti-parallel, and bind the two strands (each cardboard strip) together with three rubber bands.

- b. Primers: The 10 two-inch cardboard strips represent the primers. These have the following sequences (written one letter per quarter inch):
5' TGATGCCG 3'
3' TCGAGGCC 5'

Make at least 10 copies of each primer. Note that for demonstration purposes, these sequences are much smaller than ones that would normally be used in PCR.

3. Amplify the target sequence by PCR:

Step 1: Denaturation, at 94°C

The double-stranded DNA is split into two. To simulate denaturation, remove the rubber bands from the cardboard strips.

Step 2: Annealing, at 55°C

The primers anneal to complementary strands of the target DNA. To simulate this, arrange the primer cardboard strips to target the DNA cardboard strips that have complementary sequences. Wrap a rubber band around the two.

Step 3: Chain Elongation, at 72°C

DNA Polymerase fills in the missing sequence. To simulate this, securely tape a 10-inch cardboard strip to the primer. Fill in the missing nucleotides, using the complementary sequence of the other strand as a template. When you are done, wrap two more rubber bands around the strands. You have duplicated the target DNA sequence.

Continue, with one person acting as the denaturer, one person acting as the annealer, and one acting as the polymerase. The fourth person is the thermocycler, who tells the other three when to carry out their respective activities. In the interest of time, the thermocycler can help the other three.

Go through three cycles of the process. You should have eight copies of the target sequence after the three cycles. After completing the exercise, talk through the discussion questions with your teammates.

Discussion Questions and Microbial Diversity Chapter Excerpt

1. What are the similarities between PCR and cellular DNA replication? What is different?
2. Assuming an unlimited supply of primers and free nucleotides, how many copies of the target sequence would you expect to have after 10 cycles of PCR? After 20? After 30? (Note: After three cycles you had 2^3 .)
3. What factors might limit the numbers you obtained above?
4. Look at your target sequence again. Imagine that you used primers with the following sequences:
5' GCCGTAAG 3' and 3' TGGCATCG 5'
What section of the target DNA would be amplified? After three cycles, how many copies of the end sequences, not covered by the primers, would there be?
5. Explain how PCR is used in microbial ecology to discover new organisms, even if they cannot be cultivated in the lab. How can it be used to identify new organisms, and characterize them with respect to related organisms?
6. PCR uses a DNA polymerase called Taq polymerase (so-named because it comes from a bacterium called *Thermus aquaticus*). Why not just use DNA polymerase from a well-known laboratory bacterium like *E. coli*?

After answering these questions, read the rest of the excerpt from the Microbial Diversity chapter.

Studying Unculturable Microbes with PCR

Imagine yourself on a team studying archaea at a deep-sea hydrothermal vent at the Galapagos Rift (an area known for its hydrothermal activity). You've found a new microbe. What do you want to know about it? What metabolic class does the microbe fall within? Does it make certain proteins? How does it survive the volcanic heat? Traditionally, asking such questions involved growing microbes in the laboratory. Unfortunately, replicating the conditions in which many bacteria and archaea grow is very difficult. For this reason, only a small fraction (perhaps only as few as one percent) of the microorganisms in nature has been cultivated. To identify and compare unculturable organisms microbiologists have turned to molecular genetic techniques.

Polymerase chain reaction (PCR) is one technique for studying organisms that cannot be grown in the laboratory. When only a small quantity of DNA is available from a particular source, PCR can be used to amplify that DNA and produce billions of copies of a designated gene-sized fragment. The technique has many applications, including the amplification of DNA from crime

scenes, analysis of cancer genes, and identification of pathogens. When an environmental sample contains unculturable organisms, scientists can use PCR to generate copies of microbial genes suitable for comparison.

To replicate DNA in vitro, PCR takes advantage of a special property of the molecule: the hydrogen bonds. These bonds, which bind the complementary strands of DNA together in a double helix, are broken at elevated temperatures (about 95°C). Each single-stranded piece of DNA (**ssDNA**) is then built upon to form a new, double-stranded molecule (**dsDNA**). To initiate this, short “primers”—specific ssDNA fragments called **oligonucleotides**—must anneal to complementary regions on the single-stranded DNA. Deoxynucleotides (A,T,G and C) and DNA polymerase are added and, in a process called primer extension, the complementary copy of the ssDNA fragment is built. The result is two double-stranded DNA molecules identical to the original. Repeating these steps thirty times can result in a 10⁹-fold amplification of the original molecule.

Careful thermal cycling is required for PCR to proceed. For the primers to anneal to the ssDNA fragments, the temperature is reduced to about 55°C. However, at this temperature the original complementary ssDNA fragments will begin to re-anneal with each other. A high concentration of primers, and the tendency of the shorter primer strands to anneal more readily, ensures primer binding. The temperature is then raised again to about 72°C for primer extension. Underscoring the importance of microbes, the thermophilic bacteria *Thermus aquaticus* is the major source of the heat-tolerant DNA polymerase, which catalyzes primer extension and facilitates PCR.

In order to amplify a particular gene, specific primers, unique to that gene, are used. Two oligonucleotide primers (oligos) are constructed to flank a region of interest. One oligo will be complementary to a region on one strand of DNA, and the other oligo will be complementary to a region downstream on the homologous strand.

Back home, after your trip to the deep-sea hydrothermal vent, you want to determine what genus of bacteria you have in hand. You can use PCR to amplify the gene for ribosomal RNA (the gene isolated and sequenced by Woese from so many organisms when he constructed his “Tree of Life”). Then, you can choose conserved regions of the rRNA gene for primers. With adequate DNA from PCR, you could sequence the gene and compare it with millions of known rRNA gene sequences using a computer database. (See the *Genomics* unit.)

Alternately, you might want to ask if a microbe carries out a particular form of metabolism. Given the DNA sequence for a protein involved in a particular metabolic strategy—photosynthesis, for example—you could construct oligos so that the presence of that gene could be detected using PCR.

7. Besides its use in microbe detection and forensic analysis of crime scenes, what other uses do you know of, or can you think of, for PCR?

To view the *Rediscovering Biology* PCR animation, go to <http://www.learner.org/channel/courses/biology>.

Activity 3: Biofilm Stars

Based on video and online text content

15 minutes

Setup

Although we think of bacteria as single-celled organisms, increasing attention is being paid to the multicelled biofilms formed by many types of bacteria. Biofilms are ubiquitous, so information about how they form and how they function is relevant to medicine, environmental science, industry, and commerce. Working in teams of three, read about biofilms in the Microbial Diversity online text chapter, and consider some applications for this information.

Materials

- One copy of the Microbial Diversity online text chapter per three people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Discussion Questions

See Tips and Suggested Answers for answers to some questions.

1. Make a list of places where you would encounter a biofilm on an average day. (Check the Microbial Diversity chapter for general ideas.)
2. List the medical implications of biofilm formation. Consider both biofilms that form in the body and outside the body. (Again, consult the text chapter for ideas.)
3. In 2003, Dr. Søren Molin and Dr. Michael Givskov's labs synthesized a drug that might be useful for treating infections of *Pseudomonas aeruginosa*, an opportunistic pathogen of cystic fibrosis patients. The drug was modeled on a compound from the seaweed *Delisea pulchra*. The drug did not inhibit the growth of bacteria in culture; however, it did help infected mice clear the bacteria more efficiently. How do you think this drug works? Why would a seaweed produce an anti-bacterial compound?
4. What might be some advantages of using a quorum sensing inhibitor to treat *P. aeruginosa* infections, instead of traditional bacteriostatic antibiotics that inhibit bacterial growth or bacteriocidal compounds that kill bacteria?

Source: Hentzer, M. et al. 2003. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J.* 22:3803–15.

Discussion Question Answers

3. How the anti-*Pseudomonas* drug works:

The drug interferes with quorum sensing systems, so it prevents the formation of biofilms.

Seaweeds do not have an immune system, but their tissues are just as susceptible as other organisms to degradation by bacteria. They produce the compound to protect against bacterial attack. To quote the article that describes this work:

"...the Australian red macro-alga (seaweed) *Delisea pulchra* is largely unfouled in nature due to the production of biologically active halogenated furanones (de Nys et al. 1993). These secondary metabolites are released at the surface of the plant at concentrations that inhibit colonization by both prokaryotes and eukaryotes."

4. What might be some advantages of using a quorum sensing inhibitor to treat *P. aeruginosa* infections, instead of traditional bacteriostatic antibiotics that inhibit bacterial growth or bacteriocidal compounds that kill bacteria?

When bacteria have formed a biofilm, higher doses of normal antibiotics are required to affect them because the cells in the biofilm protect each other from exposure to antibiotics. Some bacteria within the biofilm might be dormant and not as susceptible to the antibiotics. Quorum sensing inhibitors (QSI) prevent the formation of biofilms, so they do not have these limitations. QSI drugs inhibit virulence rather than growth, so they are less likely to provide a pressure that would select for resistance.

Activity 4: The Fall of Biosphere 2

Based on video and online text content

15 minutes

Setup

In the late 1980s, a glass-enclosed structure called Biosphere 2 was constructed outside of Tucson, Arizona. It was an attempt to create a sealed, self-sustaining ecosystem that included areas of ocean, desert, agriculture, and tropical rainforest, as well as humans. The human sustainability phase went from September 1991 to September 1993 and was terminated because of atmospheric irregularities. The structure is now used as a facility for research on climate change. In this exercise, read about the Biosphere experiment and discuss its successes and failures.

Materials

- One copy of the Discussion Questions per person
- One transparency of the Table of Terms from the Microbial Diversity online text chapter (master copy provided; see Activity 1)

Discussion Questions

1. The plant and animal components of Biosphere 2 were carefully considered, but the microbial components were unknown. Where do you think the microbial activity that altered the atmospheric conditions came from? What was the activity and how did it cause problems?

After answering the question, read the following:

"...the rich soil was the major factor in causing the experiment to become unsustainable. Soil respiration was so high, and soil reserves of carbon were so great, that the atmospheric composition changed rapidly. Oxygen was absorbed from the air by soil microbes, and these released huge amounts of CO₂ from the soil back to the air. The buildup of CO₂ exceeded the photosynthetic capacity of plants to assimilate it and to regenerate O₂. While some of the excess CO₂ was absorbed by the fresh, unsealed concrete of the structure, forming limestone, the CO₂ concentration remained elevated above desired levels. More importantly, O₂ levels continued to decline rapidly, and additional O₂ had to be added to enable the eight human occupants to survive."

2. What types of microorganisms (chemotrophs, autotrophs, etc.) were responsible for the activities that changed the Biosphere 2 atmosphere? (Review the Table of Terms from Activity 1, if necessary.)
3. One solution might be to mix microbes with autotrophic activity into the soil. Do you think this would work? What might be some drawbacks to this approach?

After answering this question, read the following from the same source:

"...the impact of disturbance whenever new soils are introduced results in large changes in physical structure of the soil (aggregation, peds, natural pores and cracks, horizon formation, fragipans and duripans) that develop over thousands of years in nature and simply cannot be reconstituted...such disturbances cause large increases in soil organic matter decomposition, which can cause a pulse of CO₂ and nitrogen release for up to several years. This is essentially the plowing effect, wherein wildland soils converted to agriculture typically lose 40 percent of their organic matter and nitrogen over time. This was the major reason that the first 'human experiment' failed in B2C."

Another problem is achieving the proper balance of organisms. One problem involving macro-organisms is stated here.

"...Ants and other soil fauna are known to play a critical role in nutrient cycling, but all the terrestrial biomes are dominated by one exotic ant species that was able to survive while almost 20 other ant species originally placed in the biomes did not persist."

4. If you were to set up the next Biosphere project, what would you do differently?
5. What kind of parallels can you draw between the problems within Biosphere 2 and environmental problems in Biosphere 1 (which is earth)?

Source: U.S. Department of Energy. Biological and Environmental Research, Advisory Committee. *An Evaluation of the Biosphere 2 Center as a National Scientific User Facility*. March 2003.

Activity 1: Emerging Diseases: Causes and Effects

Based on video content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

This unit is about emerging infectious diseases, but what exactly does that mean? What makes an infectious disease “emerging”? What makes a disease reappear as a “re-emerging” disease? Warm up before watching the video by making group lists of your ideas about these questions. At the end of this session, you’ll review the lists as part of a summary discussion.

Materials

- One copy of the Instructions per person (master copy provided)
- Tips and Suggested Answers

Instructions

1. Come up with a group list of any and all diseases that might qualify as an emerging or re-emerging infectious disease. At this point, just brainstorm. You'll sort out the ideas later. (The session facilitator can write them down on a board or transparency.)
2. When all ideas are on the board or transparency, go through the list more carefully, reasoning through why some should be included and some might not.
3. Do the same exercise of brainstorming and then going carefully through your list, but this time name factors that make an infectious disease emerging or re-emerging. What contributes to the introduction and spread of a disease into the general population?
4. Compare the list of diseases made by the group and the list of factors. Do the diseases you thought of fit your list of criteria?
5. After the video, look at your lists again.
6. Keep a copy of your lists! After watching the entire video and doing some of the activities, you'll compare your list against new information from the video and activities.

Emerging Diseases: Causes and Effects

Definitions of “emerging infectious diseases” might include:

- any that have recently been introduced into the human population
- diseases that have gone from localized to global in the past few years or decades
- diseases that have recently escalated their threat to humans, for example, because of antibiotic resistance

Diseases might include:

- SARS
- HIV
- antibiotic or drug-resistant diseases, like tuberculosis and malaria
- Hantavirus
- West Nile virus
- Ebola and other hemorrhagic fevers
- Dengue fever
- cholera
- Lyme disease
- new strains of influenza
- anthrax, smallpox, or other bioterrorism possibilities

Causes and contributing factors might include:

- rapid urbanization so humans live in close contact with each other
- poor sewage and water systems
- lack of hospital facilities
- penetration of remote areas and new habitats to expose humans to new pathogens
- global distribution of food
- drug or antibiotic resistance
- human contact with animal reservoirs
- rapid global transportation

Activity 2: Lifecycles of the Infectious and Famous

Based on video and online text content

30 minutes

Setup

Finding a treatment for an infectious disease requires detailed knowledge about the cause of the disease. Developing a prevention or eradication plan requires detailed knowledge about how the disease is maintained in hosts or carriers, and how it is transmitted. For some infectious diseases, this task is complicated by a complex replication cycle and multiple hosts or reservoirs.

In this exercise, work in pairs to sort out the replication steps of the cause of four complicated infectious diseases: influenza, malaria, Lyme disease, and West Nile virus. Each team should take one set of papers with the steps of the replication cycles of the diseases. Take a few minutes to arrange the steps into the proper cycles for the disease agents. Then, using the diagrams you've made, go over the discussion questions in teams or as a group.

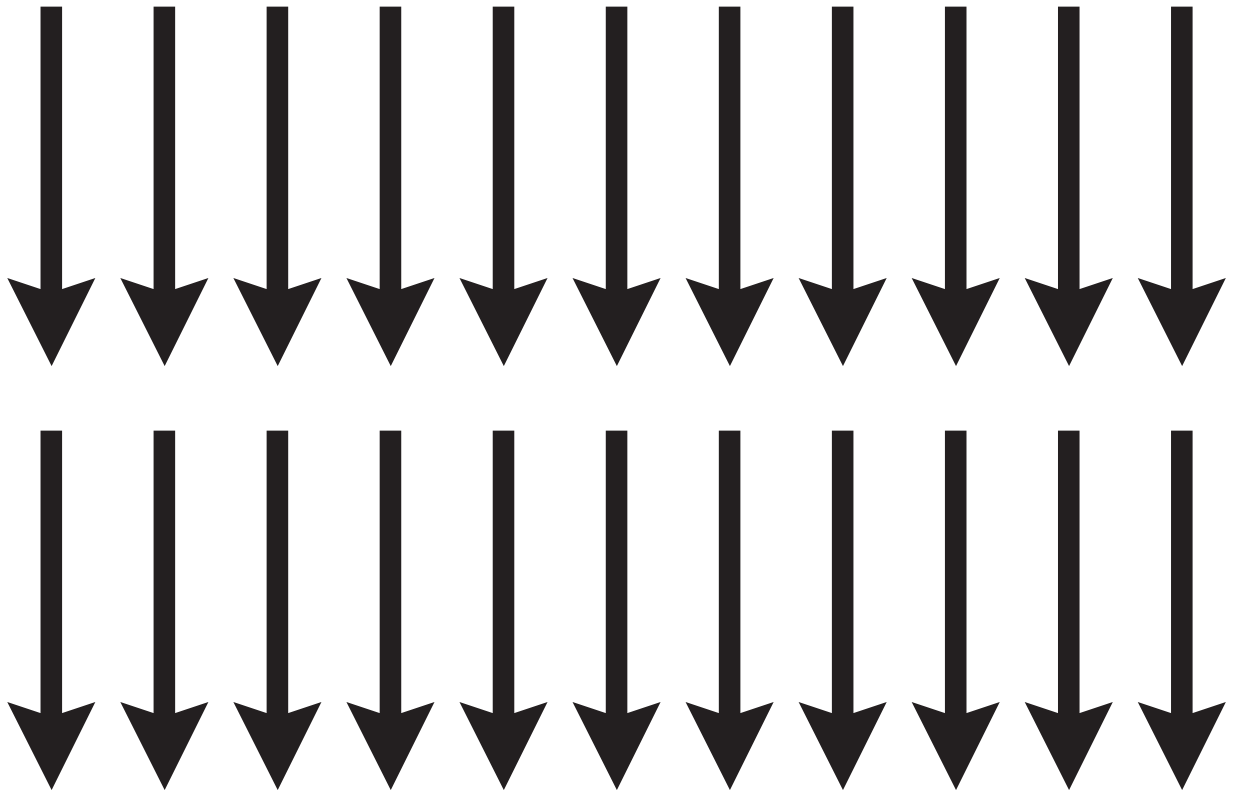
Materials

- One set of Replication Cycle Steps and Arrows per two people (master copy provided; to make a set, cut out the arrows to separate them, cut on the dotted lines of the lifecycle steps and scramble them)
- One set of Discussion Questions per person (master copy provided)
- One copy of the Emerging Infectious Diseases online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Replication Cycle Steps and Arrows

After copying, cut to separate the arrows. Then cut on the dotted lines to separate the lifecycle steps, and scramble them.

See the Tips and Suggested Answers for Replication Cycle answers.



Parasite sexual cycle occurs
in mosquito gut.

Occasionally, a mosquito
vector transmits the virus to
an animal or a human.

A mosquito carrying the
parasite bites a human. The
parasites (as sporozoites)
enter the blood.

In humans, the virus
undergoes small mutations
to create new viral strains
(antigenic drift).

A tick that is infected or
uninfected mates and lays
uninfected eggs.

Uninfected tick larvae feed on
a bacterially infected animal.

A pig is infected with multiple viruses. (e.g. a combination of human, swine or avian viruses).

Parasite form that can infect humans (sporozoites) migrates from the mosquito gut to the salivary glands.

Mosquito vector acquires the virus by biting an infected bird.

Infected tick transmits bacteria to human, deer, mice, or birds.

Gametocyte form is taken up by a mosquito that feeds on an infected human.

New viral subtype infects humans.

Viral genes from different sources (human, avian or swine) mix to create a new virus subtype (antigenic shift).

Human viruses might infect pigs.

Blood cells are infected, and undergo cycles of bursting, release and infection of new cells. Some gametocyte forms are released.

Parasites enter the liver, where they change form and are released as a form (merozoites) that can infect blood cells.

A bird is infected by a mosquito vector that is carrying the virus.

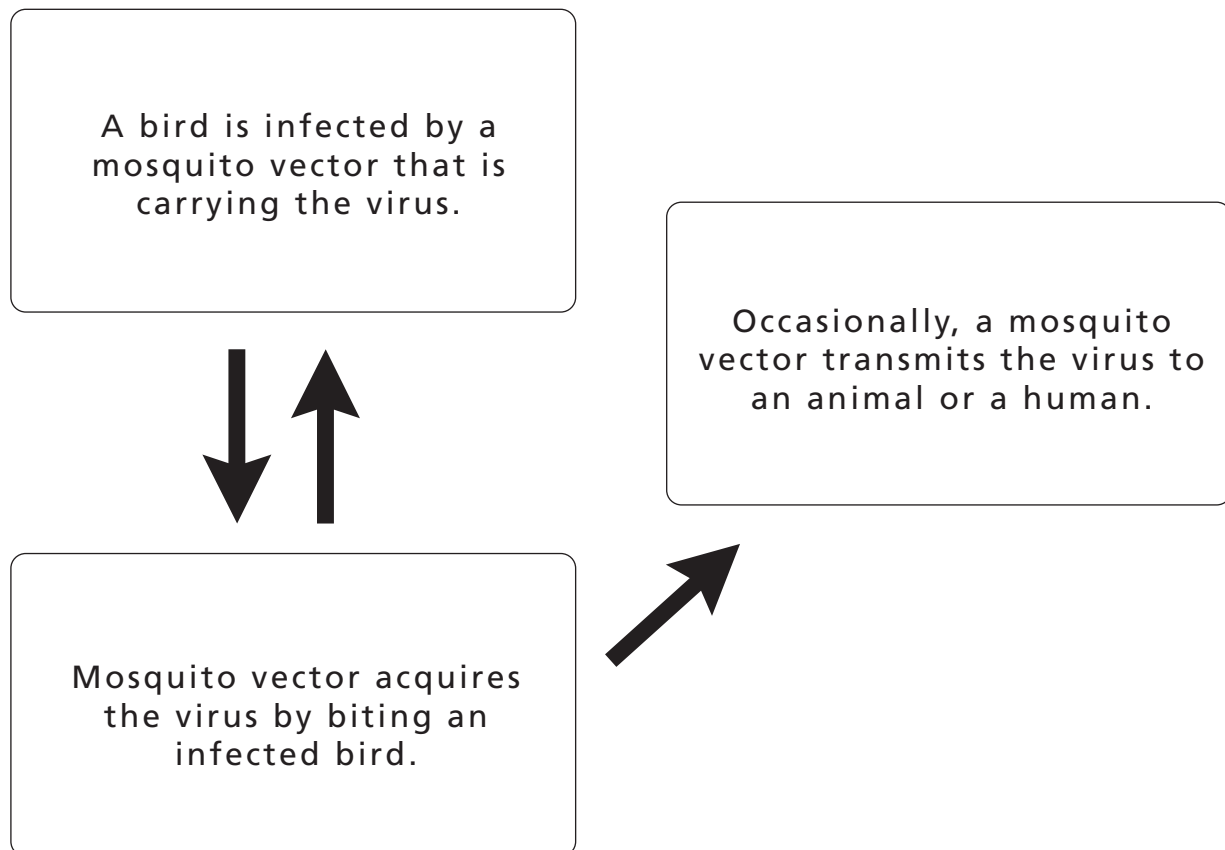
Discussion Questions

See the Tips and Suggested Answers for possible answers.

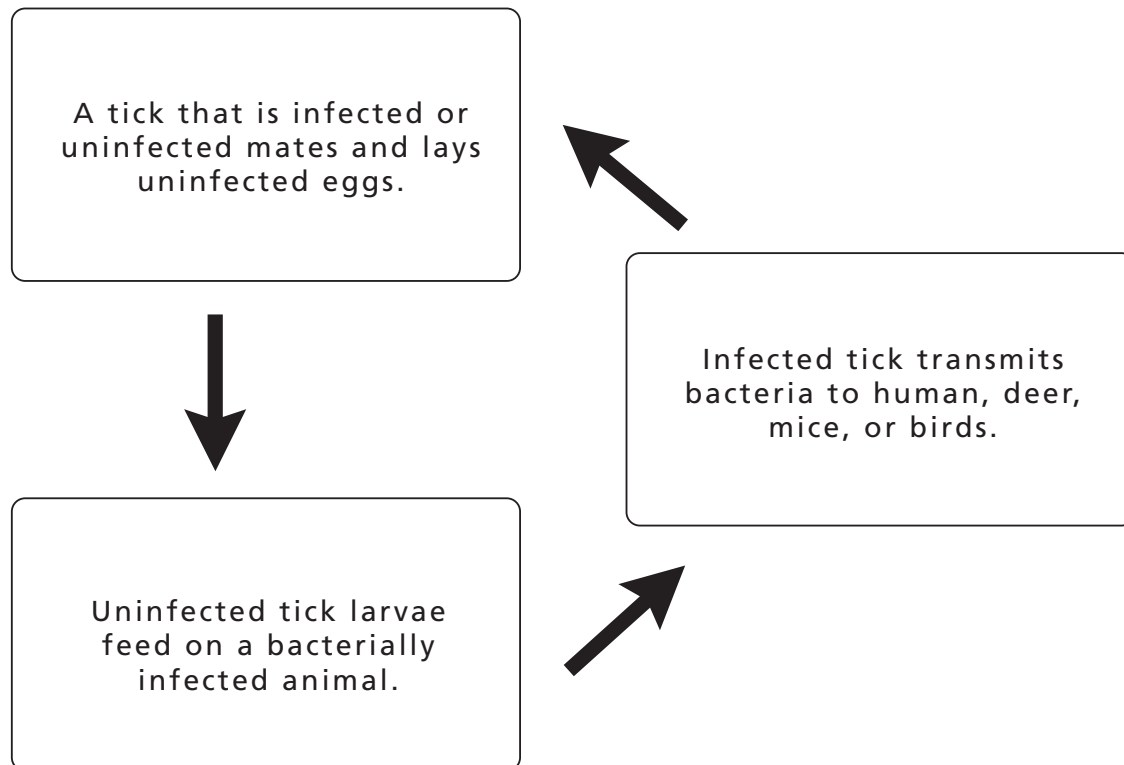
1. What are the direct causative agents of influenza, malaria, Lyme disease, and West Nile virus: a virus, a bacterium, or a eukaryotic organism?
2. Are antibiotics like penicillin or tetracycline effective against any of these diseases? Why or why not?
3. Is there a vaccine against influenza? malaria? Lyme disease? West Nile virus?
4. What treatments or strategies are used to treat or control each disease?
5. What is the role of animal or insect vectors and reservoirs in the replication cycles of these pathogens? Can the microbe exist solely in humans?
6. For each of these diseases, is transmission directly from human to human, or from human to animal?

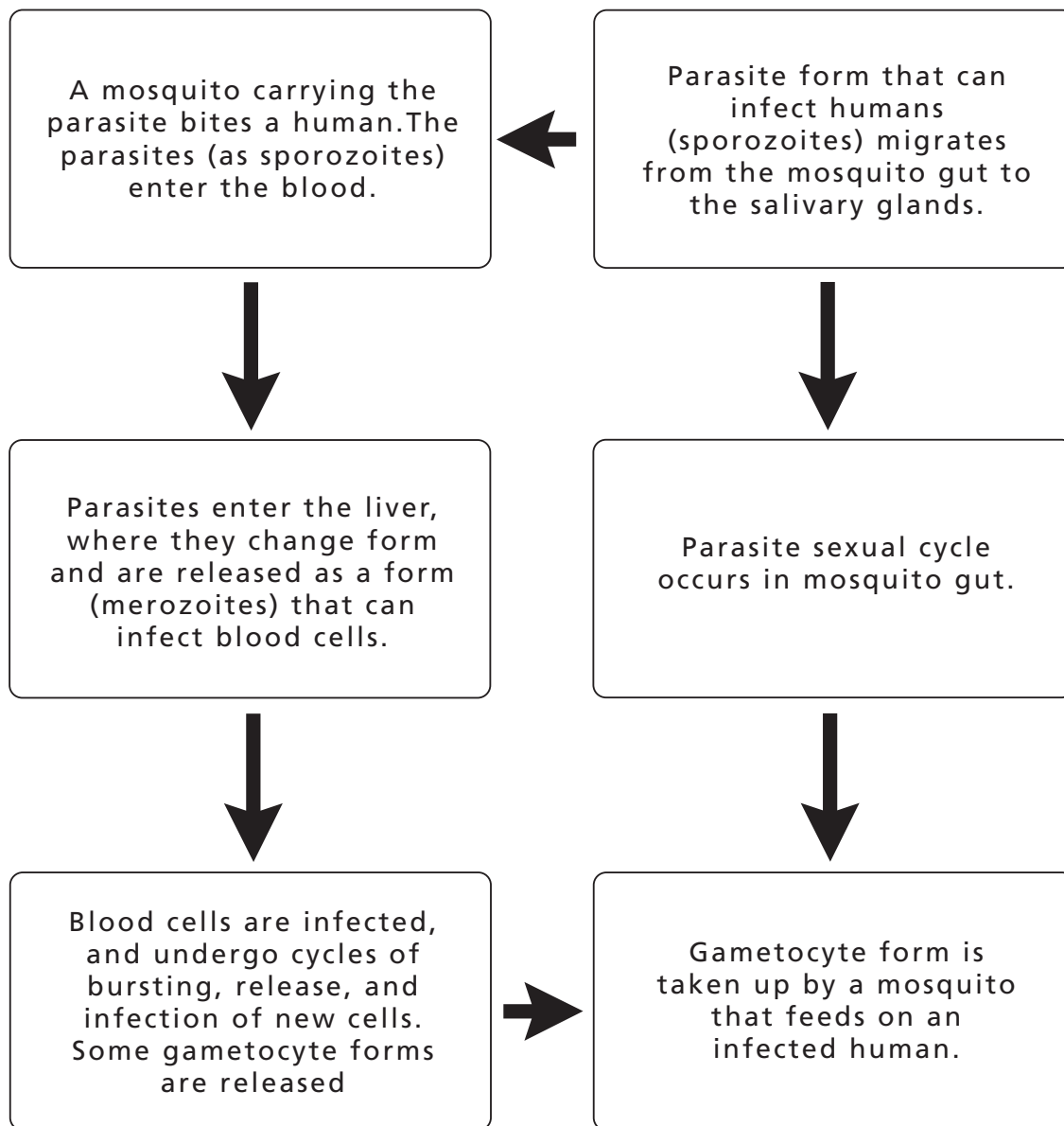
Replication Cycles

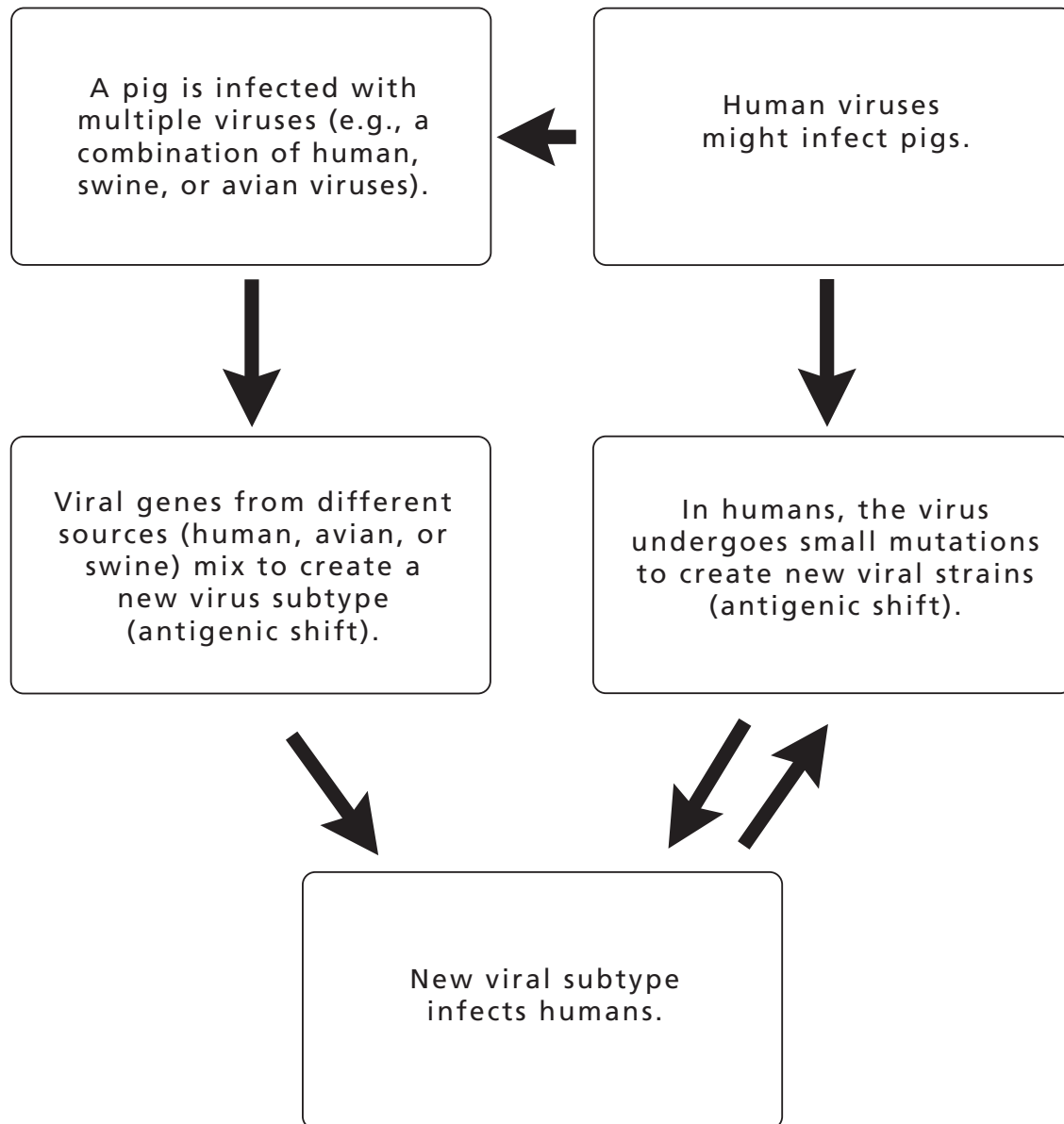
West Nile Virus



Lyme Disease



Malaria

Influenza A

Discussion Questions

1. What are the direct causative agents of influenza, malaria, Lyme disease, and West Nile virus: a virus, a bacterium, or a eukaryotic organism?
influenza: virus; malaria: eukaryotic microbe in the *Plasmodium* genus; Lyme disease: Spirochete bacterium; West Nile: virus
2. Are antibiotics like penicillin or tetracycline effective against any of these diseases? Why or why not?
Antibiotics like penicillin and tetracycline are effective only against bacteria, so antibiotics can be prescribed for the early stages of Lyme disease. Antiviral and anti-eukaryotic drugs can be prescribed for influenza, malaria, or West Nile virus, although they are less common than antibacterial drugs and often have more severe side effects. This is because antibacterial drugs can target structures or processes unique to bacterial cells. There are fewer unique targets when developing drugs to combat infections of viral and eukaryotic pathogens.
3. Is there a vaccine against influenza? malaria? Lyme disease? West Nile virus?
There is a vaccine against influenza A and B, but it must be administered every year because of antigenic drifts and shifts in the virus. As of 2003, vaccines for malaria are currently in development. There is a vaccine against Lyme disease but prevention by minimizing exposure to ticks, is more common. There is a West Nile virus vaccine for livestock, and human vaccines are in development.
4. What treatments or strategies are used to treat or control each disease?
Antiviral drugs can be used for serious influenza cases. Otherwise, we usually just treat the symptoms of fever, aches, chills, and nausea. Currently, quinine, chloroquine, and a few other drugs are used as anti-malarial therapy; mosquito eradication is used to control it. Lyme disease can be treated with antibiotics; control of ticks and prevention of tick bites is the primary means of prevention. West Nile virus is monitored through infected birds; vector control and animal vaccines prevent transmission.
5. What is the role of animal or insect vectors and reservoirs in the replication cycles of these pathogens? Can the microbe exist solely in humans?
Influenza B exists only in humans; influenza A is harbored by animal reservoirs and undergoes genetic exchange in them to generate new forms. Part of the lifecycle and a specific developmental stage of malaria occurs only in mosquitoes, so the microbe cannot exist without mosquitoes. The mosquitoes also act as the vector for transmission to humans. The Lyme disease bacterium is found in deer and mice reservoirs; it is acquired by the larvae of the tick vector, which transmits the bacterium to other animals and to humans. Birds appear to be the reservoirs for West Nile virus, with transmission from bird to bird through the mosquito vector. Transmission to other animals and to humans is only incidental.
6. For each of these diseases, is transmission directly from human to human, or from human to animal?
Influenza is transmitted directly from human to human and, rarely, from animal to human. Malaria, Lyme disease, and West Nile virus are all transmitted through mosquito or tick vectors and are not transmitted from human to human.

Activity 3: Koch's Postulates

Based on video and online text content

30 minutes

Setup

In the late 1800s, Robert Koch formulated a set of four criteria that must be met in order to prove that a particular type of bacterium is responsible for a disease. In 1937, T. M. Rivers expanded "Koch's postulates" to six criteria to determine if a particular *virus* is the cause of a disease.

An emerging infectious disease whose cause was determined relatively quickly was severe acute respiratory syndrome, or SARS. Koch's and Rivers's postulates were followed to establish a virus called SCV (SARS-associated coronavirus) as the cause of SARS. In this exercise, follow the instructions to go through the logic of Koch's postulates.

Materials

- One copy of the Instructions and Discussion Questions per person (master copy provided)
- One transparency of Koch's and Rivers's Postulates (master copy provided)
- One transparency of Experimental Results for SARS and SCV (master copy provided)
- Tips and Suggested Answers

Instructions and Discussion Questions

1. Individually, take a few minutes to write down how YOU would test if a particular virus caused a disease, and what criteria or tests you would perform to prove that a virus was the cause of a disease. Then, as a group, use the individual lists to make a group list of criteria that would prove that a particular virus was the cause of a disease. If anyone in the group knows Koch's and Rivers's postulates, see if the group's list matches the "official" list.
2. Check your list against the one on the transparency. Is there anything that your group missed? Is there anything that your group thought of that would strengthen the list of criteria?
3. Look at a list of results from fulfilling Koch's and Rivers's postulates for SARS. Compare the list to the postulates and match each result with the postulate that it fulfills. (See the Tips and Suggested Answers.) Do you find the evidence convincing, or is it possible that another infectious agent is responsible for SARS?
4. Talk about these discussion questions as a group. (See the Tips and Suggested Answers.)
If any of the following situations occurred, would it be sufficient to reject SCV (SARS-associated coronavirus) as the cause of SARS? Explain your answer.
 - a. The virus can be isolated from healthy individuals.
 - b. When introduced into animals that normally can be infected with the same disease as humans (e.g., macaques), the animals do not get sick.
 - c. When introduced into hosts, the isolated virus gives them the same symptoms as the disease in humans, but a test for anti-SCV antibodies is negative after three months.
 - d. In one study, evidence for the virus is not found in all tested patients.

Koch's and Rivers's Postulates

Koch's and Rivers's postulates for establishing a virus as the cause of a disease.

1. The specific virus is present and can be isolated from all diseased hosts, and is not present in healthy individuals.
2. The virus isolated from diseased individuals can be cultivated in host cells in the laboratory.
3. Proof of filterability (helps establish that the infectious agent is the size expected of a virus)
4. When an original host species or a related species is inoculated with the virus isolated from diseased hosts, it develops a comparable disease.
5. The virus can be re-isolated from the inoculated hosts.
6. A specific immune response can be detected in the inoculated hosts.

Experimental Results for SARS and SCV

Results that fulfilled Koch's and Rivers's postulates for SARS. Match each result to a specific postulate.

- a. Of 96 individuals in Hong Kong who met the World Health Organization's definition of SARS, 90 percent tested positive in a lab test for SCV infection.
- b. A PCR (polymerase chain reaction) test for SCV DNA was performed on nasal secretions from macaques infected with SCV. Two out of two infected macaques were positive for SCV DNA.
- c. Macaques that were negative in a test for anti-SCV antibodies became positive for anti-SCV antibodies after infection with SCV isolated from a SARS patient.
- d. Viruses isolated from macaques infected with SCV from a SARS patient secreted viruses that were identical, by electron microscopy, to the original infecting virus.
- e. Blood, serum, nasal secretions, and tissue samples from SARS patients were used to inoculate cultured cell lines. The cell lines that showed cytotoxic, cell-damaging effects were producing the SCV coronavirus.
- f. Two macaques were inoculated with SCV. Three to four days later, they became lethargic and developed skin rashes and respiratory distress. Autopsies on the animals showed pneumonia-like tissue damage in the lungs.
- g. Mice were infected with blood, serum, nasal secretions, or tissue samples from SARS patients. When tissue extracts from sick or dead mice were filtered, they yielded viruses that could be cultured in cultured cell lines.
- h. Of 19 patients with SARS, 19 tested positive for presence of the SCV virus, evidence of viral DNA in their tissue samples, or production of antibodies against the SCV virus.

Sources: *Nature* (2003) 423:240; *N. Eng. J. Med.* (2003) 348:1953

Experimental Results for SARS and SCV

- a. fulfills postulate 1
- b. fulfills postulate 5
- c. fulfills postulate 6
- d. fulfills postulate 5
- e. fulfills postulate 2
- f. fulfills postulate 4
- g. fulfills postulates 3 (also 4 and 5)
- h. fulfills postulate 1 (also 6, partially)

Discussion Questions

4. If any of the following situations occurred, would it be sufficient to reject SCV (SARS-associated coronavirus) as the cause of SARS? Explain your answer.
- a. The virus can be isolated from healthy individuals.
- Yes, it could be a reason to reject SCV as the cause of SARS. If people with no symptoms have the disease, it breaks the correlation between infection with the virus and symptoms of the disease.
- No, it should not be a reason to reject SCV as the cause of SARS. Perhaps these people had or have the disease in a mild form. Some people may be a “asymptomatic carriers”: there might be a form of the disease that has a long incubation period, so these people might not be showing symptoms yet.
- b. When introduced into animals that normally can be infected with the same disease as humans (e.g., macaques), the animals do not get sick.
- Yes, it could be a reason to reject SCV as the cause of SARS, especially because it seems that SARS originated from animals.
- No, it should not be a reason to reject SCV as the cause of SARS. Not all diseases infect both humans and macaques and, even if infection occurs, the symptoms are not always the same.
- c. When introduced into hosts, the isolated virus gives them the same symptoms as the disease in humans, but a test for anti-SCV antibodies is negative after three months.
- Yes, it could be a reason to reject SCV as the cause of SARS. Perhaps another virus is being isolated—undetected—with SCV, and that virus is the one that causes the disease and immune response. Perhaps the SCV isolation is not successful and the hosts are getting sick for some other reason.
- No, it should not be a reason to reject SCV as the cause of SARS. Perhaps a more sensitive test for immune response is needed, or the SCV antibodies will not be detectable for another few months.
- d. In one study, evidence for the virus is not found in all tested patients.
- Yes, it could be a reason to reject SCV as the cause of SARS, because if the virus is really not present in some people with the disease, it can’t be responsible for the disease.
- No, it should not be a reason to reject SCV as the cause of SARS. The test might not be 100 percent accurate and give false negatives. The test might be accurate but detects the presence of virus only when administered at a particular point in the disease progress. If the accuracy of the test is unknown, then negative test results in some patients might not be a reason to reject the virus as the cause of the disease. For example, the actual symptoms might appear after the virus is no longer present or is present at very low levels.

Activity 4: Shifting Antigens

Based on video and online text content

30 minutes

Setup

In 1918–19, 20 million–50 million people died of the Spanish flu, including approximately half a million in the U.S. This deadly flu pandemic was caused by a particularly virulent type of influenza A that “goes around” every winter. How did this version of influenza suddenly appear? Could this type of pandemic occur again? This exercise will provide some explanation.

The influenza virus changes continuously, so health organizations need to monitor it constantly. They do this by piecing together information on which types of flu virus are present in the population, and which are likely to appear next. In this exercise, you will also piece together bits of information to come up with a model of how the influenza virus changes and why some pandemics like the Spanish flu are so deadly.

The session facilitator has a set of clues to the influenza puzzle to distribute. Each person should have at least one clue. Begin by reading the “starter” questions. Then, read your clue if it seems to contribute to the answer to the question. When all the questions have been read, try to put together a model of influenza virus that explains how it changes continuously, what this means for vaccination programs, and how a particularly deadly version could appear without warning.

Materials

- One set of Clues to distribute to the group (master copy provided; after copying, cut to separate the clues)
- One transparency of the List of Questions to start the discussion of how influenza pandemics occur (master copy provided)

Clues

Cut into separate slips of paper and pass around until they are all distributed.

The Spanish flu was influenza A(H1N1).

A person who has antibodies against one type of influenza A, for example A(H1N1), may not be protected against another type of influenza A, for example A(H3N2).

The deadly Hong Kong influenza pandemic was caused by influenza A(H3N2).

A person who has never encountered an influenza A subtype that has a unique combination of hemagglutinin and neuraminidase will not have antibodies against it.

All types of influenza (A, B, and C) undergo “antigenic drift,” in which small changes occur to viral genes. This results in small changes to the viral proteins, including hemagglutinin and neuraminidase.

Influenza A undergoes “antigenic shift.” This occurs when influenza A viruses from different species (e.g., bird and human) co-infect a pig. Viral genes mix and re-assort in the pig cells, resulting in a new virus with a mixture of human and, possibly, avian or pig genes.

There are three types of influenza: A, B, and C.

Influenza C does not cause pandemics and is not included in the vaccine.

Hemagglutinin and neuraminidase are proteins on the surface of influenza that are recognized by the immune system.

Influenza A virus can be found in humans, ducks, chickens, pigs, whales, horses, and seals.

The current subtypes of influenza A in humans are A(H1N1) and A(H3N2).

Influenza B passes only from human to human, but it is possible for influenza A to pass from animals to humans.

In subtyping influenza A, “H” stands for the type of hemagglutinin and “N” stands for the type of neuraminidase.

When antibodies against hemagglutinin and neuraminidase bind to these proteins, the virus is neutralized and cannot infect cells.

Pigs can be infected by avian, human, and pig influenza viruses.

The genes for 15 different hemagglutinins and 9 different neuraminidases can be found in the influenza A viruses of wild birds.

List of Questions

1. An influenza vaccine stimulates production of antibodies against the Influenza subtypes that are expected to be common in the next flu season. How do they protect against the flu?
2. Why do we need to get an influenza shot every year in order to be protected?
3. When influenza viruses are identified as A(H1N1), what do the H and N stand for?
4. Why are some flus called “swine flu” or “bird flu”?
5. When flu scares occur—for example, swine flu in 1976 in New Jersey, or avian flu in 1997 in Hong Kong—why do health authorities impose large-scale vaccination programs or large-scale animal extermination programs?
6. How were the great influenza pandemics (worldwide epidemics) like the Spanish flu and Hong Kong flu different from the flu that goes around every year?
7. Was the Spanish flu so deadly because there were no antibiotics? If it occurred today, could we prevent it by treating everyone with an antibiotic like penicillin?

Source: <http://www.cdc.gov/ncidod/diseases/flu/viruses.htm>

Activity 5: Wrapping It Up

Based on video and online text content

15 minutes

Setup

In the first activity, the group generated lists of diseases that could be considered emerging or re-emerging infectious diseases, and a list of factors that cause this phenomenon. Take a look at your lists again now that you have thought about this issue a little bit and discuss the provided questions.

Materials

- The lists made by the group in Activity 1 (List 1: Diseases that might be considered emerging or re-emerging infectious diseases; List 2: Factors that affect the emergence or re-emergence of a disease)
- One copy of the Discussion Questions per person (master copy provided)
- One transparency of the Factors That Affect the Emergence of Disease (master copy provided)

Discussion Questions

1. Look at the list you made of factors that contribute to the emergence or re-emergence of an infectious disease. The transparency shows a table from Smolinski et al. (from the online text for this unit) with the same type of list. Compare the table to your list. Is there anything your list doesn't have? Are there additional factors that you would add to Smolinski's list?
2. Look at the list of diseases you thought might be considered emerging or re-emerging. Is there anything that you now think doesn't fit the definition? Is there any disease that you would add, either from the video or that you have thought of since starting this unit?
3. Was anyone surprised by any of the diseases that the scientists in the video considered emerging infectious diseases? If so, why? What is our perspective, in industrialized countries, on the threat of these diseases? Do we get enough information about diseases that might become a threat in the future?
4. Should diseases that might be used as bioterrorism weapons be treated as re-emerging? How much attention, research, and prevention should we devote to this category of diseases? For example, in December 2002, President George W. Bush announced a smallpox vaccination plan, with military and first responders vaccinated first, and a vaccination available to all Americans by 2004. Is this an appropriate response to the possible re-emergence of smallpox as a bioterrorism weapon?
5. If a disease is occurring on the other side of the world, sometimes it is difficult to imagine its impact and seriousness. When teaching high school students about emerging infectious diseases like cholera or malaria, what are some successful strategies for describing the seriousness of a disease that they might have no direct experience with?
6. On the other hand, if we suddenly hear a great deal of alarming information about a previously unknown disease, it can cause a fear that is not in proportion to the actual risk to the population; think of some of the reactions in the U.S. to mad cow disease, Ebola, and SARS, for example. When teaching high school students about these diseases, what are some successful strategies for providing information, while giving a balanced perspective on the risks?

Factors That Affect the Emergence of Disease

Factors that affect the emergence of disease (Smolinski et al.)

Human behavior and demographics

Microbial adaptation and change

International travel and commerce

Human susceptibility to infection

Technology and industry

Changing ecosystems

Climate and weather

Breakdown of public health measures

Poverty and social inequality

Economic development and land use

War and famine

Lack of political will

Intent to harm

Activity 6: A Picture's Worth a Thousand Words

Based on video and online text content

15 minutes

Setup

You might have played charades before, in which one person makes his or her partner say the name of a person, place, thing, or title by wordlessly acting out clues. In a variation of this game, the clues are not acted, but drawn with pencil and paper.

We'll play the game in which clues are drawn; however, in this version, the items to draw are diseases that might be considered "emerging." The game is played in teams of two. For each round of the game, one person from each team will see the term and will have 60 seconds to get the other person on the team to say the word by drawing key elements of the disease. Suggested clues to draw are the disease symptoms, the vector (carrier) of the infectious agent, area of origin, mechanism of transmission, or any other hint that suggests the disease. No words or letters are allowed!

When one of the team members guesses the disease from his or her partner's drawings, the round is over. For the next round, the team members switch the drawing and guessing roles.

Materials

- Clock with second hand or stopwatch
- One set of the List of Items to Draw (master copy provided; to make a set, cut on the dotted lines after copying)

List of Items to Draw

Hantavirus	malaria	spongiform encephalopathies
SARS	influenza	tuberculosis
Dengue fever	anthrax	antibiotic resistance
HIV	smallpox	bioterrorism
Legionella	cholera	West Nile virus
Lyme disease	hemorrhagic fevers	

Notes

Activity 1: Multiple Choice

Based on video and online text content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

We hear so many statistics and read so much information about HIV and AIDS, it is difficult to keep the facts straight. Sometimes we even hear conflicting information. As a warm-up to this unit, go through the following multiple choice questions. Don't think about them too much—just check your first impulse. The answer you are expecting might not be there because you may have read or heard something different. As you watch the video or read the text, watch for information that is relevant to these questions. After the video, look at the Multiple Choice Answers sheet, which has answers from the video. We often hear conflicting numbers about HIV and AIDS, so are there any answers for which you have heard other information?

Materials

- One copy of the Multiple Choice Questions per person (master copy provided)
- Transparency of the Multiple Choice Answers (master copy provided)

Multiple Choice Questions

Note: Data are from 2003.

1. Each day, how many people die from AIDS, worldwide?
 - a. 125 million
 - b. 75 million
 - c. 8,000
 - d. 400
2. Each day, how many new HIV infections are there, worldwide?
 - a. 15,000
 - b. 6,000
 - c. 2,000
 - d. 150
3. How many people, worldwide, are infected with HIV?
 - a. 500 million
 - b. 65 million
 - c. 5 million
 - d. 300,000
4. How many people in sub-Saharan Africa are infected with HIV?
 - a. 100 million
 - b. 25 million
 - c. 2 million
 - d. 500,000
5. In what year were HIV DNA vaccines first tested in people?
 - a. 1982
 - b. 1991
 - c. 1999
 - d. 2002
6. In parts of Africa, what is the highest proportion of the adult population that is infected with HIV?
 - a. nearly one in five
 - b. approximately one-third
 - c. up to one-half
 - d. three-fourths
7. What percent of Russians are projected to be infected with HIV by 2010?
 - a. 50%
 - b. 30%
 - c. 20%
 - d. 12%

8. What kind of virus is HIV?
 - a. rotavirus
 - b. retrovirus
 - c. rhinovirus
 - d. coronavirus
9. Which of the following is NOT one of HIV's enzymes?
 - a. reverse transcriptase
 - b. ribosome
 - c. integrase
 - d. protease
10. What cells carry out the "humoral" immune response?
 - a. macrophages
 - b. neutrophils
 - c. T cells
 - d. B cells

Multiple Choice Answers

1. Each day, how many people die from AIDS, worldwide?
c. 8,000
2. Each day, how many new HIV infections are there, worldwide?
a. 15,000
3. How many people, worldwide, are infected with HIV?
b. 65 million
4. How many people in sub-Saharan Africa are infected with HIV?
b. 25 million
5. In what year were HIV DNA vaccines first tested in people?
c. 1999
6. In parts of Africa, what is the highest proportion of the adult population that is infected with HIV?
c. up to one-half
7. What percent of Russians are projected to be infected with HIV by 2010?
d. 12%
8. What kind of virus is HIV?
b. retrovirus
9. Which of the following is NOT one of HIV's enzymes?
b. ribosome
10. What cells carry out the "humoral" immune response?
d. B cells

Activity 2: According to WHO?

(An alternate to Activity 1)

Based on video and online text content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

The World Health Organization's (WHO) 2002 document "The World Health Report" lists 10 risk factors for health. The WHO believes that, together, these factors account for more than one-third of all deaths worldwide. Risk factors associated with genetic diseases or infectious diseases, like the existence of pathogenic viruses and bacteria, are not included, although some of the risk factors are associated with the transmission of pathogens. Everything on the list is a risk factor that could, in principle, be reduced, and if it were reduced; the expected life span of the population would increase.

What do you think is on the list of global top 10 health risks? Make a list of the 10 largest health risk factors you can think of. One starting point is to think of the greatest global risk factor for HIV transmission. What are some other health problems and what is the greatest risk factor for these diseases? After you have made your list, compare it to the list from the 2002 World Health Report and discuss the discussion questions.

Materials

- Transparency of the list of Top 10 Risks for Health from The World Health Report 2002 (master copy provided)

Top 10 Risks for Health (The World Health Report, 2002)

According to the report, these are “real risks to health—for which the means to reduce them are known.”

- underweight
- unsafe sex
- high blood pressure
- tobacco consumption
- alcohol consumption
- unsafe water, sanitation, and hygiene
- iron deficiency
- indoor smoke from solid fuels
- high cholesterol
- obesity

Discussion Questions

Because this unit is about HIV, concentrate on unsafe sex as a risk factor.

1. What might be some effective strategies for reducing this risk in developing countries?

2. What might be some effective strategies for reducing this risk in developed countries?

Activity 3: The Mighty Immune System

(An activity for those who want a better understanding of the basic immune system)

Based on video and online text content

45 minutes

Setup

The key to understanding HIV is understanding the immune system—but our defense against diseases is one of the most complex systems in biology. Although it is a bit artificial, sometimes it helps to divide and conquer; that is, categorize and organize the parts of a complex system and think about them separately.

Working in pairs, follow the activity instructions to make diagrams and tables that organize the immune system parts. Use the HIV and AIDS online text chapter and the beginning of Dr. Jay Levy's interview transcript as references. Then make a big picture diagram that integrates the parts. If time permits, use the diagrams and tables to talk about one or more of the diseases in the discussion questions at the bottom of the activity instructions sheet.

Materials

- One copy of the Activity Instructions per person (master copy provided)
- One copy of the HIV and AIDS online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of Dr. Jay Levy's interview transcript per two people (to approximate marker 11:20:55; available online at <http://www.learner.org/channel/courses/biology>)

Activity Instructions

Step 1: Make a Non-Specific/Innate vs. Specific List

- Make two columns so two long lists can be made. Label one column "non-specific/innate" and the other "specific." Start by listing all the mechanical defenses in the "non-specific/innate" column. For each, write a sentence or two about how it works (e.g., "Ciliated cells keep mucus moving so bacteria and viruses that are inhaled can't hold fast to cells, or can't infect them.")
- Next, list the cells that are involved in the non-specific response. Again, write a sentence or two about what they do (e.g., "Phagocytes engulf foreign cells and particles.")
- Next, list the general types of cells of the specific immune system, with a sentence about what they do.
- Finally, draw lines between cells of the non-specific immune system that interact with, or stimulate, the specific immune system cells.

Step 2: Make a Specific Immune System List

- Make two columns. Label one "B lymphocytes/humoral" and the other "T lymphocytes/cellular." List the types of B lymphocytes and their functions. (Which are involved in short-term response and which are involved in long-term response?)
- List the types of T lymphocytes, giving a short description of the job of each one. Mark those that are CD4 and those that are CD8. Which are infected by HIV?
- Finally, draw lines between cells that interact with each other.

Step 3: Make a Big Picture Diagram

- Divide a paper, section of the blackboard, or a transparency into three parts. Label them "non-specific," "specific B lymphocytes," and "specific T lymphocytes." Put all the cell types from Steps 1 and 2 into the diagram and draw lines to show interactions between cell types.
- Draw arrows from one cell to a cell that it stimulates. Draw a line that ends in a short perpendicular line from one cell to a cell that it inhibits or down-regulates.

Step 4: Discussion Questions

Choose one or more of the following diseases, and describe its encounter(s) with the non-specific defenses and specific immune systems.

- common cold
- *Staphylococcus* skin infection
- cancer
- influenza
- malaria
- tuberculosis
- lupus
- pollen, in an allergy

Activity 4: Miracle Drugs?

(An activity for those who want to focus on HIV)

Based on video and online text content

45 minutes

Setup

When HIV infections begin to progress to AIDS, they are treated with a combination of sophisticated anti-HIV drugs. New drugs and new treatments are continuously being developed, so this exercise will focus on the types of drugs that are available or are in development. In pairs, do a brief presentation on one type of anti-HIV treatment. Your sources of information will be the video, the online text, transcripts from scientists interviewed for the video, and transparencies of helpful figures that are available for your presentations.

Start by spending a few minutes reviewing the immune system, because some of the drugs do not target the virus but manipulate the immune system. Especially note the roles of cytokines, interferon, and interleukins. Then review the mechanism of HIV infection. Focus especially on the proteins of HIV, because any protein is a potential drug target for a drug. Then each pair will choose one or more of the drugs to explain to the rest of the group. If time permits, discuss the additional discussion questions.

Materials

- Video available for people to review
- One copy of the HIV and AIDS online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the interview transcript with Dr. Jay Levy per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the interview transcript with Dr. David Weiner per two people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the Guide to the Presentations plus Discussion Questions per person (master copy provided)
- Tips and Suggested Answers
- Transparency of the HIV infection cycle (master copy provided)
- Transparencies of the HIV drugs and the molecules they resemble to use for presentations (optional, master copies provided)

Guide to the Presentations plus Discussion Questions

Guide to the Presentations

Here is a list of drugs or treatments that could be investigated and presented:

- protease inhibitors
- nucleoside reverse transcriptase inhibitors
- non-nucleoside reverse transcriptase inhibitors
- integrase inhibitors
- interferons and interleukin
- CAF and other cytokines
- anti-HIV vaccines
- presentations could also be made on the innate/non-specific immune system, the specific immune system, and the replication and infection cycle of HIV

Here are some questions to try to answer in the presentations:

1. Does the treatment inhibit the virus or does it boost the infected person's immune response?
 - a. If it targets the virus, which step in replication and which viral protein does it target?
 - b. If it targets the virus, does it prevent the virus from becoming established in the cell, or does it inhibit its replication once it is integrated?
 - c. If it targets a viral protein, is it a "competitive inhibitor" that looks like a molecule the protein normally interacts with, like a substrate of an enzyme? If not, what is its mechanism of action?
 - d. If it targets the immune system, does it affect the innate (nonspecific) or the specific response? What kind of cells does it affect and how does it affect them?
2. Is the treatment currently available?
 - a. If so, how long has it been available and how effective is it?
 - b. What are the side effects?
 - c. If it is not yet available, what is the state of research and development on this therapy?

Discussion Questions

1. Why are drugs taken in combination with each other? (Note: See the Tips and Suggested Answers.)
2. What points in the HIV replication cycle are not being targeted yet?

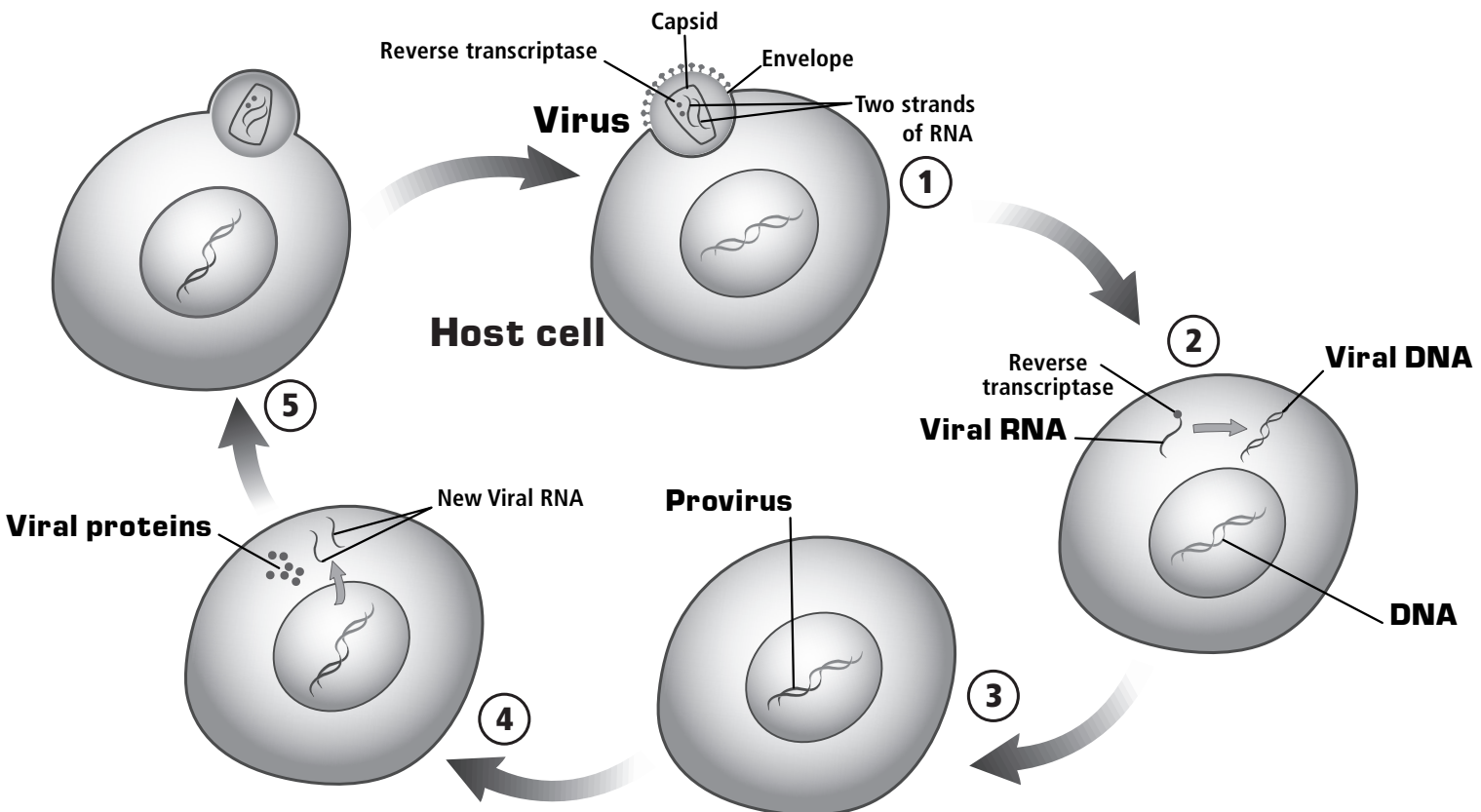
After discussing the above questions, read the following:

HIV encodes a protein called viral infectivity factor (Vif), which is absolutely required for HIV-1 to infect certain cell types. These cells express a protein, called APOBEC3G, that is a viral defense mechanism. APOBEC3G works on unusual, viral DNA, like the RNA-DNA intermediate that forms while reverse transcriptase is converting the HIV RNA chromosome into double-stranded DNA. It deaminates cytosine (C) residues in the unusual DNA, converting them to uracil (U), which does not belong in DNA. The U residue in DNA can cause it to be destroyed (or impairs its ability to be replicated) or cause the introduction of many deleterious mutations.

Source: *Nature*. 2003. Jul 3; 424(6944):21–2.

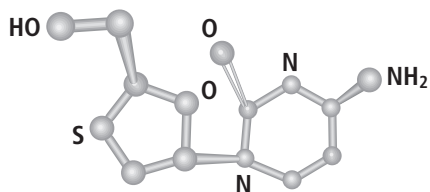
3. How might this information be used to generate new anti-HIV drugs?
4. With what you've seen so far, do you have an opinion on what kind of drugs seem more promising for long-term management of HIV infection: drugs that interfere with viral replication or drugs that regulate the innate immune system? Why?
5. Developing new drugs takes millions of dollars, and years of research and development time. Who should pay for this? Do you think most people would be willing to pay an extra dollar for a bottle of aspirin if it would lower the cost of anti-HIV drugs? What strategies could a pharmaceutical company use to raise money for this kind of drug development?

The HIV Infection Cycle



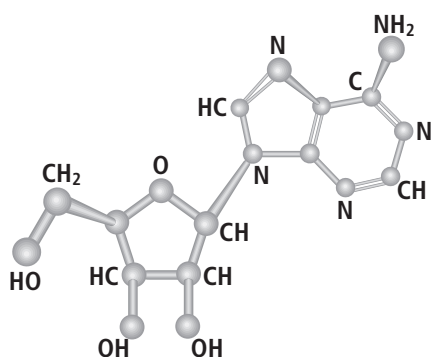
Nucleoside Reverse Transcriptase Inhibitor (3TC)

(from 2003 Web site <http://www.medicine.mcgill.ca/mjm/issues/v05n01/v05p060/v05p060main.htm>)



Nucleoside

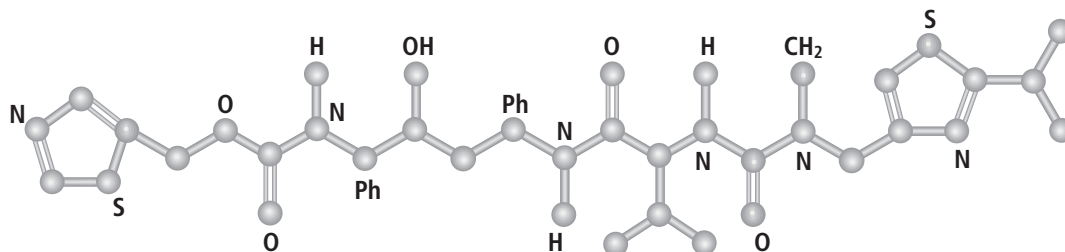
(from 2003 Web site <http://www.web-books.com/MoBio/Free/Ch3A4.htm>)



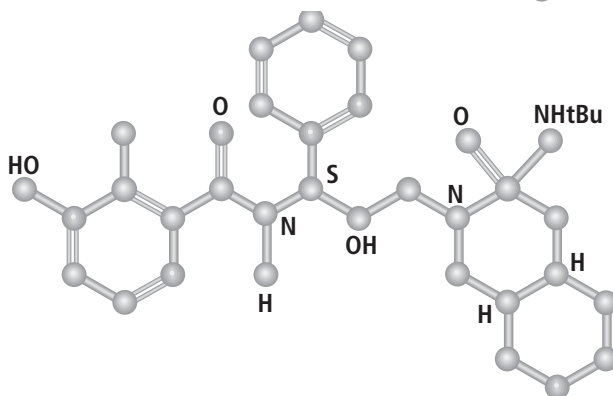
Protease Inhibitors

(from 2003 Web site NEJM via <http://cmgm.stanford.edu/biochem118/Projects/2002/ro.pdf>)

Ritonavir



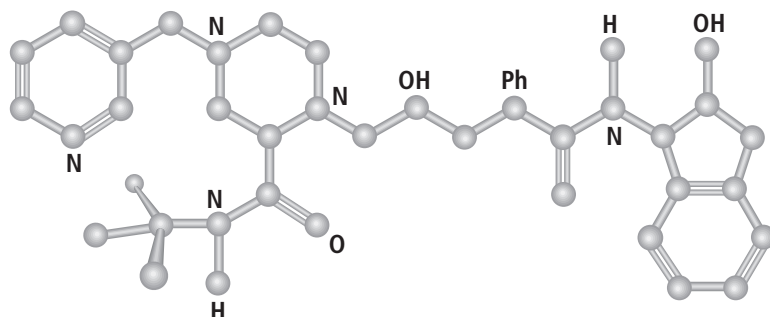
Nelfinavir



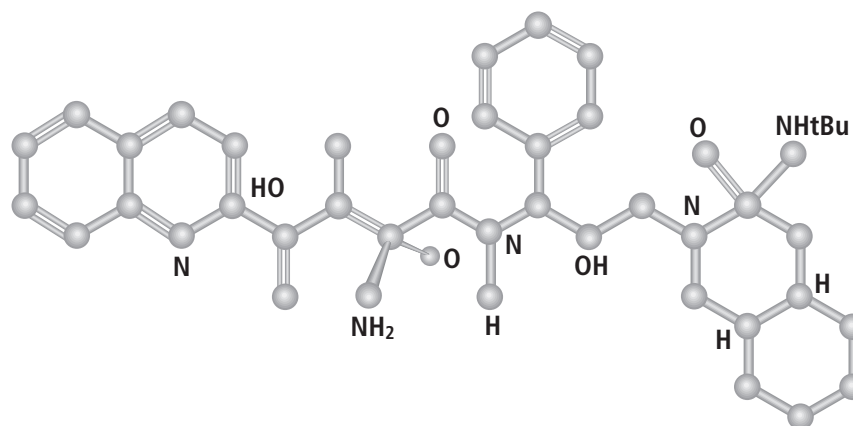
Protease Inhibitors (continued)

(from 2003 Web site NEJM via <http://cmgm.stanford.edu/biochem118/Projects/2002/ro.pdf>)

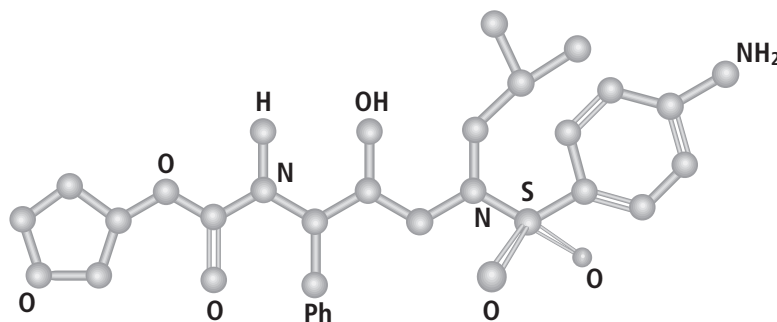
Indinavir



Saquinavir

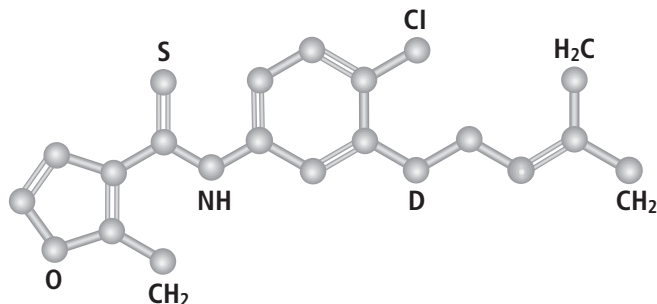


Amprenavir



Non-Nucleoside Inhibitor (UC781)

(from 2003 Web site <http://aidsscience.org/Articles/aidsscience010.asp>)



Answer to the Discussion Question

1. Why are drugs taken in combination with each other?

When HIV is exposed to just one drug, resistant viral strains quickly appear. Much more time is required for resistance to multiple drugs.

If two drugs separately can slow HIV, together they can have an even greater impact on slowing the replication.

HIV can infect many different cells. The drugs differ in their abilities to reach different types of cells, so a combination is more likely to reach all the types of infected cells.

Activity 5: DNA Vaccines

Based on video and online text content

10 minutes

Setup

DNA vaccines are a relatively new technology. Compare them to traditional vaccines by answering the following questions.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. A person who receives the oral polio vaccine is actually infected and produces virus, although they should not get polio. A person who receives the polio vaccine as a shot is not infected and does not produce the virus. Which one of the polio viruses is a “live” but “attenuated” (weakened) virus? Which is an intact but chemically “killed” virus? How does each vaccine stimulate an immune response and protect against the virus? How is the antigenic protein for this vaccine produced: by the virus, in a cell outside the vaccinated person, or in the cells of the vaccinated person?
2. The Hepatitis B vaccine is a viral protein produced in, and then purified from yeast cells. How does this vaccine stimulate an immune response and protect against the virus? How is the antigenic protein for this vaccine produced: by the virus, in a cell outside the vaccinated person, or in the cells of the vaccinated person?
3. What is injected in a vaccination with a DNA vaccine? How does this vaccine stimulate an immune response and protect against the virus? How is the antigenic protein for this vaccine produced: by the virus, in a cell outside the vaccinated person, or in the cells of the vaccinated person?

Activity 6: Lesson Plans

Based on video and online text content

20 minutes

Setup

Many of us have discussed HIV and AIDS in a biology class. All of us have presented information that had both academic and practical value to students. The goal of this unit is to learn more about HIV and AIDS. The goal of this exercise is to focus especially on academic and practical information about HIV and AIDS that you would like students to know.

Work in pairs to come up with five facts or pieces of information about HIV and AIDS that you have presented to a high school class, or have considered presenting. If you have not covered HIV and AIDS in your classes, think of other information related to this topic that you have covered. Next, write down five new ideas that you have gained from the video, online text, or activities of this unit that you might present to a high school class.

As a group share ideas, with a word or two about whether this is something you want students to know for practical (meaning health and safety) reasons, or something you want them to know because it is a good example of a biological principle. If time permits, discuss the additional discussion questions.

Materials

- Transparency of the Activity Instructions and Discussion Questions

Activity Instructions and Discussion Questions

Activity Instructions

- List five facts or points related to HIV and AIDS that you *have* or *have considered* presenting to a high school class.
- List five facts or points from this unit that you might present to a class.
- Are these points important for practical reasons or as an example of a basic biological principle?

Discussion Questions

1. What are the three most important points to emphasize when teaching about HIV/AIDS to high school students?
2. What are the three most important points about the *biology* of HIV to emphasize to high school students?
3. What are the three most important points about *HIV infection and prevention* to stress to high school students?

Activity 7: Public Opinion, Public Policy

Based on video and online text content

20 minutes

Setup

HIV can be transmitted through unprotected sex or shared needles during intravenous drug use. This is one of the reasons for the stigma of being HIV-positive, and the stigma has had an impact on prevention programs and the distribution of treatment. As a group or in pairs, discuss the following questions about the public perception of HIV infection and how public opinion affects policy about HIV treatment.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. When an HIV vaccine is approved for general public use, it will probably be available in limited quantities at first.
 - a. Who should be vaccinated first? Here are some suggestions:
 - children
 - adults who are at high risk for infection, like prostitutes and intravenous drug users
 - people who live in sub-Saharan African countries, where the infection rate can be as high as 30% and the availability of anti-HIV drugs is low
 - health care workers
 - b. Who should decide who receives the first vaccinations? Here are some suggestions:
 - the World Health Organization or the Red Cross
 - the United Nations
 - the company that produces the vaccine
 - c. Sometimes, post-infection vaccination can slow down or prevent the development of disease. If this turns out to be the case with the HIV vaccine, should we first vaccinate people who are already infected? If so, would it matter if they were infected “accidentally” (e.g., through blood transfusion) or if they were infected through sex or drug use?
2. Hepatitis B is transmitted the same way as HIV, yet a Hepatitis B infection does not have the same stigma as an HIV infection. Why?
3. Dr. Jay Levy from the University of California—San Francisco was interviewed for the *Rediscovering Biology* project (you can read a transcript of the entire interview at the Web site). In answer to questions about how to distribute treatments, he said:

I want to emphasize that we should not lose sight of prevention. And what’s happening is all the funds are going for treatment, and we’re saying, “Fine, we just treat, that’s it.” We aren’t looking at blocking this epidemic. So I’m not sure how it can be done, but we must not lose sight of the fact that we need to prevent it through education, distribution of condoms, needle exchanges, and, of course, a vaccine.

 - a. Do you agree or disagree with Dr. Levy’s statement?
 - b. The three suggestions that are possible as of 2003 are education, distribution of condoms, and needle exchanges. Given public sensibilities, are we doing all we can in these areas in the U.S.? If you think we could do more, how? If you think we are doing everything possible in these areas, do you think it is working to prevent HIV transmission?

4. Dr. Levy also said:

Of course, the big question now—and it's vying groups against one another—is, do you encourage prevention or do you encourage treatment? There is no question that now that the world knows that there are drugs that can help people who advance to disease; we cannot deny them the drugs. There is evidence to suggest that if you give drugs to people, it lowers the amount of virus in their blood, and also in genital fluids, so you may actually reduce transmission. Doesn't mean that if you're on drugs you can go and not worry about transmitting it, but there is some evidence to suggest that. So we've got to do something worldwide, and it's being recognized. It's as if one says, "Yeah, we're slow, but it's going to happen." You can't look at the world and see what's happening with 15,000 new infections every day and 8,000 deaths every day—that we can just sit back and be oblivious and not respond. So that's a clear thing.

Source: Sharon Lerner, "How To Advise Youth in the Age of AIDS,"
International Herald Tribune, 8 August 2003.

- a. Are we doing all that we can to prevent HIV transmission worldwide?
 - b. Are we doing enough to treat HIV worldwide?
 - c. How are prevention and treatment related? How might transmission of HIV be affected, either positively or negatively, if people in a community see that effective treatment is available?
5. At the 2003 National HIV Prevention Conference at the Center for Disease Control in Atlanta, Claude Allen, the deputy secretary for the U.S. Department of Health and Human Services, said, "encouraging young people and young adults to abstain is the only appropriate initial strategy" for controlling HIV infections in young people.
- a. What is your opinion about this policy?
 - b. Are we implementing this policy in our schools? If so, is it effective? If not, what can we do to implement it?
 - c. Two alternatives to "abstinence only" policies are "stand-alone" classes in safe sex; or intensive, individualized programs that integrate sex education with components that encourage self-expression in arts, sports, and other "above-the-waist" activities. Of these two alternatives, which seems the most practical? Which seems the most likely to work?

Activity 1: Fact or Fiction?

Based on video and online text content

15 minutes (10 minutes before and 5 minutes after the video)

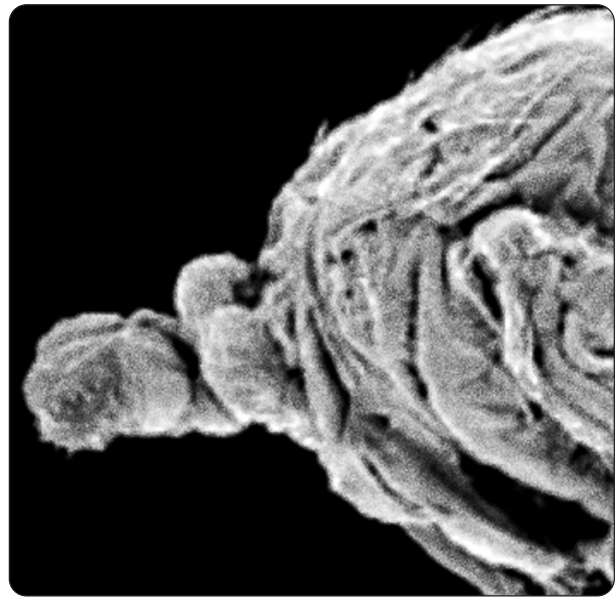
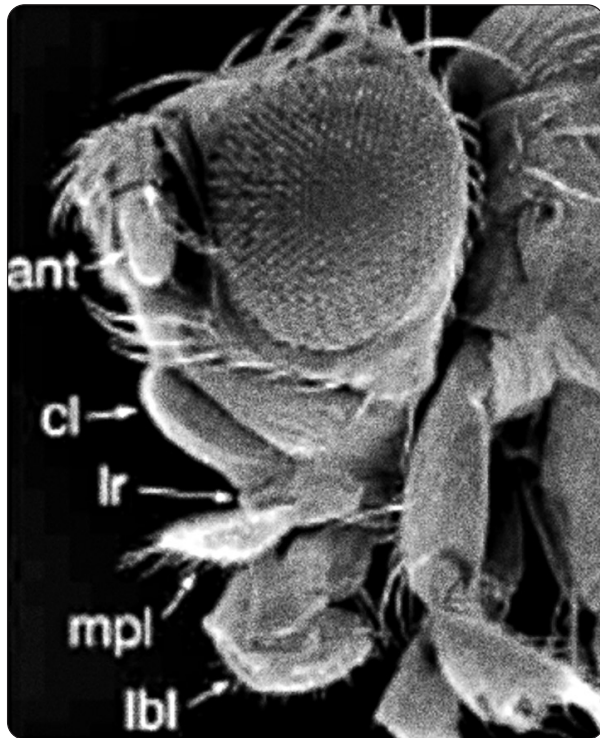
Setup

Recent results in developmental genetics have been eye-popping—literally. The electron micrographs on the overhead transparency show a normal fruit fly on the left; and, on the right, a fruit fly that has developed without eyes and several other head structures. In this unit, we'll see more of the revelations that have come from the study of developing organisms. Before viewing the video, look at 10 statements about developmental genetics. All sound surprising, and half are actually true. Just based on your instincts, which do you think are the true statements? After watching the video, review your choices. Information on some statements can be found in the online text chapter.

Materials

- One transparency of the *Drosophila* Head Micrographs
- One copy of the Statements per person (master copy provided)
- One copy of the Genetics of Development online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Drosophila Head Micrographs



Statements

All of these statements about developmental biology sound unbelievable, but five are actually true. Can you choose the five statements that are *entirely* true? Even if you don't know anything about this subject, just take a educated guess. Answers, found in Tips and Suggested Answers, will be discussed after watching the video.

1. The phenotype of the fly in the electron micrograph on the left is caused by altering the expression of a single gene, called *eye/less*.
2. One of the most commonly used organisms for developmental research is the zebra, because of the universality of its developmental processes and its surprisingly brief gestation period.
3. In animals, most genes are not grouped together by function. However, the Hox genes that regulate development are grouped together by function, and arranged in order of the body segment they control and the time they are expressed.
4. A compound made by the corn lilly plant blocks a receptor required for signaling during animal development. Sheep that are exposed to the compound in utero have a cyclops phenotype.
5. Women who are trying to become pregnant are advised not to take an anti-acne drug called Accutane because it triggers a response similar to fertilization, resulting in a haploid embryo that cannot complete development.
6. The most promising medical feature of embryonic stem cells is that they can be transplanted into any adult without being rejected.
7. A gene called Hedgehog is a crucial signal-transduction component of animal development. The gene got its name because mutations cause fruit fly embryos to have a spiny appearance.
8. There are some developmental genes, encoding MADs box transcription factors, that are so highly conserved that they are found in both plants and animals.
9. The *C. elegans* species of nematode worms is an excellent model organism for development: each worm develops in a slightly different way and ends up with a different number of cells, just like a human.
10. After the age of about 25, a human no longer has any stem cells.

Source of micrographs: Jiao, R. et al. 2001. *Development* 128:3307

Fact or Fiction? Answers

1. True.
2. False, but *zebrafish* are a commonly used organism for developmental research.
3. True.
4. True.
5. False. Accutane is contraindicated for pregnant women, but it is because it triggers incorrect expression of Hox genes during development.
6. False. Embryonic stem cells come from an embryo that resulted from fusion of a sperm and an egg. As a result, it has a unique genetic makeup and novel combination of cell surface antigens that could be rejected by the immune system of a transplant recipient.
7. True.
8. True.
9. False. The nematode worm *C. elegans* is used as a model organism because the developmental fate of every cell has been determined, and every non-mutant individual has a precise number of cells: 1031 in the males and 959 in the hermaphrodites.
10. False. Adults have stem cells that are continually dividing to generate replacements for skin, sperm, blood, and other cells.

Activity 2: Mommie Dearest

Based on video and online text content

45 minutes

Setup

A breakthrough in the genetics of development was recognized with the 1995 Nobel Prize in Physiology and Medicine, given to Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric F. Weischaus for their work on maternal effect genes. Although inherited as nuclear genes, their phenotypic effects depend on the genotype of the mother, not the individual. In this exercise, work in pairs to read about these genes and their role in development from the Genetics of Development online text chapter. Then, set up some fruit fly crosses that involve mutations in maternal effect genes. Follow several generations in the crosses to see how maternal effect genes are inherited and how they exert their phenotypic effects.

Materials

- One copy of the Readings, Worksheet, and Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Readings, Worksheet, and Discussion Questions

Part 1: Readings

Before starting on the problems, familiarize yourself with maternal effect genes by reading this excerpt from the Genetics of Development online text chapter (available online at <http://www.learner.org/channel/courses/biology>).

Establishing the Gradient and Coordinate Genes

Development is a process where the products of some genes turn other genes on or off. But how does the process start? Even before fertilization, development is occurring. We normally think of an egg as a storehouse of energy supply and nutrients that the embryo will use as it develops. While this is true, the egg also supplies information to establish a molecular coordinate system. This coordinate system provides a way to tell “which end is up”; in other words, the location of the embryo’s head is determined even before the egg is fertilized.

Coordinate genes are named because they establish the primary coordinate system for what will become the embryo. One important example of a coordinate gene is *bicoid*, which is involved in establishing the anterior-posterior polarity in *Drosophila*. How does *bicoid* do this? To understand this process, we need to first discuss how *bicoid* gets to the anterior part of the egg. Nurse cells surround the anterior region of the egg in *Drosophila* and other flies. Cytoplasmic bridges allow various substances—in this case mRNA from *bicoid*—to be transported from the nurse cells into the egg. The *bicoid* mRNA is then trapped by proteins produced by other genes. The result is a concentration gradient of *bicoid* mRNA: the anterior end has the highest concentration and the posterior end lacks it. Translation of *bicoid* is inhibited until after fertilization, leading to a *bicoid* protein concentration gradient.

In addition to *bicoid*, other coordinate genes help establish an anterior-posterior polarity. Still other coordinate genes allow the establishment of a dorsal-ventral gradient. These coordinate genes, like *bicoid*, are sometimes called maternal effect genes. **Maternal effect** occurs when the phenotype of the individual is dependent on its mother’s genotype, not its own. In cases of maternal effect, the transmission pattern of the alleles is the same as in standard Mendelian genetics but the action of the gene occurs a generation later. For example, consider a maternal effect gene where the mutant allele (*m*) is recessive to the wild-type allele. In the cross of homozygous, wild-type females to homozygous, mutant males, all the F1 offspring are heterozygotes and appear normal. In the reciprocal cross, all of the F1 offspring are heterozygotes but have the mutant phenotype. Although the F1 offspring are genotypically identical in the reciprocal crosses, they are phenotypically different. This is because phenotype is due to the action of the mothers’ genotype. Maternal effect is not the same thing as maternal inheritance, such as in mitochondria, where the genetic material is transmitted only across maternal lines.

Responses to the Concentration Gradient

Coordinate genes such as *bicoid* lay down the grand plan, so to speak, upon which the genes downstream will act. The pattern of the developing embryo arises as these downstream genes are activated or repressed.

Like many of the other coordinate genes, *bicoid* encodes a transcription factor; thus, there is a concentration gradient of a transcription factor. The next genes in this developmental cascade, the “gap genes,” possess binding sites for this transcription factor. Gap genes are so named because mutations in these genes can produce larvae with “gaps” (missing several segments). These genes differ in how many *bicoid* binding sites they have and, thus, vary in their sensitivity to this transcription factor. Some gap genes will become active at low concentrations of *bicoid*, while the activation of others will require higher concentrations. Due to the concentration gradient, different regions of the developing embryo will activate different gap genes.

Unlike the coordinate genes, the gap genes are not maternal effect genes. The activities of the embryo's gap genes (and not those of the mother's genes) determine the phenotype. Gap genes also encode for transcription factors, and these affect the transcription of genes that further refine the patterning of the *Drosophila* embryo.

Part 2: Worksheet

Now that you are familiar with maternal effect genes, work through these problems. Some answers can be found in Tips and Suggested Answers.

1. An example of a maternal effect coordinate gene that is expressed in the anterior section of the fly embryo is *bicoid*. Null alleles of *bicoid* have recessive effects, and *bicoid* is an autosomal locus.
 - a. Draw the cross between a female fly that is a heterozygote of a null and a wild-type allele, with a male that is also a heterozygote. (Write the genotypes of the parents and all possible offspring.) The null allele is designated *bcd* and the wild-type is designated *bcd*⁺.
 - b. What are the expected genotypes of the offspring (and in what ratios) from such a cross?
 - c. What are the expected phenotypes of the embryos from this cross?
 - d. What would you expect for the phenotype of an embryo that is the result of a homozygous *bicoid* null mother mated to a homozygous wild-type male? Draw out this cross as well.
2. The protein from the *bicoid* gene is a transcription factor that turns on expression of *hunchback*, a gap gene. Like *bicoid*, *hunchback* is also on the third chromosome.
 - a. Draw the cross of a female that is homozygous for the null allele of *bicoid* and wild-type for *hunchback*, with a male that is homozygous for the wild type allele of *bicoid* and homozygous for the null allele of *hunchback*. The wild-type allele of *hunchback* is designated *hb*⁺ and the mutant is *hb*.
 - b. Given a *Drosophila* embryo with a mother that was homozygous for a *bicoid* null allele, what would you expect for the expression pattern of the hunchback protein?
 - c. What would be the expression pattern of bicoid protein in a *Drosophila* embryo that was homozygous for the wild-type allele of *bicoid*, but was homozygous for a null allele of *hunchback*?

Part 3: Discussion Questions

1. How is maternal effect inheritance different from mitochondrial inheritance? In particular:
 - a. Which genes are nuclear? Which are cytoplasmic?
 - b. In which type of inheritance do the mother and offspring always share phenotypes?
 - c. In which type of inheritance are alleles inherited equally from the mother and the father?
2. Challenge question (may be more difficult): Two species of fruit flies each have very similar patterns of abdominal bristles; however, hybrids between these species often have deformed bristles. Recent studies have found that the gene (*shaven baby*) involved in making those bristles has fourfold lower expression in the hybrids than in the pure species.

Based on what you have learned developmental genetics, propose an explanation for these results. Hint: Think about what might be different in the two species.

Mommie Dearest, Suggested Answers

Part 2: Worksheet

1.
 - a. 25% *bcd+/bcd+*, 50% *bcd/bcd+*, 25% *bcd/bcd*
 - b. The gene is maternal effect, and null alleles are recessive. Given that the mother is heterozygote, all embryos should be phenotypically normal.
 - c. Even though the offspring is a heterozygote, its phenotype is determined by the maternal genotype, and the mother is a homozygous null. Individuals without any *bicoid* expression would lack anterior segments—the head and thorax—and would likely have two tails.
 - d. Even though the offspring is a heterozygote, its phenotype is determined by the maternal genotype, and the mother is a homozygous null. Individuals without any *bicoid* expression would lack anterior segments—the head and thorax—and would likely have two tails.
2.
 - b. The embryo would not express hunchback protein, because it lacked the bicoid protein.
 - c. The expression of bicoid would be normal—expressed in the anterior of the fly and not the posterior—because hunchback expression doesn't affect *bicoid* expression.

Part 3: Discussion Questions

2. The species have diverged in both the promoter of *shaven baby* and the transcription factor(s) that bind to that promoter. In each of the pure species, binding between the transcription factors and the promoter is normal. The hybrids, however, have the wrong combinations of transcription factors and promoter.

Activity 3: Small Cells, Big Controversies

Based on video and online text content

60 minutes

Setup

Stem cell research has yielded key insights into the fundamentals of development. It has also promised great medical advances, especially in tissue transplants. Because this area of research includes work on stem cells that come from embryos, this field is also the center of much controversy. *Rediscovering Biology* interviewed a leading stem cell researcher, Dr. Markus Grompe of Oregon Health and Sciences University. Read excerpts of his interview and, in teams of three or four, discuss the scientific facts and the public opinions about stem cells.

Materials

- One copy of the Discussion Questions per person (master copy provided)
- One copy of the Interview Transcript Excerpts per person (master copy provided)

Discussion Questions

1. Read Excerpt 1, and then discuss these questions:

- a. What are the developmental differences between totipotent and pluripotent stem cells? How are they different in potential medical functions?
- b. How would you explain the differences between embryonic, prenatal, and adult stem cells?
- c. Dr. Grompe, in his interview for *Rediscovering Biology*, says that embryonic stem cells are “not really little human beings” because they are not totipotent—they cannot generate a placenta. Is he splitting hairs when he distinguishes between embryonic and prenatal, totipotent, and pluripotent? Or are these useful distinctions for scientific and public policy discussions?
- d. As we just read, embryonic stems are obtained directly from embryos. One way to obtain human embryos is to combine a donated sperm and a donated egg in a test tube, and let the zygote begin to divide in vitro. State all possible opinions in favor and against generating embryos this way for the purpose of obtaining embryonic stem cells. If you feel comfortable doing so, state your own personal opinion.

Stem cells have also been taken from embryos that were generated as part of an infertile couple’s therapy. State all possible opinions in favor and against taking stem cells from the unused embryos of an in vitro fertilization procedure. If you feel comfortable doing so, state your own personal opinion.

2. Read Excerpt 2, and then discuss these questions:

- a. What are the political and ethical connections between the issues of embryonic stem cells and human cloning? Do you think people have difficulty seeing the difference between these procedures? If so, what can be done to make the issues more clear?
- b. In August 2001, President George W. Bush announced that federally funded labs could work with only those embryonic stem cell lines that were established before the announcement. Of course, privately funded labs and labs in countries with other regulations are not subject to these rules: they can generate new embryonic stem cell lines from embryos that were not used after an in vitro fertilization, or from embryos generated solely for the purpose of extracting stem cells. What factors were involved in this decision by President Bush’s administration?
- c. How can the government effectively regulate the use of stem cells in research? Who should decide which cells should be made available for research? Is this an issue for state, federal, or global authorities to decide and regulate?

- d. Does the policy accurately reflect public opinion about embryonic stem cells and their uses?
 - e. What effect, if any, has this policy had on public health in the U.S.? Will basic research be affected?
 - f. In September 2000, several celebrities with diseases and disabilities that might benefit from stem cell research—such as Mary Tyler Moore, Christopher Reeve, and Michael J. Fox—asked Congress to allow federal funds to be used for embryonic stem cell research. Do you think this kind of publicity affects public opinion or federal policy?
 - g. What diseases and medical conditions could potentially be treated or cured through stem cell therapy?
3. Read Excerpt 3, and then discuss these questions:
- a. Which do you think is more promising for research on development: adult or embryonic stem cells?
 - b. Which do you think is more promising for medical treatments like transplants: adult or embryonic stem cells?
 - c. Is it necessary to use embryonic stem cells? Or can adult stem cells serve the same research purposes?
 - d. Would you be able to discuss these topics in your high school biology classes? Or are the issues too sensitive? If you would be able to discuss it, would you allow the expression of personal opinions? If so, how would you moderate the discussion?

Interview Transcript Excerpts

Excerpts from a 2003 interview with Dr. Markus Grompe of Oregon Health and Sciences University, for the *Rediscovering Biology* Genetics of Development unit. The entire interview transcript is available online at <http://www.learner.org/channel/courses/biology>.

Excerpt 1:

Q: Can you please define a stem cell?

A stem cell is a cell in the body that is responsible for renewing other tissues. It is not a differentiated functioning cell, but it is a cell that's sort of the reservoir for other cells that are needed in the body. Stem cells exist both before birth—prenatal stem cells—and they continue to exist in the adult organism.

An “embryonic” stem cell is actually a cell that doesn't naturally exist in humans or in animals. It's actually a kind of a laboratory stem cell that has been used extensively since the late 1980s for experimental biology. This very specific kind of cell [is] derived from early embryos and is used in the lab. Those are now available for human and mouse and a variety of other species. I tend to refer to the cells that naturally exist in the early developing organism as “prenatal” stem cells, because the term “embryonic” stem cell is already basically very narrowly defined as that particular kind of cell.

It all starts with the fertilized embryo when the sperm and the egg come together and that's the only cell really that one would describe as a totipotent stem cell, in the sense that that first one- or two- or four-celled embryo has the ability to give rise to the entire fetus as well as the placenta. So the difference between a totipotent and a multi- or pluripotent cell is that only the totipotent cells can give rise to both the placenta and the embryo both.

The embryonic stem cells that have been talked about so much are actually cells that can give rise to virtually all the tissues of the fetus and the adult organism, but they do not make placenta. So those embryonic stem cells that are talked about quite a bit in the newspapers and so forth, are not totipotent, they're not really little human beings. They have the capability to making all the tissues of the fetus, but not the placenta.

Q: And the placenta is necessary for the fetus?

For complete development, yes. The very earliest cells are the ones with the most developmental potential. The embryonic stem cells that people are beginning to use for tissue repair studies and so forth are derived from the very early embryo at the so-called blastocyst stage, which is where the embryo has first developed a cavity within it and there's a group of cells in there called the inner cell mass. What people do for mouse and human and primate embryonic stem cells, is pick out those inner cell mass cells and then grow them in the laboratory. What's been learned from the mouse in particular is that you can actually culture these cells in a dish for many many generations and then inject them back into blastocysts and they have the capacity to develop back into a full adult mouse, which is the basis of a lot of the genetic manipulation of mice that we use in the laboratory.

I think that the distinction between totipotent and pluripotent is important in regards to the ethics of this discussion, because some people are under the impression that embryonic stem cells are little people being grown in the tissue culture dish in the lab. These are not embryos; they're embryo-derived. So in the process of generating embryonic stem cells an embryo is destroyed, but it's not the same thing as cloning or actually having the equivalent to a human conceptus in the lab.

Q: What's the process to obtaining embryonic stem cells?

The procedure by which embryonic stem cells are made differs a little bit from species to species, but basically fertilized embryos are taken in the tissue culture dish and they are allowed to develop to the blastocyst stage, which consists of 32 to 64 cells. You can see that under the microscope, it takes several days for that to happen. At the blastocyst stage there will be a group of cells on the inside of the embryo called the inner cell mass. They are dissected out under the microscope and then they are dispersed and grown on a group of cells that we call feeder cells. They essentially then start to grow like bacteria, doubling and doubling and doubling again with virtually unlimited capacity for that process.

The nice thing about embryonic stem cells is, though, they haven't forgotten how to go back and be differentiated and stop growing. So that if they are put back into an environment such as a developing embryo where they get the right signals, they stop growing uncontrolled and they start behaving like a proper inner cell mass again.

Excerpt 2:**Q: Because there has been controversy about this and there are ethical questions, are there some labs that have been using the totipotent cells? Is there advantage to doing research with that and has it happened?**

Well, there are labs that work with actual human embryos. Those are particularly—in terms of the research applications—the people interested in cloning. Those cells actually grow very very poorly and so you really don't have the ability to propagate them extensively and use them for tissue repair studies. What people have been using them for is to basically try to put into these embryos the nuclei from adult cells and that's called cloning.

That's what we've been hearing about here the last few weeks or months, is basically killing a human embryo and transferring the nucleus of an adult cell into it. I think the ethics with embryonic stem cells come from the fact that to get them you have to destroy an embryo. But once they're there and exist and can be grown, there are no further ethical problems at that point.

I think this is where the policy of the Bush government has come from, is the fact that [it is OK to use] the already existing lines.

Q: So the ethical question for using the pluripotent cells arises because the embryo at that early early stage is essentially destroyed?

Yes. So, basically, to get embryonic stem cells, the embryo is allowed to progress to the blastocyst stage, which is 32 or 64 cells, and is harvested essentially at that point, or a portion of the embryo is cut out. Basically that's the end of that embryo except for the cells in the inner cell mass that can then essentially grow indefinitely; they're in a sense immortal.

Excerpt 3:**Q: Now can you talk about adult stem cells?**

Adult stem cells are, for the most part, tissue-specific adult stem cells; meaning that for a certain tissue, you need in some instances a stem cell that continuously divides and spawns new cells to stay alive. There are tissues that turn over all the time and you basically need stem cells—so that would be blood, skin, intestine, or sperm production. Those are all examples of tissues where there's continuous cell division going on and all of those tissues have stem cells that are responsible for tissue renewal.

People have known and written about [these] for a considerable length of time. It's now become apparent that most, if not all, tissues actually have stem cells. Although most tissues are not continually dividing all the time, there are cells that are responsible for tissue repair in the case of injury. So for example, let's say your liver gets hurt by a virus or something like that. Even though liver cells on average only divide once a year in a normal person, you can wipe out your liver with a virus or an injury quite drastically and then you might need stem cells. These are adult stem cells that we call facultative, meaning they are only activated when they're needed as opposed to those in the tissues like blood that are always activated and continuously dividing.

Finally there's a new concept in terms of adult stem cells, which is the idea that adult organisms, including people, continue to harbor some of these very early stem cells like the embryonic stem cells, basically pluripotent stem cells. That's really a concept that's only emerged in the last five, six years and has generated a lot of excitement in terms of the therapeutic potential of those cells.

Q: What are the advantages of using embryonic stem cells or prenatal stem cells for research?

The reasons embryonic stem cells are advantageous for research are many-fold. The main advantage that I see in terms of practical use is the fact that these cells can grow virtually indefinitely in the tissue culture dish. It's possible to share cells between laboratories, it's possible to have comparison of results between different laboratories. But just the sheer fact that they are what we would call immortal allows very very easy experimental manipulation of the cells.

Embryonic stem cells have the other advantage that they are multipotent and can turn into many different types of tissue in culture, and therefore it's possible to learn from studying embryonic stem cells how differentiation occurs. Basically, how you go from multipotent to developmentally restricted and finally differentiated. People are using them for that purpose.

Q: Are there advantages of using adult stem cells for the same type of research?

The adult stem cells have the advantage of the fact that you can have many more cell donors; it's easy to get the cells. For example, it should at least in theory be possible to get adult stem cells from any person who wishes to donate cells. In terms of research, they have really not many advantages over embryonic stem cells, with the possible exception that what we learn about differentiation from embryonic stem cells could potentially only pertain to embryonic differentiation.

There's a paradigm in developmental biology that says that the way a liver stem cell becomes a hepatocyte—for example, in the embryo—is the same process in principle that happens later in life when a liver stem cell becomes a hepatocyte. But that is only a hypothesis at this point. We really don't know in most cases whether adult stem cell differentiation mimics embryonic stem cell differentiation. It's possible that what you would learn from embryonic stem cell differentiation would not apply to adult stem cells.

Q: Can you summarize in a "compare and contrast" fashion the use of embryonic versus adult stem cells?

Embryonic stem cells and adult stem cells can be both used for research. There are several advantages and disadvantages to both. Again, the most important advantage of embryonic stem cells is that they can be easily grown to large numbers; it takes a very short period of time to grow a lot of them, which makes it easy to study. Also the fact that they can develop into multiple tissues makes it possible to study those processes.

The disadvantage of adult stem cells in comparison is that they grow much more slowly and at lower density, so it takes a long time to make lots of adult stem cells. However, the advantage of using the multipotent adult progenitor cells is that they would potentially teach you about developmental processes in the adult, which could be very different from embryonic stem cells. So, in that sense, even though they grow in a more difficult system, they have the advantage of probably reflecting the status of adult stem cells more readily.

In terms of clinical application, there are also quite important differences between embryonic stem cells and adult progenitor cells. The most important one is that embryonic stem cells are derived from donor fetuses that are immunologically mismatched to the person you'd want to be treating. You would have the same issues as you currently have with organ transplantation or with blood transfusions, where you might have to actually use immunosuppression to be able to use those cells. With the adult stem cells it should be possible to derive those cells from the patient herself, so you at least in theory may be able to get away completely from using immune suppression. So the immunological mismatch is a problem.

The other issue with embryonic stem cells is that when they're injected or when they're transplanted without additional modification, in animals at least they form tumors very easily. There is a significant safety issue with embryonic stem cells. Whereas the adult stem cells as they've been studied to date, do not form tumors in any kind of setting. So there are differences for clinical use in that regard.

Q: Would another disadvantages of using adult stem cells for clinical use be that the genetic makeup of that cell is going to be the same as the person you're putting it back into and the same mutations may be there?

Adult stem cells are thought by some to have the disadvantage of being a problem with genetic disease. For example, if you derive adult stem cells from a person with a lung genetic disease, the cells that you derive from that person are also of course going to have that same genetic disorder. Even though they're immunologically matched, you couldn't do a cure with them because they have the same genetic defect. However, that problem is easily surmounted, because like embryonic stem cells, adult stem cells grow extensively in tissue culture. It is possible to correct the genetic defect first *in vitro* and then use those genetically modified cells. The only difference between the stem cells and the patient are that the stem cells have been cured of the genetic defect.

Q: Can you talk about the pros and cons of a patient receiving his or her own cells?

There are disadvantages and advantages to using the patient's own cells for the treatment of Type I diabetes in terms of stem cell therapy. It's important to note that Type I diabetes comes about by immunological rejection of the patient's own beta cells. So, the thinking is that if you were to generate beta cells from that patient's own tissue, you would basically restart the entire process. In other words, the immune system would come after those beta cells again unless you use immune suppression. If you use embryonic stem cells from a donor that's not tissue matched to the person with diabetes, it could have both a positive or a negative effect. The negative effect would be that the immune system might also attack those cells because they are foreign. The positive effect might be that that immunological rejection may be more easily managed than the original immune rejection of the beta cells.

Until those kind of experiments or studies are done, it's really impossible to know whether that would be an advantage or disadvantage to use tissue matched or non-matched cells. But the immune rejection is going to be an issue with Type I diabetes whether you use the patient's own cells or not.

Activity 1: Classifying Life

Based on video and online text content

35 minutes (30 minutes before and 5 minutes after the video)

Setup

Over time, the way that organisms have been classified into kingdoms has changed significantly. The extensive genus and species classification system developed by Linne had only two kingdoms: plants and animals. The familiar five-kingdom system was proposed by Whittaker; it classifies all organisms into plants, animals, protista, fungi, and monera (bacteria). Based in part on molecular data, Carl Woese proposed a three-domain system, in which all organisms are classified as Eubacteria, Archaea, or Eukarya.

In this activity, the group will be divided into six teams. Each team will be given characteristics for one organism. Using this information and the online text chapter, compare the three classification systems by discussing how the organism would be classified in the Linne, Whittaker, and Woese systems. Then explain your organism and its classification to the rest of the group.

After watching the video, talk about the Discussion Questions.

Materials

- One set of the Organisms to Be Classified (master copy provided, cut along dotted lines after copying)
- One copy of the Evolution and Phylogenetics online text chapter per team (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the discussion questions per person (master copy provided)
- Tips and Suggested Answers

Organisms to Be Classified

For answers, see Tips and Suggested Answers.

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 1: *Anabaena sp.*

Characteristics:

- Lacks a true nucleus
- Cytoplasm contains no organized membrane-bound organelles
- Possesses photosynthetic membranes

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 2: *Sulfolobus*

Characteristics:

- Lacks a true nucleus
- Inhabits hot sulfur springs of Yellowstone
- Obtains energy by oxidizing sulfur
- Optimal conditions for this organism are 60–80°C and pH 2–4 (thermoacidophiles)

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 3: *Laminaria* (kelp)

Characteristics:

- Possesses a true (membrane-enclosed) nucleus
 - Multicellular, marine species
 - Thallophytes (i.e., lack true vascular tissue)
 - Photosynthetic
-

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 4: *Physarum* (Myxomycota; "slime mold")

Characteristics:

- Possesses true nucleus
 - Terrestrial
 - Possesses hyphae
 - Obtains nutrition by engulfing bacteria and small bits of organic matter
-

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 5: *Jasminum sp.* (Jasmine)

Characteristics:

- Possesses true nucleus
 - Terrestrial
 - Photosynthetic
 - Multicellular
-

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 6: Nematode worm (*Caenorhabditis elegans*)

Characteristics:

- Possesses true nucleus
 - Terrestrial
 - Dependent on external nutritional sources
 - Multicellular
-

Discussion Questions

1. Of the three classification schemes used in this activity, which is currently favored and why?
2. Researchers such as Carl Woese have integrated the use of molecular and phenotypic tools to re-evaluate the classification of life. Do you think one set of information is more reliable for determining relationships than the other? If so, why? What other tools might be used in the future for classification of organisms?
3. Biologists have long classified organisms according to similarities of form. They usually name them this way too. More recently, some biologists have asserted that the names/classifications that we give organisms should reflect their evolutionary histories. Why might this be a good idea? What additional data are now available to make this a reasonable standard?

Organisms To Be Classified

Classifications according to the Linne two-kingdom system of Plantae and Animalia:

- Plantae – Organisms 1–5
- Animalia – Organism 6

Classifications according to the Whittaker five-kingdom system: Monera, Protista, Plantae, Fungi, and Animalia:

- Monera – Organism 1, 2
- Protista – Organism 3
- Plantae – Organism 4
- Fungi – Organism 5
- Animalia – Organism 6

Classifications according to the Woese three-domain system: Eubacteria, Archaeobacteria, and Eukaryota:

- Eubacteria – Organism 1
- Archeobacteria – Organism 2
- Eukaryota – Organisms 3–6

Activity 2: Construction of a Phylogenetic Tree

Based on video and online text content

60 minutes

Setup

Swordtail fish in the genus *Xiphophorus* exhibit substantial variation in male body size as the result of genetic variation. Some species, however, are either uniformly large or small. In addition, males of the various species exhibit differences in the length of the sword and in mating rituals. These observed differences elicit various responses in females with regard to sexual mating. Based on these physical characteristics and molecular information (fabricated for this activity), see if you can build a phylogenetic tree for four different *Xiphophorus* species.

Work in pairs to build the best phylogenetic tree, first using physical information, and then using genetic characteristics of the related species. The Evolution and Phylogenetics online text chapter explains the logic used to generate a phylogenetic tree and provides step-by-step instructions.

Materials

- One copy of the Instructions and Physical Information per person (master copy provided)
- One copy of the Genetic (rDNA) Information and Discussion Questions per person (master copy provided)
- One copy of the Evolution and Phylogenetics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Instructions and Physical Information

Step 1:

First, construct a draft of an unrooted phylogenetic tree of swordfish, based on the provided physical information. (Unrooted means it will show only relatedness, not the direction of evolution). Use the logic of Hennig and the systemists presented in the text to construct this first-draft phylogenetic tree.

Step 2:

Now consider that parts of the 12S rDNA have been sequenced from these species. Construct a phylogenetic tree based upon the molecular data and compare it to the tree made based on physical data. Are the trees different? What would explain these differences?

Species 1 — *X. cortezi*

Physical Information: sympatric with *X. pygmaeus*; share characteristics with larger morphs of *X. nigrensis* (larger than *X. pygmaeus*, have deep bodies and swords); males perform courtship displays; loss of sword in *X. pygmaeus* may be due to avoidance of *X. cortezi*; UV component in sword to attract females

Species 2 — *X. multilineatus*

Physical Information: can be a threefold difference in mean sword length across male populations; polymorphic characteristic of swords (many shapes); sword variation may be a result of predation pressure (predator preference for males with long swords appears to be ancient); UV component in sword to attract females

Species 3 – *X. nigrensis*

Physical Information: females prefer large males with long swords; size inherited paternally; one of the most studied species because size is controlled by pituitary gene (*P*); males court females based on size; polymorphic characteristic of swords (many shapes); sword variation may be a result of predation pressure; UV component in sword to attract females

Species 4 – *X. pygmaeus*

Physical Information: Name refers to unusually small size of males (and swordless); parsimony suggests large size alleles lost in this species; could have been lost due to predatory pressure; females still prefer larger size males (retained ancestral preference); males do not court; UV component in sword to attract females

Genetic (rDNA) Information and Discussion Questions

For possible answers, see Tips and Suggested Answers.

12S rRNA sequences (shortened and fabricated for activity)

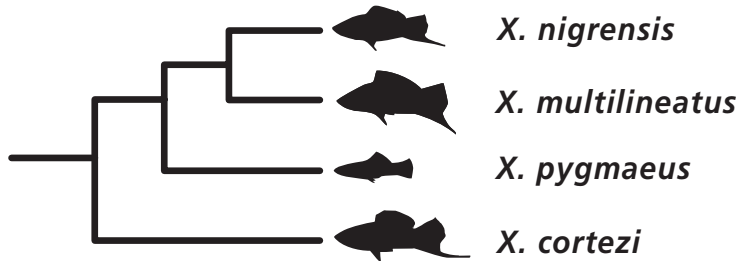
Species	12S rRNA Sequence
<i>X. multilineatus</i>	ATTAGCGCATCGGCATTTAACGGCCAATGCATTGCCCATCGTACGGCACTGTT
<i>X. nigrensis</i>	ATTAGCGCATCGGCATTGCGCTAATCGGCGTTTGCCCATCGTATTACATCATT
<i>X. cortezi</i>	ACTACCAATATGGATTACGGCTAATACGGCATAACGACGGCAATTAGGCAGAT
<i>X. pygmaeus</i>	ATTAGCGCGCGCAATATAAACGGCCTTACGCCTATGCATTTCGACGGCACTGTT

Discussion Questions

- The trees made in this exercise were unrooted. What additional step would be required to make a rooted tree? What additional information would be shown in a rooted tree?
- What are the major steps involved in generating a phylogenetic tree using a cladistic approach?
 - In cladistic analyses, shared, derived characteristics are more informative than shared, ancestral characteristics. Why?
 - Give an example from the text or video of homoplasy. What are two different explanations for the existence of homoplastic characteristics, and what does each say about the relatedness of the groups being compared?
- How do scientists use the genetic data to determine a cladistic tree?
- Groups of animals (nematodes, for example) are nearly identical morphologically, but have considerable genetic variation. What does this imply? Why might this occur? How might a scientist deal with this problem in making classifications?

Construction of a Phylogenetic Tree

Actual Tree:



Answers to Discussion Questions:

1. The trees made in this exercise were unrooted. What additional step would be required to make a rooted tree? What additional information would be shown in a rooted tree?
Making a rooted tree would require adding information from a distantly related outgroup. In addition to relatedness, a rooted tree would show the progression of evolution.
2. What are the major steps involved in generating a phylogenetic tree using a cladistic approach?
 - a. In cladistic analyses, shared, derived characteristics are more informative than shared, ancestral characteristics. Why?
 - b. Give an example from the text or video of homoplasy. What are two different explanations for the existence of homoplastic characteristics, and what does each say about the relatedness of the groups being compared?

Major steps in generating a tree include the assumption that evolution is a branching process, establish relationships based on taxa, taxa organized into clades based on derived character states, and so on. Cladistic analyses favor shared, derived characteristics over ancestral because primitive characters do not reveal information about which groups share more recent common ancestors (primitive character states only add noise to the system). An example of homoplasy could be wings on both birds and bats (see the online text chapter). Two different explanations of homoplasy are convergence and reversal. Convergence means different lineages do not share a common recent ancestor but evolve the same character. Reversal means mutation or selection causes the derived character state to revert back to ancestral character.

3. How do scientists use the genetic data to determine a cladistic tree?
Scientists use genetic data to back-up the similarities observed in morphological characters. (It is assumed that there is no difference between the analysis of morphological and molecular characters.)
4. Groups of animals (nematodes, for example) are nearly identical morphologically, but have considerable genetic variation. What does this imply? Why might this occur? How might a scientist deal with this problem in making classifications?

It implies that they have adapted to environmental factors in similar ways, yet reached the same goal in different ways. In other words, nematodes that evolved in different regions have had the same adaptive obstacles to overcome. So, although they are similar morphologically, genetically they did not breed to pass on traits, and relied on mutations within their species for evolutionary change. Another possibility here is that most of the genetic differences that we observe are not functionally too important, whereas similarities (strict homology) at the molecular level are still responsible for the similarities at the morphological level. To deal with problem, scientists must take DNA and phenotype information into account. not just one or the other.

Activity 3: HIV and the Dentist

Based on video and online text content

25 minutes

Setup

One of the uses of comparative evolution is in epidemiology, tracing the source of an infectious agent. In the early 1990s, a young woman in Florida died of AIDS, even though she had no known risk factors for HIV infection. Comparative genetic analysis determined that she had been infected by her dentist, who may have also infected other patients during invasive dental procedures.

To do the analysis, virus samples were isolated from each of the patients, from the dentist, and—as a control—from HIV-positive individuals from the local community. The gene sequences for the HIV-1 outer-envelope protein was determined for viruses in each sample and the degree of genetic similarity was compared. Some of the data are provided. Working in pairs, examine the data and discuss the accompanying questions.

Materials

- One copy of the Comparative Genomics Data and Questions per person (master copy provided)
- Tips and Suggested Answers

Comparative Genomics Data and Questions

For answers, see Tips and Suggested Answers.

Questions:

1. First, look at the Table of Sequence Variation. Note that *intraperson* variation, which is the genetic variation seen within HIV viruses extracted from one person, can be quite high, as in patient D.
 - a. What do you think causes the *intraperson* variation? What qualities of HIV would cause genetic variants of the virus to appear within a single individual?
 - b. What kind of factors would contribute to high *intraperson* variation? What factors about the infected individual or the environment of the virus would cause the emergence of variant viruses?
2. Now look at the Phylogenetic Tree of the different HIV quasispecies from the dentist and patients. *x* and *y* stand for two different genetic variants of HIV, called subtypes, from the same person. For example, HIV samples from the dentist had several HIV subtypes. The two used in this study are called *x* and *y*.
 - a. All the samples—except those from D and F—clustered into a single clade with the sequences from HIV taken from the dentist. On what basis were D and F excluded from the cluster of infections linked to the dentist?
 - b. From the tree, can you tell who was infected by only one of the dentist's viral subtypes and who was infected by both subtypes *x* and *y*?

The Applications:

1. Do you think it is always important to trace the origin of transmitted diseases?
2. Can the techniques used to determine the origin of HIV infection be applied to other infectious diseases? What characteristics of HIV make it more amenable to this type of analysis than other viruses—like polio or smallpox, for example?
3. What would be the advantages of tracing diseases in terms of vaccine development?

The Ramifications:

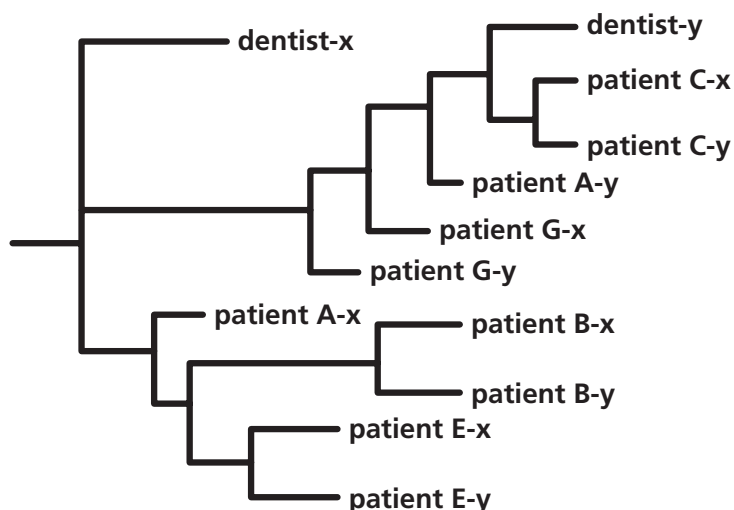
1. The dentist in this case performed invasive dental procedures for three years after he was diagnosed HIV-positive and two years after he was diagnosed with AIDS. What kind of legal ramifications should there be in this situation? What about for transmission of a disease if the person is unaware that they are infected? What if they are aware that they are infected, but believe that they have taken precautions to prevent infecting others?
2. How could information on the evolution of bacterial or viral strains be helpful in developing vaccines or drugs?

The Data:

Table of Sequence Variation. This table shows percent sequence variation for sections of the HIV *env* gene from an HIV-positive dentist, and several patients who were found to be HIV-positive after invasive dental treatments.

Sample	Intraperson Variation (%)	Intraperson Variation (compared to dentist, %)	Intraperson Variation (compared to local HIV+ persons, %)
dentist	3.3	—	11
patient A	2.0	3.4	10.9
patient B	1.9	4.4	11.2
patient C	1.2	3.4	11.1
patient D	7.5	13.6	13.1
patient E	2.1	3.4	10.8
patient F	3.0	10.7	11.9

Phylogenetic Tree. The Dentist and Patients are the same as in the Table of Sequence Variation.



Sources: Ou et al. 1992. Molecular epidemiology of HIV transmission in a dental practice. *Science* 256(5060):1165–71.

HIV and the Dentist, Suggested Answers

Questions:

- 1a. What do you think causes the *intraperson* variation? What qualities of HIV would cause genetic variants of the virus to appear within a single individual?
- 1b. What kind of factors would contribute to high *intraperson* variation? What factors about the infected individual or the environment of the virus would cause the emergence of variant viruses?
HIV has a high mutation rate. Factors that affect the number of variant (quasi-species) viruses a person has include duration of infection, selective pressures caused by the host immune system, disease stage, and therapy (for example, selection by drugs).
- 2a. All the samples—except those from D and F—clustered into a single clade with the sequences from HIV taken from the dentist. On what basis were D and F excluded from the cluster of infections linked to the dentist?
D and F were excluded because the viruses in their samples were as distantly related to the dentist's viruses as they were to viruses from the control group, which contained infections that were epidemiologically unlinked to the dental clade. D and F also had other risk factors that could explain how they were infected.
- 2b. From the tree, can you tell who was infected by only one of the dentist's viral subtypes and who was infected by both subtypes x and y?
Patient A was probably infected with both of the dentist's viral subtypes. In patient A, the subtype y shares a common ancestor with dentist y; this suggests infection with subtype y, with subsequent divergence of the virus in both patient A and the dentist. A's subtype x has a common ancestor with the dentist's other viral subtype.
B and E were infected with a single viral subtype that diverged into their different subtypes. C and G were infected with the other subtype, which diverged into their own subtypes.

The Applications:

What characteristics are required for this type of analysis?

This technique requires a virus like HIV with a high mutation rate and high genetic variability.

Notes

Activity 1: What's the Relationship?

Based on video and online text content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

The field of human evolution touches on all other fields of biology: genetics, physiology, phylogeny, cell biology, and more. The list of terms below demonstrates the breadth of the study of human evolution.

In pairs, discuss the possible meaning of the terms in this list, their relationship to each other, and their significance in studying human evolution. After the video, take a few minutes to see if these terms are used the way you thought, or if their meanings in the field of human evolution are different from what you expected. For terms whose meaning or significance were not fully explained in the video, have someone check the Human Evolution online text chapter and give a short explanation to the rest of the group.

Materials

- One copy of the List of Terms per person (master copy provided)
- One copy of the Human Evolution online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

List of Terms

1. What might these terms mean? What is their relationship to each other?
 - a. Mitochondrial Eve and Y chromosome Adam
 - b. multi-regional hypothesis and replacement (out-of-Africa) hypothesis
 - c. SNPs (single nucleotide polymorphisms) and genetic variation
 - d. molecular clock and DNA sequence divergence
 - e. gene expression and the regulatory hypothesis (about differences between humans and chimps)
2. What is the relationship between these groups?
 - f. humans, chimpanzees, and gorillas
 - g. Homo neanderthalis and Homo sapiens

Activity 2: Molecular Clock

Based on video and online text content

60 minutes

Setup

When evolutionists work with a fossil, they determine its age using radiometric dating, which tests the amount of radioactive decay that has occurred since the fossilized organism was living. When evolutionists work with molecular data, they need a different method to measure time. A molecular clock uses changes in the DNA sequences of a common gene to measure the time since related organisms diverged.

In this exercise, you and a partner will follow step-by-step instructions that will show you how to calibrate a molecular clock and use it.

Materials

- One piece of graph paper per person
- One copy of the Worksheet of Instructions and Sequences per person (master copy provided)
- Transparency of Molecular Clock (master copy provided)
- Tips and Suggested Answers
- One copy of the Human Evolution online text chapter (available online at <http://www.learner.org/channel/courses/biology>)

Worksheet of Instructions and Sequences

Part 1. Calculating Sequence Divergence

To set, or calibrate, a molecular clock, we need to calculate how much DNA sequences for common genomic regions have diverged over a known period of time for the organisms we are studying. To see how this works, try this exercise.

Below are sequences of an imaginary gene from a single human, chimpanzee, and baboon. Most genes consist of several thousand nucleotides, but, for demonstration purposes, the imaginary sequences here are much shorter. Calculate the percentage sequence divergence between (a) the human and chimp sequence, (b) the human and baboon sequence, and (c) the chimp and baboon sequence.

	1	21
human	ACTAGCAACGGATACCATAG	GTATATCTAGGCTACATTGT
chimp	ACTAGCAACGGTTACCACAG	GTATATCAAGGCAACATTGT
baboon	ACTGGCTGCGGATGCCTCAG	ATTTATCAAGGCTACAATGT
	41	61
human	TAGCTTACCGATAGTACTGG	TGACTCTAGAATGCCTAGTC
chimp	TACCTTACCGCTAGTACTGG	TGACTCTCGAATGCCTTGTC
baboon	TACCATAGCGATACTTGTGG	TGACTGTGCGATTGCCTAGTG

Steps to follow:

1. Examine the sequences in question and count the differences between them.
2. Divide the number of differences between the two sequences by the total length of the sequence to get the proportion sequence divergence. To get the percent sequence divergence, multiply the proportion by 100.

Example: If the sequences are each 80 nucleotides long and there are 6 differences between them, then the sequences have diverged by $6/80$ or 0.075 or 7.5%.

Part 2. Using the Molecular Clock To Infer Divergence Times

The major assumption of the molecular clock is that sequence divergence increases more or less linearly as species diverge. Species that diverged 20 million years ago should have twice as much sequence divergence as ones that have diverged only ten million years ago. If one knows when some pairs of species diverged, one can use that information to calibrate a molecular clock. That clock can then be used to determine when other species diverged, provided that we know how much sequence divergence there is between those species.

Exercise A. Using graph paper and the data below, plot the sequence divergence on the x-axis and the known age on the y-axis for each of the three species pairs. Draw the slope line.

1. Calculate the ratio of the sequence divergence to the known age. What answer do you get? That is the calibration of that molecular clock.

Species Pair	Sequence Divergence	Known Age
Human-Chimp	9.8%	5.5 million
Human-Gorilla	12.0%	7.0 million
Chimp-Gorilla	12.8%	7.0 million

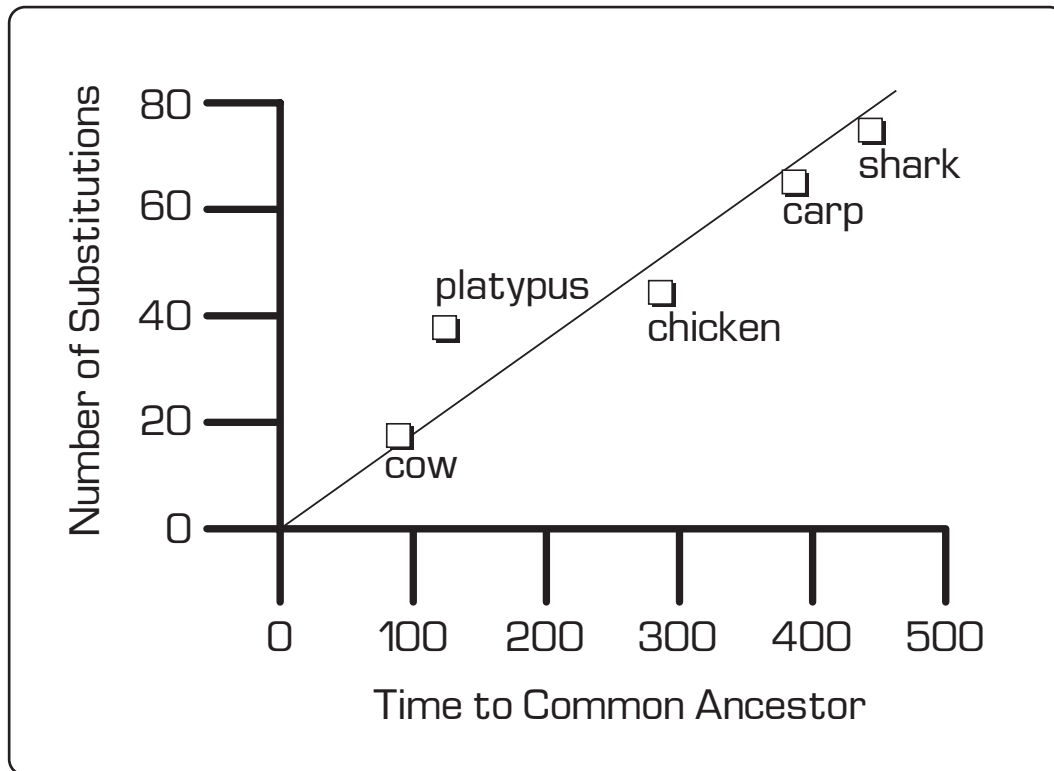
2. Note that the figure just obtained represents the rate of sequence change in both lineages. We are assuming that the rates of evolution along the two lineages are equal. To get the rate of change that occurs in one lineage, divide that figure by two.
3. Compare your result with a more detailed molecular clock seen on the overhead. What sequence was used? Why do you think this sequence was chosen? What similarities and differences do you see between your clock and this example? What accounts for the differences?

Exercise B. The sequence divergence between chimpanzees and bonobos at this gene is 4.2%. Using the calibrated molecular clock, calculate the estimated age of the divergence between chimps and bonobos.

Exercise C. The sequence divergence between humans and Neanderthals (based on DNA obtained from a fossil) is 1.2%. Using the calibration of the molecular clock, what would you estimate the age of the split between humans and Neanderthals?

Suppose that other data showed that the Neanderthal fossil was only 0.35 to 0.40 million years old. What are possible reasons for the discrepancy between this age and the age obtained by the molecular clock method?

Molecular Clock



Source: http://departments.oxy.edu/biology/Franck/Bio326_2000F/lectures/Image12.jpg

Part 2 Answers

Exercise A.

1. Calculate the ratio of the sequence divergence to the known age. What answer do you get?

That is the calibration of that molecular clock.

The answer would be about 1.8% sequence divergence for each million years. There's some slop in this clock—anywhere from 1.7% to 1.9% per million years would be acceptable.

2. Note that the figure just obtained represents the rate of sequence change in both lineages. We are assuming that the rates of evolution along the two lineages are equal. To get the rate of change that occurs in one lineage, divide that figure by two.

About 0.9% change per million years per lineage.

Exercise B. The sequence divergence between chimpanzees and bonobos at this gene is 4.2%. Using the calibrated molecular clock, calculate the estimated age of the divergence between chimps and bonobos.

The answer would be 2.4 million years. One can obtain that either by dividing 4.2% by the calibration (1.8% per million years) or by interpolating the graph.

Exercise C. The sequence divergence between humans and Neanderthals (based on DNA obtained from a fossil) is 1.2%. Using the calibration of the molecular clock, what would you estimate the age of the split between humans and Neanderthals?

The answer would be about 0.67 million years.

Suppose that other data showed that the Neanderthal fossil was only 0.35 to 0.40 million years old. What are possible reasons for the discrepancy between this age and the age obtained by the molecular clock method?

One answer: Genetic divergence data tells how much divergence there has been since the last common ancestor of the two species. This figure may be much greater than the age of the fossil. Moreover, fossils don't continue to evolve.

Another possible answer: Saturation—that clock appears to be ticking slower for the more diverged pairs of species because it doesn't take into account multiple changes in the sequence. If a sequence changed from A to G and then to T in one lineage, it would be scored as only one change. If both lineages changed from A to T, then it would be scored as no change, even though there were two changes. There are various algorithms that evolutionary geneticists use to take saturation into consideration.

Another possible answer: Selective forces are altering the rate of evolution, or for some other reason; the clock is ticking erratically.

Activity 3: Genealogies

Based on video and online text content

15 minutes

Setup

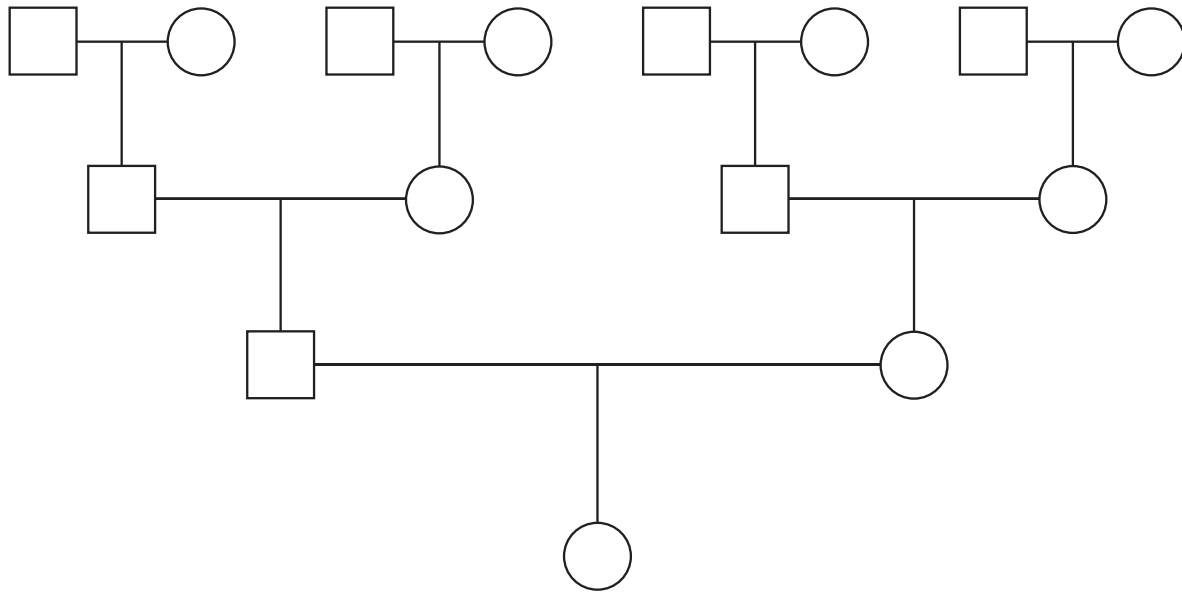
As we saw in the video, Iceland has genealogical information going back to the time when it was first settled, 1,100 years ago. One objective of this exercise is to give us some perspective on how many generations that can mean. The other is to answer a common question about how there could have been only one mitochondrial Eve, by showing how mitochondrial DNA and Y chromosomes are lost and retained in a lineage.

In this exercise, each person will trace his or her genealogy back three generations (to great-grandparents). First look at the simple example on the overhead. Then make a real, extended genealogy of your own family or use the example to answer the questions.

Materials

- Transparency of the Example Genealogy and Questions (master copy provided)
- Tips and Suggested Answers

Example Genealogy and Questions



Examining mitochondrial and Y chromosome transmission in human genealogies.

1. Of the four great-grandmothers, how many have transmitted their mitochondrial type to any of their great-grandchildren?
2. Of your four great-grandfathers, how many have transmitted their Y chromosome type to any of their great-grandchildren?

Note: See the Tips and Suggested Answers.

Answers

Even though all of these great-grandparents had at least one great grandchild, it is likely that their mitochondrial or Y chromosome type would be lost.

Activity 4: Icelandic Perspectives

Based on video content

25 minutes

Setup

In the video we saw that the company Decode is using the genealogies of Icelandic citizens to identify genes involved in complex diseases. This exercise will explore the issues that this practice raises about using the genetic information of individuals to learn about the population.

Divide into teams of four and then into pairs. One pair will represent the perspective of a company like Decode and one pair will represent the perspective of citizens of the local community. Each pair will spend a few minutes coming up with answers to the provided discussion questions that reflect the perspective of the group they represent, either the company or the citizens. After a few minutes working in pairs, share your answers with the others in your team of four.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

Remember to answer these questions *from the perspective of the company or of the citizens*.

1. What advantages and disadvantages are provided to your group by this type of research?
2. Should we allow this information to be used as a basis for patents or other profit-making ventures? State your reasons.
3. What potential problems do you foresee regarding privacy issues?
4. Can national legislation effectively handle privacy concerns?
5. Should a company be able to acquire the rights to the DNA sequence of an individual citizen if it can benefit the entire population? Why or why not?
6. In the course of the company's research, sensitive information about individuals or families might be uncovered. For example, DNA testing might uncover discrepancies in a pedigree that suggest undisclosed adoption or infidelity, or it might discover information about a genetic disease that is currently untreatable. How should this information be handled?

Activity 5: Roots

Based on video and online text content

15 minutes

Setup

According to a *Sacramento Bee* article from 2003, genealogy is America's second largest hobby (gardening is first). The traditional sources of genealogical information have been civil records, family bibles, letters, and oral history. In recent years companies have been helping people find their roots using DNA testing. An individual's DNA fingerprint pattern for mitochondrial or Y chromosome markers is matched against databases that collectively contain information for over 100,000 people. A match indicates a shared common maternal ancestor (for mitochondrial DNA) or paternal ancestor (for Y chromosome). Discuss the implications of this work as a group.

Materials

- One copy of the Discussion Questions per person (master copy provided)
- Transparency of More Quotes and Perspectives (master copy provided)

Discussion Questions

After discussing the questions, see More Quotes and Perspectives.

1. Here is a quote from the article in the *Sacramento Bee* about DNA testing that tries to determine a person's ancestral background: "...in effect this science is starting to debunk the idea of pure anything," said Bennett Greenspan, who in 2000 started FamilyTree DNA, the largest commercial roots-testing firm in the country."

Source: Stephen Magagnini, "DNA Helps Unscramble the Puzzles of Ancestry,"
Sacramento Bee, August 3, 2003.

- a. What have we seen in this unit about human origins that would debunk the idea of pure races?
 - b. What have we seen that would support the idea of separate, pure races?
2. The non-profit African American Roots project offers free testing for African Americans and Caribbean blacks who want to know where in Africa their family lineage began.
 - a. What are some personal reasons for knowing this information?
 - b. Since most African Americans came to America as slaves, what are potential problems with tracing lineage with DNA testing?
 - c. What societal benefit might come from African Americans knowing the geographical origin of their ancestors?
3. Do you think this kind of testing and matching to race is scientifically valid? Would you be interested in testing your own genealogy this way?

More Quotes and Perspectives

1. Arguing against the notion of separate, pure races is the theory that all modern humans descended from, for example, a mitochondrial Eve. Another argument is the amount of genetic mixing that occurs between populations. Jason Eshleman of another DNA testing firm called Trace Genetics, said:

There's a mother and a father of us all—everyone on Earth can trace their maternal or paternal lineage to them, and their offspring gradually populated the world. We're so closely related that there's no good reason to split up the human race into all these categories.

Another quote from the article also argues against separate, pure races:

"Most of us are generally the result of some sort of mixing of peoples, so in effect this science is starting to debunk the idea of pure anything," said Bennett Greenspan, who in 2000 started FamilyTree DNA, the largest commercial roots-testing firm in the country.

2. On the other hand, the spread of humans out of Africa and into many different areas suggests the idea of race could be valid.
 - a. Here is what Bruce Jackson, who is in charge of the African American Roots project, says about wanting to know where his own ancestors came from. "My history goes back thousands of years in Africa, but most of it has been blotted out. What holidays did my ancestors celebrate? What were their marriage customs? Their politics? Their names? What were the great things they did, and the things that aren't so great?"

Donald Black of Santa Clara said "When I opened the envelope and started to read, I almost cried because it gave me a sense of wholeness. It tells me we didn't start here, my folks weren't always slaves, they didn't always have to step off the sidewalk and say, 'Yes sir, no sir.' In Mali, Greeks and Romans sent their sons to study at the feet of black scholars...folks like me.
 - b. Apparently, nearly a third of African Americans have a European male ancestor. Based on family history and the results of his mitochondrial DNA test, Bruce Jackson believes one of his ancestors was a servant from Ireland who married a freed slave.
 - c. Here is what he said about the greater impact of his project: "If African Americans can link ourselves to our nations of origin, we will be more invested in the fate of Africa and could have a tremendous impact on its future."

Notes

Activity 1: Connections

Based on video content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

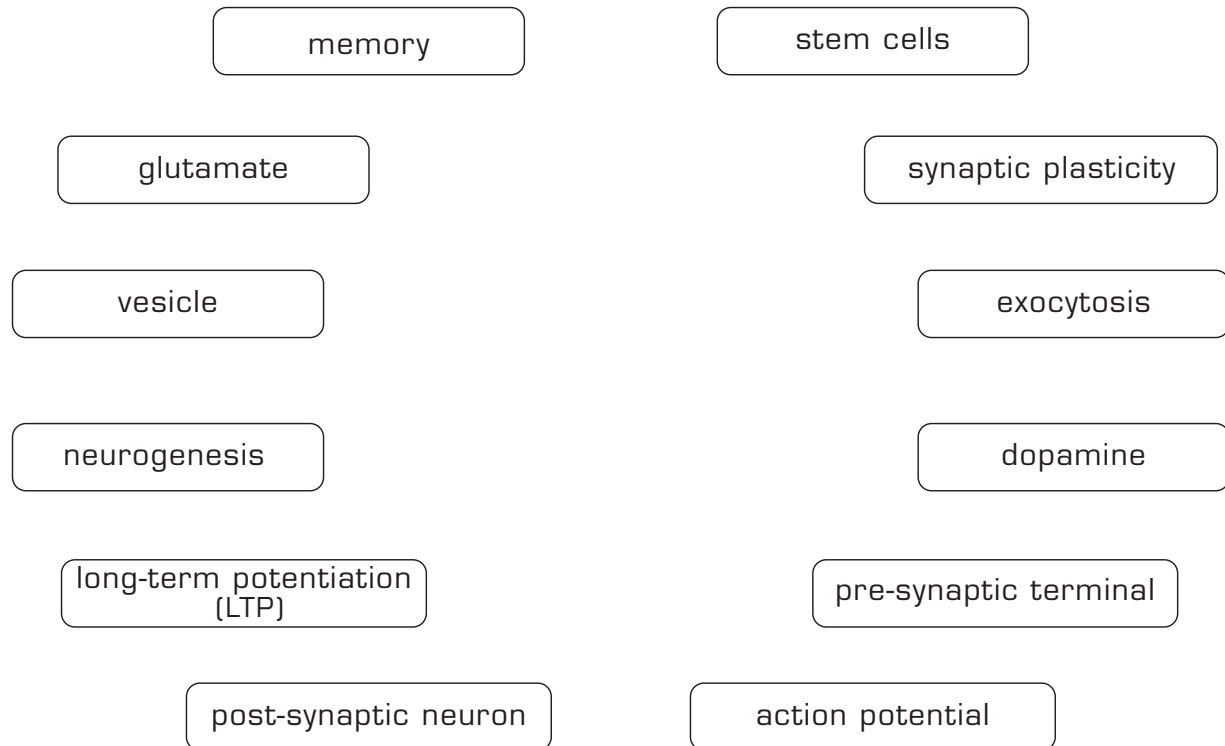
Neurobiology is all about connections. So, to begin this unit, try making connections between some of the topics that will be covered in the video. Work in pairs on the diagram with terms for this unit. Draw a line between terms that have a connection. As you draw the line, explain to your partner what the connection is between the terms. If you don't know the meaning of a term, either leave it out of the network until after the video or see if you can deduce how it is connected.

After viewing the video, look at your diagram and see if there are any new connections you would make between the terms.

Materials

- One copy of the Diagram of Terms per person (master copy provided)

Diagram of Terms



Activity 2: Penny for Your Thoughts

Based on video content

10 minutes, during a break in watching the video

Setup

We handle coins nearly every day, but the features of a common coin (like a penny) can be difficult to recall if those particular details have not been placed in long-term memory. In the video, we'll see a little about neural connections and the molecular basis of memory. But just for fun, before watching the video try this exercise: Working in pairs, take one set of the parts of a penny and, from them, put together a replica of the front and back of a penny from memory. The sets contain extra parts, so you will not use them all.

As you make your replicas, think about why it is easy or difficult for you to recall details about an object you've seen many times. Are the details that you remember connected with the memory of a specific event? Is it hard to remember details because you've never really paid attention to the features of a penny? Are facts like the details of a coin important to remember? When you and your partner are finished, check your replicas against a real penny.

Materials

- One set of the Parts of a Penny per two people (master copy provided; to make a set, cut on the dotted lines after copying)
- One penny per two people (as the answer key)

Parts of a Penny

UNITED STATES OF AMERICA

GOD BLESS AMERICA

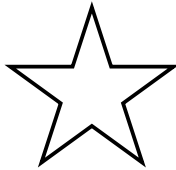
IN GOD WE TRUST

ONE CENT

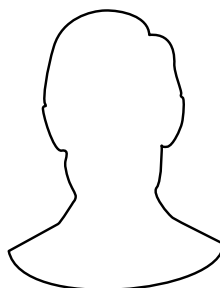
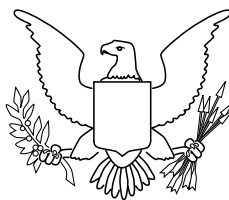
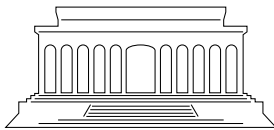
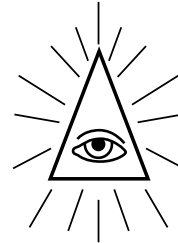
FREEDOM

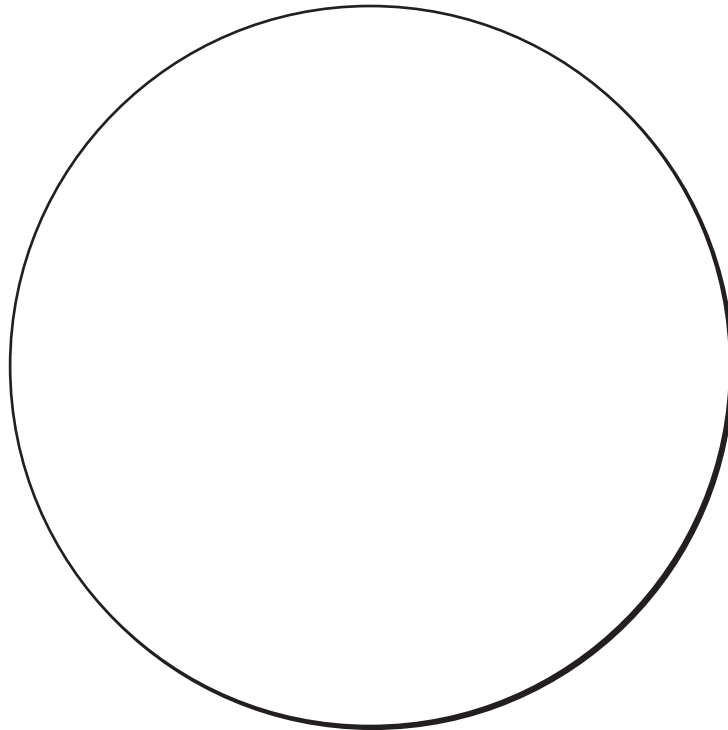
LIBERTY

**E PLURIBUS
UNUM**

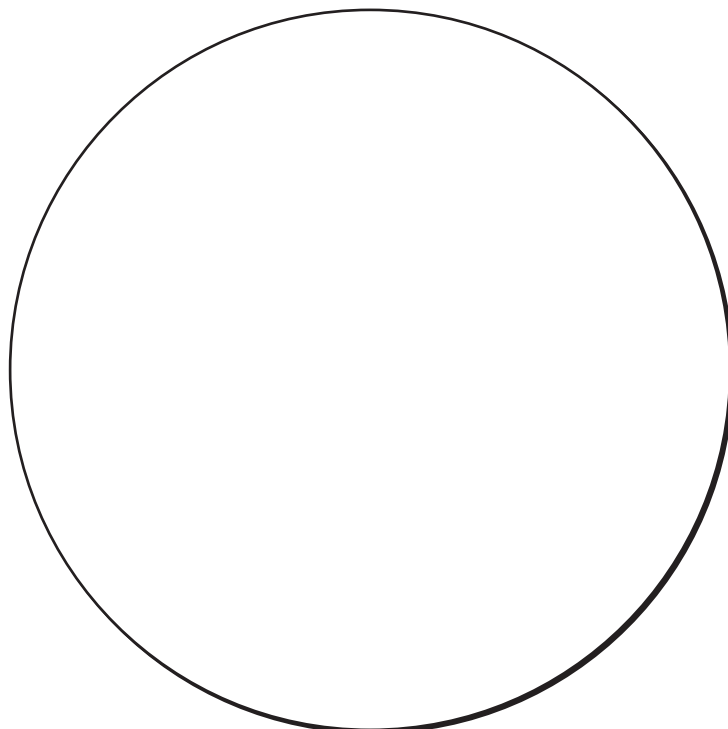


2003





Front



Back

Activity 3: Action Potentials

Based on video and online text content

60 minutes

Setup

Action potentials are the key to neural activity. Within a few milliseconds of receiving a chemical or physical stimulus, a neuron responds by changing the balance of ions across its cell membrane. This change in membrane potential is propagated along the neuron and ultimately leads to a response that might be the stimulation of another neuron, a gland, or a muscle.

In this activity, you will simulate an action potential using a box to represent the neuron, and small colored objects to represent the ions that generate the resting membrane potential and move across the membrane during an action potential. The purpose is not to build a realistic model of a neuron, but rather to illustrate key principles about action potentials. This activity is quite involved, so please read over all of the instructions carefully before starting. This activity will be done in teams of four: one person acts as a scorekeeper, one as gatekeeper, and two as ball handlers.

Materials

- One copy of the Instructions per four people (master copy provided)
- One copy of the Neurobiology online text chapter per four people (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the Discussion Questions per person (master copy provided)
- One set of 100 small colored objects per four people. (The objects can be balls, chocolate-covered candies, jellybeans, coins, or gumdrops. Each set should have 30 blue, 49 red, and 21 green objects.)
- One box for every four people. The boxes should be open at the top to allow visualization and manipulation of the colored objects. Make two panels in the sides of each box that can be opened and closed, and are sufficiently large that they permit the addition and removal of the objects. For Exercise 2, have at least three boxes.
- One piece of graph paper per person
- Tips and Suggested Answers

Instructions

Exercise 1: Simulating a Neuron and an Action Potential

1. Reviewing action potentials

Briefly review the membrane potential of a resting neuron and the changes that occur during an action potential by reading the appropriate section in the chapter text.

2. Setting up the neuron

In this exercise, colored balls are used to represent ions of potassium (blue), sodium (green), and chloride (red). If colored balls are not available, use colored candies, coins, or other small objects. The boxes represent neurons. Each ball will contribute enough of the ion to change the voltage in a neuron by 5 mV. Potassium and sodium ions are positively charged and will increase the voltage by 5 mV each, while chloride is negatively charged and will decrease the voltage by 5 mV.

The neuron itself will be represented by the open box. The box should be constructed so that the 100 balls representing the ions can be visualized and manipulated. The box has two panels that open and close to permit addition and removal of the balls. One represents the sodium channels and the other represents the potassium channels.

The “resting potential” of the neuron is -70 mV. We will represent this in our simulated neuron as 30 potassium ion balls, 5 sodium ion balls, and 49 chloride ion balls. Check that this results in a net voltage of -70 mV.

3. The rules of the simulation

Sodium channels open when the potential of the neuron is above -50 mV; they close when the potential is above +25 mV. They are otherwise closed.

Potassium channels open when the potential reaches +25 mV and they remain open until the concentration is below -75 mV.

The gatekeeper of each group is responsible for determining whether the sodium and potassium channels should be open or closed. The ball handlers are responsible for adding and removing the sodium and potassium ions. The scorekeeper is responsible for determining and recording the potential of the neuron at each time unit.

4. The simulation

- a. At a signal provided by the facilitator, five sodium balls are added to each box to represent an increase in positive charge. (These do not go through the sodium channels, but have entered through another channel, for example a neurotransmitter-gated channel.)

What is the charge now inside the neuron? The scorekeeper should record the charge and mark this as time period one.

- b. Because the charge is now above the threshold for the sodium channels to open, they do so. The opening of the sodium channel permits three sodium balls to enter the neuron for each time period. What is the voltage within the neuron now? Record that value as the potential at time period two.
- c. For each time period, continue adding two sodium balls, unless the potential goes above +25 mV.
- d. When the potential goes above +25 mV, the sodium channels close and no further sodium balls are added to the neuron. Moreover, the potassium channels open when the potential goes above +25 mV. In our simulation of a neuron, the opening of the potassium channels results in the removal of three potassium balls for as long as the potassium channels remain open. The potassium channels will close when the potential goes below -75 mV.

5. The results

Once the data have all been collected, graph the changes in potential as a function of time and discuss the discussion question for this exercise.

Exercise 2: Propagating an Action Potential

1. The background

An action potential must be propagated down the length of the neuron, from its input source at the dendrites, to the cell body, and then down the axon to the synaptic terminals. How is this achieved? This propagation also involves the movement of ions. When the sodium channels in one part of the neuron are opened, sodium ions rush in. Once inside, they cause nearby regions of the neuron to become depolarized by moving laterally through the axon. This in turn causes the opening of more voltage-gated sodium channels in those regions. Thus, the sodium channel activation moves in a wave-like fashion down the neuron.

2. Setting up the neuron

We can simulate the propagation by arranging the boxes in a linear array. Now, each box represents part of the neuron. A box at one end will be designated the dendrite of the neuron.

As before, each box will start with 30 potassium ion balls, 5 sodium ion balls, and 49 chloride ion balls, giving an initial potential of -70 mV.

3. The simulation

The rules of this simulation will be the same as the one above except in every time period that one box has more than 20 sodium ion balls, it will pass one sodium ball to its nearest neighbor away from the dendrite. This is how we will simulate the lateral movement of sodium ions. At some point, these additional sodium ion balls will cause the potential of that region of the neuron to rise above the threshold that will open the sodium channel.

At time period one, the dendrite will receive 5 sodium balls which will cause its potential to rise above the -50 mV threshold and will open the sodium channel.

Discussion Questions

See the Tips and Suggested Answers.

After finishing Exercise 1:

1. Although the membrane potential of the neuron has returned to near the resting potential, there is much more sodium and much less potassium inside the neuron than before the action potential. How does the neuron return to having low sodium and high potassium concentrations?

After finishing Exercise 2:

2. Compare the amount of time it took your team to simulate or propagate an action potential with the time required for a neuron to perform this task. What mechanisms increase the speed at which action potentials are relayed?
3. After finishing the propagation exercise, consider what happens when the action potential reaches the end of the neuron. List the effects on the neuron and on cells with which the neuron may communicate.

Discussion Question Answers

Exercise 1:

1. Although the membrane potential of the neuron has returned to near the resting potential, there is much more sodium and much less potassium inside the neuron than before the action potential. How does the neuron return to having low sodium and high potassium concentrations?

The sodium-potassium pump, which actively transports potassium in and sodium out, restores the ionic balance.

Exercise 2:

2. Compare the amount of time it took your team to simulate or propagate an action potential with the time required for a neuron to perform this task. What mechanisms increase the speed at which action potentials are relayed?

Besides the propagation along the axon demonstrated in this exercise, gaps in the myelin insulation of myelinated neurons allow the action potential to jump from one gap to another, resulting to signal speeds of over 100 meters per second.

3. After finishing the propagation exercise, consider what happens when the action potential reaches the end of the neuron. List the effects on the neuron and on cells with which the neuron may communicate.

From neuron to neuron, the signal may be transferred electrically through a gap junction or chemically by a neurotransmitter. Neurons may also communicate chemically with muscles or other cells.

Activity 4: Sex, Drugs, and Neurobiology

Based on video and online text content

25 minutes

Setup

We have all witnessed or experienced the effects of alcohol, nicotine, and other drugs on the central nervous system. As we learn more about the nervous system, we learn more about the mechanisms and effects of these drugs. However, knowing how the drugs affect neurons and synapses does not answer questions about how the drugs should be used, if they should be used at all, and who should decide these issues. In this discussion, read a few paragraphs from the online text about the action mechanisms of different drugs. Then, in teams of three, discuss some of the physiological and societal aspects of the drugs and their use.

Materials

- One copy of the Neurobiology Online Text Chapter Excerpts per person (master copy provided)
- One copy of the Discussion Questions per person (master copy provided)

Neurobiology Online Text Chapter Excerpts

Section 1: Neurotransmitters, Psychoactive Drugs, and the Reward Pathway

Drugs that have effects on the central nervous system are known as psychoactive drugs. The mode of actions of both therapeutic drugs (e.g., Ritalin, Prozac, and Paxil) and recreational drugs (e.g., alcohol, cannabis, cocaine, and nicotine) affect the firing of certain neurons by changes in various neurotransmitters or receptors. Not all drugs have specific modes of action; alcohol, for example, has many and varied effects. We will focus, however, on a few examples of those drugs that have specific effects.

Humans and many other animals engage in many activities from which they derive pleasure. Researchers working with various animals have shown that there are regions of the brain, such as the ventral tegmental area, that are more active when animals engage in pleasurable acts. When researchers stimulate these areas experimentally, the animals will perform various tasks in order to receive further stimulation. Hence, the neural pathway comprised of those regions has been called the **reward pathway**.

Like many drugs, nicotine from tobacco products acts on the reward pathway. This drug, however, is unusual in that it directly affects the dopamine receptor in the reward pathway's neurons. Unlike the action of most drugs, no intermediary steps are involved: nicotine binds to the receptor and stimulates the postsynaptic neuron. The overstimulation of the postsynaptic cell, however, also has effects at the cellular level. Over time, it leads to a decrease in the number of dopamine receptors being expressed and inserted to the membrane, as well as a change in the shape of the cell. The reduction of receptors is referred to as "desensitization." When the nicotine is removed, because there are fewer receptors on the postsynaptic cell, more dopamine than normal is required for proper stimulation of postsynaptic neuron. Addiction can result because nicotine becomes needed just to maintain the normal stimulation of the postsynaptic cells.

Allelic variation at the dopamine receptor gene appears to affect one's likelihood of becoming addicted to nicotine. Individuals who have the A1 allele have fewer dopamine receptors than those that do not have the allele. These individuals also have more difficulty in quitting smoking and are more likely to exhibit other addictive and compulsive behaviors. The genetic components of many types of addiction are the topic of intensive research—and often heated debate.

Cocaine also works on dopamine and the reward pathway but does so in a different way. Recall that some neurotransmitters are normally taken up by the presynaptic neuron by reuptake receptors, or transporters, in the presynaptic membrane. The molecular structure of cocaine is such that it can block the binding site for dopamine on its reuptake receptor. Because this cell is now impaired in the reuptake of dopamine, an excess of dopamine builds up in the synapse. This excess leads to overstimulation of the postsynaptic neuron. Because the action is occurring in the reward pathway, overstimulation leads to euphoria. The effects of overstimulation of the postsynaptic cell by cocaine are much the same as those of nicotine: the reduction of the number of receptors leads to desensitization and the possibility of addiction.

There have been concerns that Ritalin (methylphenidate), used for treatment of attention deficit and hyperactivity disorder (ADHD), is chemically similar to cocaine. Indeed, Ritalin increases dopamine levels by interfering with reuptake. Moreover, Ritalin and cocaine compete for the same receptor site. One crucial difference between these two drugs is that Ritalin acts much more slowly than cocaine. While cocaine's effects on dopamine levels occur within seconds, the response from Ritalin (when administered in pill form) takes about an hour. Some studies suggest that, far from leading to addiction, Ritalin treatment in childhood may be associated with *decreased* risk of drug and alcohol use later on. Other studies, however, suggest that Ritalin may be a gateway drug: by using it, teens may be more willing to experiment with other drugs. As of 2003 the consequences of Ritalin treatment remain unresolved.

Before reading further, discuss the questions for Section 1.

Section 2: Cannabis, the Cannabinoid Receptors, and Endocannabinoids

The active ingredient of marijuana, from the cannabis plant, is THC (delta-9-tetrahydrocannabinol). This chemical exerts its effects on the brain by binding to receptors called the cannabinoid receptors. Scientists have identified two cannabinoid receptors (CB1 and CB2), and evidence suggests that there may be others. Although CB1 is found in many regions of the brain, CB2 is present only in certain cells of the immune system. Because the receptor is present in several brain regions, THC can have manifold effects. For instance, THC may affect memory formation. CB1 is prevalent in the **hippocampus**, a region of the brain strongly associated with memory. By binding to and activating CB1, THC decreases activity of neurons in the hippocampus and interferes with the proper function of that region, which may translate to an interference with memory formation.

The human body does not produce THC, so why would there be receptors that can bind it? During the 1990s, researchers discovered that the body makes chemicals, such as anandamide, that can bind to the cannabinoid receptors. The function of these chemicals, called endocannabinoids, and their receptors is still unknown. To investigate the role of the CB1 receptor, scientists have studied mutant mice that lack the receptor. Compared with normal mice, these mice have a decreased appetite, are less active, and have a reduced lifespan; however, the mice have an enhanced memory.

The CB receptors have recently been associated with some beneficial actions, such as pain relief and extinguishing some fear behaviors. THC has even been prescribed as medication in some states for pain relief for various diseases, including glaucoma, AIDS, and cancer.

Discuss the questions for Section 2.

Discussion Questions

Questions for Section 1:

1. For what purpose might a plant like tobacco or coca make a chemical compound that acts on the reward pathway of animals?
2. All three of the drugs discussed here—nicotine, cocaine, and Ritalin—operate on the same basic dopamine receptor and reuptake system. Why is nicotine legal, but cocaine is not? Why is Ritalin a prescription drug, while the others are not? What societal and historical factors determine which drugs are used socially, which are used medically, and which are forbidden? What makes one type of drug acceptable for medical use while others are not?
3. Compare the use of the three drugs with two other legal and socially acceptable drugs, alcohol and caffeine. Of the five substances, which do you think has the greatest negative effect on society? What kind of solutions can you think of to reduce the negative effects of these drugs on individuals and on society?
4. Who should decide which drugs may be legally used and which should be illegal?
5. In your opinion, how much of addictive behavior is genetically determined, and how much is determined by environmental or other factors? If a large portion is genetically determined, how should we perceive and treat people with addiction problems?

Questions for Section 2:

1. For what purpose would the cannabis plant make a chemical compound that interferes with the memory function of animals?
2. Why do we have the system of endocannabinoids and receptors that interferes with memory formation? What might it be an advantage to forget? Think of the Penny for your Thoughts activity, for example. Do you need to remember all the details of the structure of a penny?
3. As you did with the dopamine system drugs, compare cannabinoids with alcohol and caffeine. Which do you think is the most damaging or dangerous to individuals? Which has the greatest negative effect on society? What factors have made alcohol and caffeine legal but marijuana illegal?
4. What arguments can you think of in favor of legalizing marijuana? What arguments can you think of against it?
5. Are there additional arguments for or against legalizing marijuana only for medical purposes? If marijuana were legalized for medical use, would it be possible to regulate its use effectively? Would it be acceptable to use synthetic forms of marijuana for medical treatment?

Activity 5: Fountain of Youth

Based on video and online text content

10 minutes

Setup

Our image of the brain as fully developed and unchanging by the end of childhood is being replaced by a new model. Recent research has discovered neural stem cells that can differentiate into neurons and make new connections, even in adulthood. The research by Dr. Fred Gage (described in the video) and Dr. Elizabeth Gould (described in the online text) suggests that environmental factors are critical for neurogenesis.

Materials

- One copy of the Discussion Questions per person

Discussion Questions

1. In mice and in monkeys, the animals studied by the researchers in this unit, areas with toys provided the stimulating environment that led to increased neurogenesis. What do you think would provide this environment for humans?
2. Do you know of any anecdotal evidence that supports the correlation between stimulating environments and adult neurogenesis? Have you seen evidence for it in your classroom or your personal experiences?
3. How might you use this information to make your efforts in education more effective?

Notes

Activity 1: 1 in 4000

Based on video content

15 minutes

Setup

One in approximately 4000 babies is born with intersexuality. In this condition, gender cannot be determined by a visual examination of the genitals. The video for this unit explores the biology of gender and some of the variations that can occur in gender development. Before watching the video, spend a few minutes in pairs, thinking of all the details you can recall about how gender is determined in humans. Then brainstorm as many different causes as you can think of for abnormalities in gender development, either in humans or in other animals. Finally, as a group, collect and categorize the causes you came up with and the effects they would have. Tips and Suggested Answers lists some ideas that your group may have thought of.

Materials

- Tips and Suggested Answers

Potential Answers

A few causes your group may have thought of are:

1. abnormalities of entire chromosomes
 - a. missing chromosomes
XO (Turner's syndrome, result is female development with some mental and physical differences)
 - b. extra sex chromosomes
 - i. more than two X (XXY, XXX, etc): viable, although more than two X chromosomes causes some physical differences and any Y causes mostly male development
 - ii. more than one Y (XYY etc): viable as long as there is an X; results in male development
2. abnormalities of parts of chromosomes or defects in single genes on sex chromosomes
 - a. translocation of parts of Y to X (or another chromosome)
inheritance of the male-determining gene SRY causes mainly male development, although some abnormalities can result if there is only the SRY gene without the rest of Y
 - b. mutations in single genes on Y
depending on the gene, can cause male development with sterility or other effects; or if SRY is affected, can cause female development, even though a Y chromosome is present
3. single gene mutations on non-sex chromosomes
 - a. defects in hormones, receptors, or enzymes can cause syndromes like androgen insensitivity, in which an XY develops as a female
4. other
 - a. some people may know about "freemartins" in cows: if a cow has twins, one male and one female, the female will be sterile because of the hormones produced by the male during their in utero development

Activity 2: Birds Do It, Bees Do It

Based on video content

15 minutes

Setup

Biology has a variety of ways to create different genders. In pairs, take a few minutes to brainstorm as many different sex-determination mechanisms as you can think of. Use specific examples if you can think of any. Then, as a group, list all the different mechanisms the pairs thought of and categorize them. Tips and Suggested Answers lists some ideas that your group may have thought of.

Materials

- Tips and Suggested Answers

Potential Answers

A few different mechanisms that your group may have thought of are:

1. chromosomal determination
 - a. Humans and other mammals have XX females and XY males. In humans, the gene *SRY* on the Y is primarily responsible for male development.
 - b. *Drosophila* fruit flies also have XX females and XY males, but sex is not determined by a specific gene on one of the sex chromosomes; it is determined by the ratio of X chromosomes to autosomes (non-sex chromosomes). In *Drosophila*, an X:autosome ratio of 1:0 is a female, so a diploid set of autosomes and XX is female. An X:autosome ratio of 1:2 is male, so a diploid set of autosomes and XY is male. Therefore, in humans, XO is a female; in *Drosophila*, XO is a male. In humans, XXY is male; in *Drosophila*, XXY is female.
 - c. In birds, males are the homogametic sex, meaning one type of sex chromosome, with the ZZ combination; females are heterogametic (or heteromorphic) with ZW.
 - d. Some, but not all, plants that have separate male and female plants (dioecious) have an XY sex chromosome system.
 - e. In *Caenorhabditis elegans*, a soil worm used in genetic and developmental research, most individuals are hermaphrodites with two X chromosomes. Rare males are XO.
 - f. In some insects, like grasshoppers, females are XX and males are XO.
 - g. In bees and some other insects, males come from unfertilized eggs so they are haploid (one set of chromosomes). Females come from fertilized eggs and are diploid (two sets of chromosomes).
2. environmental cues
 - a. In turtles and some other reptiles, temperature of the egg during development determines male or female development.
 - b. In some fish, the presence of other males and females determines sex. If the group loses a male, a female will change gender and become a male.

Activity 3: What About Meiosis?

Based on video and online text content

15–30 minutes, depending on the experience of the participants

Setup

The sex chromosomes represent a special situation in meiosis. In meiosis I, autosomes pair with their homologs, cross-over, and segregate. The pairing and crossing-over is an essential step; meiosis cannot proceed without it. However, in an XY male, the X and the Y do not have a homologous chromosome with which to pair. So what do they do?

Homologous chromosomes are necessary for repairing damaged chromosomes. Every time an X chromosome finds itself in a female, it has a chance to repair mutations from a homolog; however, the Y chromosome is nearly always by itself because a normal male is XY. So how does the Y undergo recombinational repair?

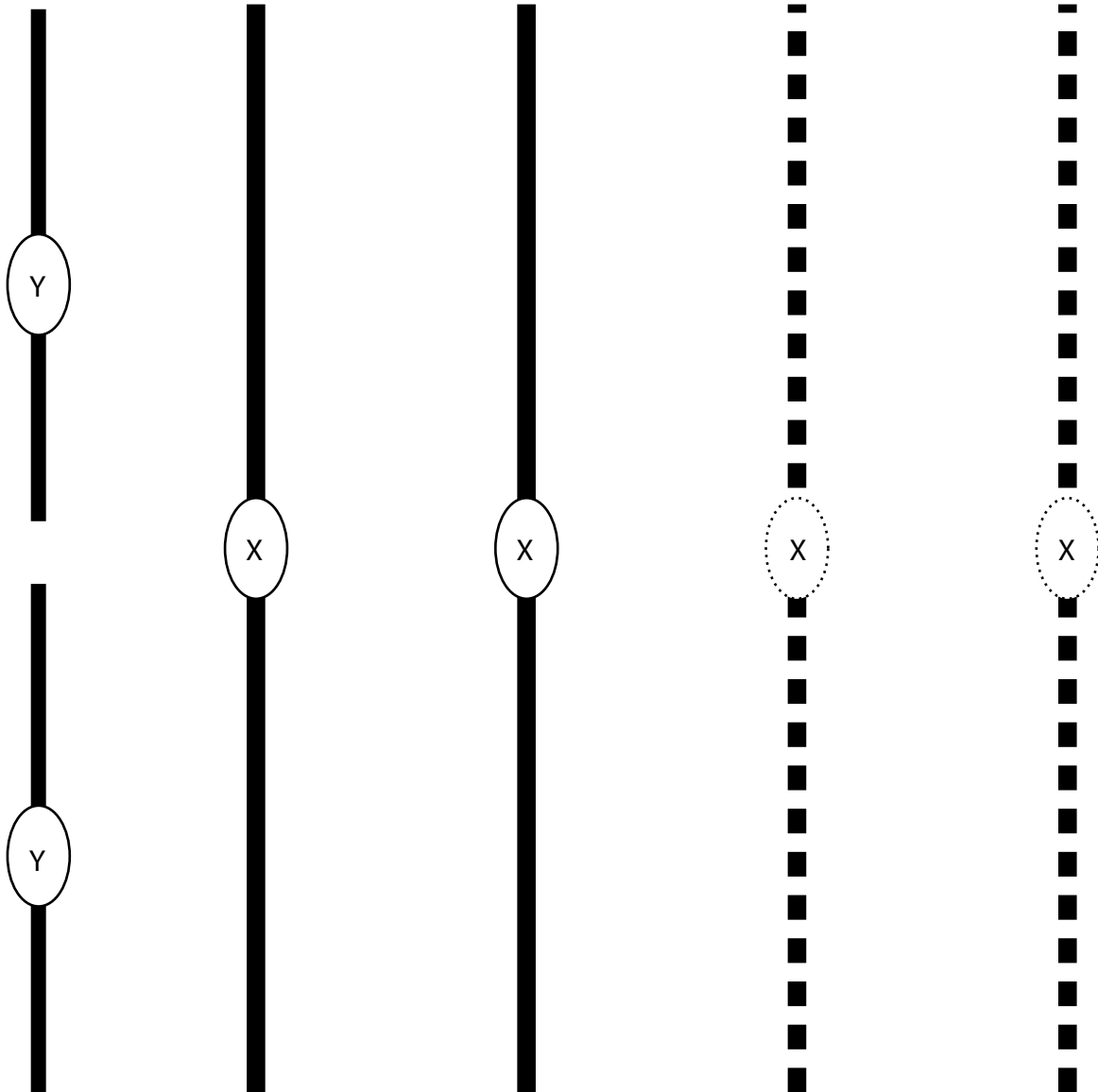
Although someone with a sex-chromosome abnormality may be infertile, there may still be germ line cells that go through meiosis—even if viable eggs and sperm are not produced. What happens if there are extra sex chromosomes or missing sex chromosomes?

We often do labs or demonstrations of meiosis using pipe cleaners or pieces of paper to represent chromosomes; we can use them here to show some of the exceptional meiotic situations of the sex chromosomes. Work through each situation in pairs. If you are used to working with demonstration chromosomes in this way and don't want a warm-up, you can skip the first few exercises.

Materials

- One set of Paper Chromosomes per two people (master copy provided; to make a set, cut after copying, so that each chromosome is separate)
- One copy of the Instructions and Situations per two people (master copy provided)
- A box of small- to medium-sized paper clips
- A roll of tape
- Tips and Suggested Answers

Paper Chromosomes



Instructions and Situations

For answers, see Tips and Suggested Answers.

Instructions:

Make sure your chromosomes have been cut so that each chromosome is separate. To make duplicated sister chromatids, paper-clip them together at the circle, which represents the centromere. There are four copies of autosome 1, so you can make paired, duplicated chromosomes during meiosis I. For meiosis II, remove the paper clips binding the duplicated chromosomes to show how they will separate, and what combinations of chromosomes can end up in the gametes.

There are four copies of X and two of Y, so all the situations can be represented. The arrows on one of the Y chromosomes show palindromic sequences needed for Situation 5.

Situation 1: Normal male

(A warm-up, so skip this one if you are used to showing X and Y segregation using demonstration chromosomes.)

To warm up and get used to using the paper chromosomes, show meiosis I and meiosis II using the sex chromosomes and one autosome from a normal male. For the autosomes, take one solid-line and one dotted-line version of chromosome 1. What is the probability of a gamete with a Y chromosome and a "dotted" chromosome 1?

Situation 2: Androgen Insensitivity Syndrome (AIS)

(A warm-up, so skip this one if you are used to showing X and Y segregation using demonstration chromosomes, and determining probabilities of segregation outcomes.)

A person with this condition is XY. The recessive mutation that causes AIS is on the X chromosome. Show all the relevant chromosomes pairing and then segregating in meiosis I; then show the sister chromatids separating in meiosis II. If a person with AIS was not infertile, what would be the chances that they would pass on a chromosome with the AIS mutation?

Situation 3: 45, XO (Turner syndrome, O stands for no other sex chromosome)

(A warm-up, so skip this one if you are used to showing X and Y segregation using demonstration chromosomes, and determining probabilities of segregation outcomes.)

In a person who is XO, there is only one X chromosome. Show what you think happens in meiosis I and II. What are the chances of a gamete that, if fused with normal sperm from a male, would result in the Turner syndrome genotype? What are the chances of an inviable zygote?

Situation 4: 47, XXY (Klinefelter syndrome)

Show what would happen to the sex chromosomes during meiosis of a cell that was XXY. What are the chances of a gamete that, if fused with normal gametes from a female, would result in the Klinefelter syndrome genotype?

Situation 5: X and Y

When the sequence of the Y chromosome was determined, investigators discovered that it contained several palindromic repeat sequences. These are regions with similar sequence but oriented in the opposite direction. The investigators suggested these regions could be used for recombinational repair by the Y chromosome, essentially allowing the Y chromosome to undergo crossing over with itself.

Start by showing exactly how the X and Y pair during meiosis and where crossing over can occur. What conformation does the X chromosome have to assume?

Next, use the Y chromosome with the white arrows to show how Y can use its palindromic regions to undergo recombinational repair. What conformation does it have to assume?

Situation 6: 46, XX male

Some males have two X chromosomes, but with part of the Y chromosome translocated onto one of the X's. The translocation mutation may occur during meiosis in the father of the 46, XX male. The father would be normal XY, but would produce a sperm that, when combined with an egg, produced the 46, XX male genotype.

Start with the normal XY chromosome combination, as they would be in meiosis I. Show how the translocation would occur by ripping off a little bit of one Y chromatid, transferring it to one X chromatid, and then securing it with tape. Then show how the rest of meiosis would proceed. What gametes are produced in the end? What part of Y has to be translocated in order to direct male development? Because translocations often occur through errors in crossing-over—and you have shown in Situation 5 how X and Y cross-over in meiosis—where do you think the critical part of Y is located?

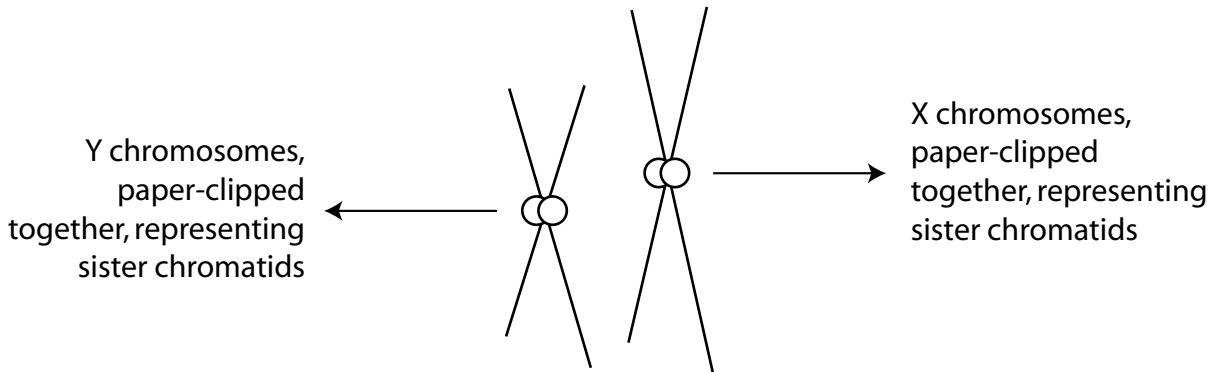
Answers

Situation 1:

The chance of gamete with Y is $1/2$. The chance of a dotted chromosome is $1/2$. $1/2 \times 1/2 = 1/4$.

meiosis I

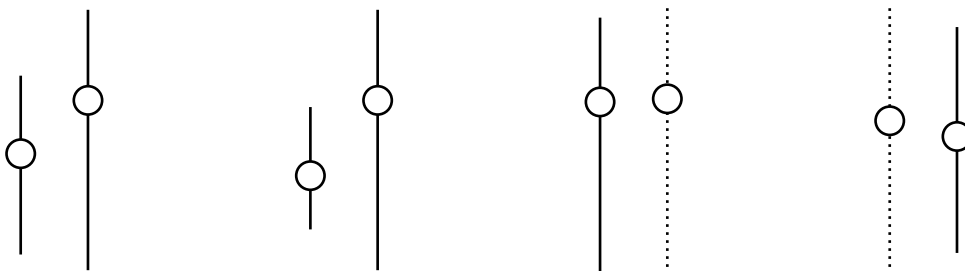
uplicated X and duplicated Y pair and then segregate, just like the homologous autosomes, even though they are not true homologs



meiosis II

sister chromatids separate

(Of course, independent assortment says these are not the only combinations of chromosomes that are possible!)



Situation 2:

Diagrams look like the sex chromosomes in Situation 1, but both copies of the X carry a recessive AIS allele, so the chance of a gamete with this allele is $1/2$.

Situation 3:

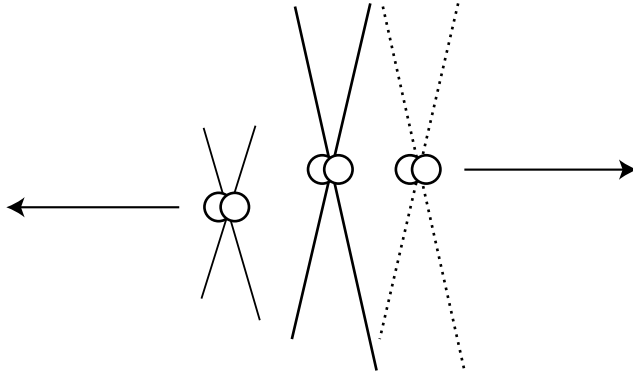
In meiosis I, there is no X or Y for the duplicated X to pair with; it segregates to one cell or the other. So at the start of meiosis II, one cell is normal and the other will give rise to two gametes with no sex chromosomes.

The chance of a gamete with no sex chromosome is $1/2$. Gametes from a male would have either an X or a Y. In order to result in a zygote with the Turner syndrome genotype, a gamete with no sex chromosome would have to fuse with a sperm with an X. The chance of a gamete with no sex chromosome from the XO person is $1/2$. The chance of a sperm with an X is $1/2$. $1/2 \times 1/2 = 1/4$.

At least one X chromosome is essential, so an inviable zygote would result from the fusing of a gamete with no sex chromosome with a sperm with a Y. The chances are $1/2 \times 1/2 = 1/4$.

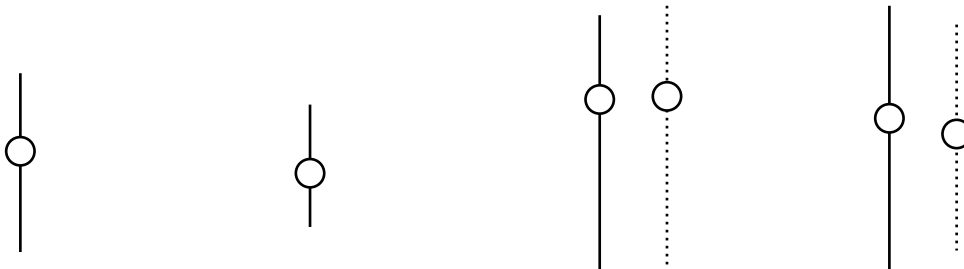
Situation 4:

XX and Y all pair, so meiosis I looks like this:



During segregation, the two duplicated X's could go into one cell, with the duplicated Y segregating into the other. Alternately, an X and a Y could segregate together into one cell, with the other X segregating into the other cell.

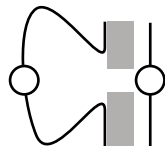
In meiosis II, sister chromatids segregate; a cell that had received two, duplicated sex chromosomes would divide into two cells, each with an extra sex chromosome. Here's one possibility for the end of meiosis II: If the chromosomes shown during meiosis I segregated so that the two duplicated X's segregated away from the Y, the end of meiosis would produce two gametes that each have 1Y, and two gametes that each have 2 X's.



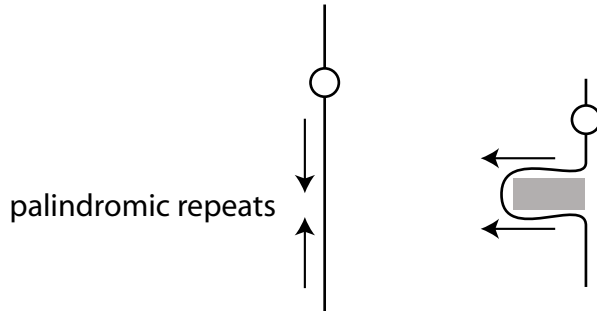
The XXY cell would produce the following gametes (with the X's marked so they can be followed separately): X^1 , Y, X^2 , X^1Y , X^1X^2 , X^2Y . Normal gametes from a female would all contain one X. The probability of a XY gamete from an XXY person is $2/6$. $1 \times 2/6 = 2/6 = 1/3$.

Situation 5:

The Y chromosome is much shorter than X, yet the homologous regions that pair and cross-over are at the ends of the chromosomes. Therefore, the X chromosome has to assume a looped conformation to pair with Y in meiosis. Crossing-over can occur in the shaded area.

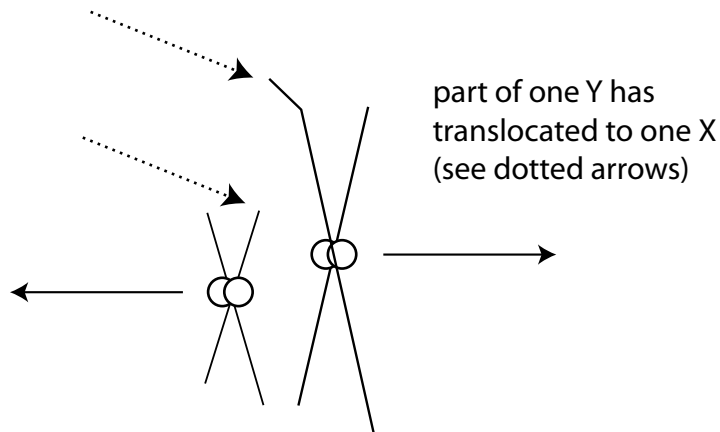


If the Y chromosome uses its palindromic repeat sequences to undergo recombinational repair, the sequences have to align along their homologous (or similar) regions. A conformation like this would have to occur. As above, regions where crossing over can occur are shown in gray.

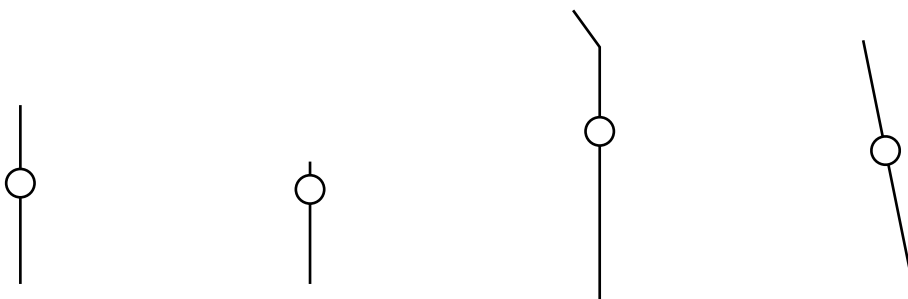


Situation 6:

In meiosis I, after the translocation, the chromosomes would look like this:



After meiosis II, they look like this:



As long as all the autosomes segregated correctly, there are two completely normal gametes: one with a X and one with a Y. There are two abnormal gametes—one with an X that has a portion of Y. If this portion contains the gene SRY, which determines maleness, this chromosome will direct male development. In fact, the SRY gene is located toward the end of the Y chromosome, close to the region where crossing over occurs with X. A slight misalignment of X and Y in meiosis can transfer SRY, and possibly more of Y to the X chromosome. The extent to which the development produces a normal, fertile male depends on how much of Y was translocated. The other gamete has a Y that is missing a segment. The viability and gender of a zygote from this sperm depends on how much of Y is missing.

Activity 4: What Are Our Roles?

Based on video and online text content

30 minutes

Setup

One of the ramifications of gender assignment is how parents, teachers, counselors, and school administrators will treat intersex children. In 2001, the television show *Friends* offended the Intersex Society of North America by treating this situation comically. Guest star Brad Pitt played a former high-school colleague who made up a rumor that Jennifer Aniston character was intersex.

In real life, the issue can create difficult situations, which this exercise will explore. Working in teams of three or four, choose at least three different roles to explore—such as mother, father, doctor, principal, the child’s teacher, the school counselor, or the child. Suggested topics for role-playing or discussion are listed below. Choose either a situation in which parents are first confronted with the birth of an intersex child, or a situation in which the child has grown and is adjusting to life in school. If a school situation is chosen, each group can choose the grade they have the most experience with, or different groups can portray the same situation at different grade levels.

As a group, discuss afterwards how the viewpoints of different parties varied or were the same. Were there some fundamental principles or ground rules that everyone agreed on?

Materials

- One copy of the Situations and Discussion Topics per person (master copy provided)

Situations and Discussion Topics

1. How might a health care professional approach parents who will give birth to, or have given birth to, an intersex baby? How might the parents react, what might their options be, and how would they make their decision?

For this situation, it might help to read the position of the Intersex Society of North America as of 2003 (<http://www.isna.org>):

- Intersexuality is basically a problem of stigma and trauma, not gender.
 - Parents' distress must not be treated by surgery on the child.
 - Professional mental health care is essential.
 - Honest, complete disclosure is good medicine.
 - All children should be assigned as boy or girl, without early surgery.
2. How might a teacher introduce a child of ambiguous gender in a classroom, at several grade levels? What might we tell students who notice that a student is different and treat them differently, or ask about him or her? Should the child have special academic consideration because of his/her personal situation?
 3. How might a teacher or counselor talk to a teenage student who is undergoing personal gender issues—for example, an intersex person whose personal gender assignment does not match the assignment determined by his or her parents? Another example might be a student who is completely male or female physiologically, but feels like a person of the opposite gender and is considering sex change after reaching adulthood. How might a teacher or counselor approach the parents in this situation?
 4. What should teachers and administrators do if an intersex or homosexual student who wants privacy is "outed" by his or her peers? What if a student's peers are uncomfortable with a student who is outspoken about his or her status as an intersexual, a homosexual, or any other sexual or gender status that might make other students or parents uncomfortable?

Activity 5: Let's Call the Whole Thing Off

Based on video and online text content

60 minutes

Setup

Gender development affects the entire person, not just development of external genitals and internal reproductive organs. In this activity, read the article on gender and intellectual ability. Then, in teams of four that are composed of two men and two women, try tests that measure skills at which men and women generally show differences and discuss the questions provided.

Materials

- One copy each of the Article Excerpt, Tests, and Discussion Questions per person (master copy provided)
- A stopwatch, watch with second hand, or timer for each team of four
- A tennis ball or other small ball for each team of four
- A wastebasket or other target for each team of four

Scientific American Article Excerpt

Women weep over *An Affair to Remember*. Men compulsively channel-surf. Women can't drive. Men can't express emotions. Do these stereotypes reflect real biological differences between the sexes?

As we saw from the Biology of Sex and Gender video, the development of sexual organs depends on the presence or absence of a Y chromosome. In the absence of a Y, ovaries develop. If a Y chromosome is present—or more specifically, the SRY gene that normally resides on Y—testes develop. The subsequent production of testosterone leads to male physiology. The assumption has been that sex hormones are also responsible for behavioral differences between males and females, with supporting evidence coming from animal studies. Newborn female guinea pigs that are exposed to testosterone become males in both appearance and behavior. Male rodents that are castrated as newborns will grow up to exhibit reduced male mating behavior and increased female mating behavior.

However, if sex hormones alone are responsible for differences in male and female behavior, then we cannot explain cases like David Reimer. As shown in the video, David's male sex organs were removed soon after birth, after an accident during circumcision. He was raised as a girl and underwent hormone therapy; yet, at 14, he voluntarily changed his gender assignment, stating that he had always felt like a boy. These cases are rare, but anecdotal evidence like this suggests that a model in which gender identity is determined solely by physiology or levels of sex hormones is too simple.

Recent experiments by Eric Vilain from UCLA suggest that differences in male and female brain development may begin even before significant amounts of sex hormones are produced. Using DNA microarrays (see the Genomics unit), Dr. Vilain discovered that 51 of the 21,000 genes that were active in early mouse embryos were expressed differently in males and females—at a stage in which gonads had not yet developed. Although not all of the genes and their functions have been identified yet, these data suggest that differences in male and female brains may be determined very early. This leaves us a long way from a biological explanation of why male mice in a maze refuse to ask directions and the females just look for landmarks. Nonetheless, studies in human cognition suggest that the general intellectual patterns of males and females might really differ.

In laboratory tests of problem solving, men are, on average, better at spatial tasks like visualizing the rotation of an object; women are better at word recall and matching. Of course, the variation within a group of men or a group of women may be quite large, with a great deal of overlap between the abilities of the groups. However, when taken together, many of the differences reflect our stereotypes about male and female abilities. Generally, men navigate a labyrinth with fewer errors than women, while women are better at remembering the location of an object. Is that why Dad usually drives, but Mom knows where the car keys are? In any case, as investigations in gender determination move into early embryonic development, the debate continues over male and female stereotypes and roles. Contribute your own analysis by doing the following activities and discussions.

Sources:

Kimura, D. 2002. Sex differences in the brain. *Scientific American*, May 13.
<http://www.sciam.com/article.cfm?articleID=00018E9D-879D-1D06-8E49809EC588EEDF>.

Dennis, C. 2004. The most important sexual organ. *Nature* 427:390.

Tests

Take only one page (one test) at a time.

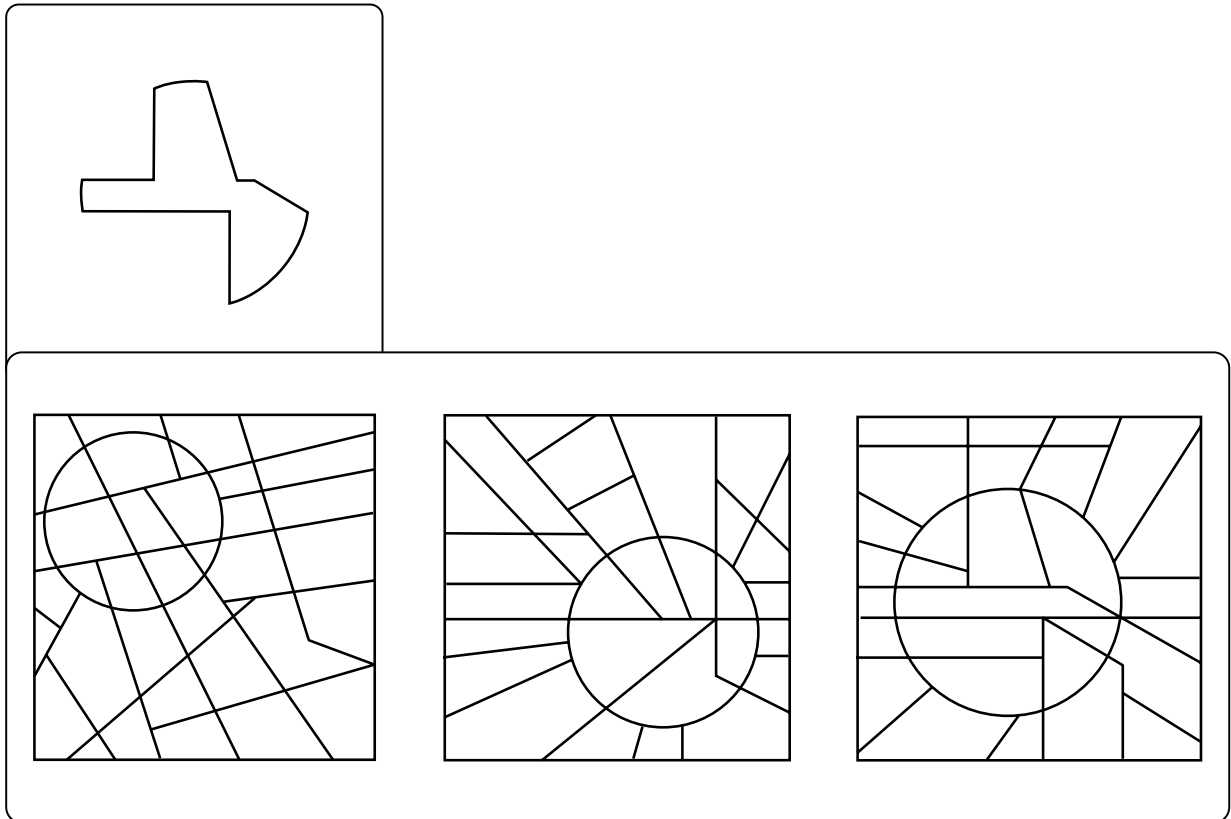
Test 1: Time each other to see how long it takes you to match the first picture in the top row with its identical copy in the bottom row.



Tests, cont'd.

Take only one page (one test) at a time.

Test 2: Time each other to see how long it takes to place the shape below into its matching shape in one of the complex line drawings.



Tests, cont'd.

Take only one page (one test) at a time.

Test 3: Give everyone 15 seconds to look at this list of unrelated words. Take away the words and have everyone write down as many as they can remember on a blank piece of paper.

dog

shadow

hamburger

cloud

flower

eyelash

opening

paper

water

light

fork

bank

lemon

fish

safety pin

key

comb

bird

paper clip

game

camera

scissors

gloves

complaint

Tests, cont'd.

Take only one page (one test) at a time.

Test 4: Set up a target, like a wastebasket or piece of paper on the wall. Take turns throwing a ball at the target and keep track of whom in your group is the most accurate.

Source: <http://exn.ca/brain/tests/>

Discussion Questions

1. Women are generally better performers on tests like Test 1, which measures “perceptual speed.” Men are generally better performers on tests like Test 2, which measures “disembedding ability.” Men are generally better at target-directed motor skills like throwing balls and other projectiles. Women are generally better at recall when read a story or list of unrelated words. Do the results of your group agree?
2. What kind of skill or ability do the tests measure? Why might women and men generally differ in their ability on these tests?
3. Do you think the tests you’ve just done are accurate measures of an innate skill? Or do they measure a learned skill or one that would improve with practice? Go back through the characteristics described in the article and see if you can think of tests that would measure these skills or abilities.
4. In the absence of a specific condition, like aberrant hormone levels, how would you explain exceptions—such as women with exceptional spatial abilities and men with exceptional word recall?
5. If the male-female differences in intellectual skill are genuine, what evolutionary reasons can you think of that might explain why women are generally better at remembering landmarks, recalling words, and performing tasks that require manual dexterity? What selective pressures might account for men generally being better at navigating mazes, detecting hidden geometric shapes, and throwing or catching projectiles?

Activity 6: Y?

Based on video content

15 minutes

Setup

The sequence of the Y chromosome has been determined, and this smallest human chromosome contains more genes than we had previously expected. Take a few moments to consider the implications of this discovery by discussing the provided questions.

Materials

- Optional: Paper Chromosomes (master copy provided in Activity 3)
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

Discussion Questions

See Tips and Suggested Answers for more information and answers.

1. At one point, the Y chromosome was thought to have a dozen genes or fewer. Now we know that it has 78—meaning that men have 78 genes that women do not have. David Page, one of the researchers who sequenced the Y chromosome, was interviewed for National Public Radio on June 19, 2003. In the interview, he said that while we say that all humans are 99.9 percent identical in DNA sequence, this statement is true only half the time—when comparing two males or two females. If we take into account the 78 genes on the Y chromosome that males have and females do not, males and females are only 98.5 percent alike in DNA sequence. A male chimpanzee and a male human are also 98.5 percent alike in DNA sequence. This leads Page to tell the interviewer "...you are about as similar to your wife as you are to a male chimp."

Do you believe this statement is true? Or, are there other factors that make male and female humans more alike than humans and chimps? In any case, what does this information about the Y chromosome tell us about the differences between sexes?

2. (If you did Activity 3, review the information relevant to this question. If not, skip to the next question.) One of the functions of crossing-over (recombination) in meiosis is to protect against the deleterious effects of mutations. The reshuffling of allele combinations during meiosis means that mutant alleles of several different genes might, by random crossing-over, all end up on one chromosome. This chromosome carrying several lethal alleles would be lost by segregation into a gamete that would be inviable.

However, crossing-over requires homologous chromosomes—that is, two chromosomes with the same genes. Except in rare cases of males with two Y chromosomes, the Y chromosome never has a homolog in meiosis with which to cross-over. What are the implications of this for genes on Y? How does the Y pair with X in meiosis if they are not homologous? Does knowing that the Y chromosome contains large repeated sections give you any clues about how it compensates for the inability to shuffle out mutant alleles?

3. The XY sex chromosome system means that men have genes that women do not. It also means that women, with two X chromosomes, have twice as many copies of the X chromosome genes as men. These include genes that encode carbohydrate metabolism enzymes, blood clotting factors, and color vision receptors.
 - a. Humans are quite sensitive to having too many or too few copies of genes. Think of the consequences of trisomy 21 (Down syndrome). What mechanism balances the difference in X chromosome gene copy number and how does it work?
 - b. What other systems are possible for balancing X chromosome gene copy number? (For example, *Drosophila* also have an XY chromosome sex determination system but they use a different mechanism than humans.)

Additional Information and Answers

1. One caveat to the genetic “differences between the sexes” is that the differences between male and female humans is in discrete genes. The differences between humans and chimps is over the entire genome, at every gene, at gene regulatory regions and in large changes in chromosome structure.
2. X and Y share enough homology at the ends that they can pair in meiosis in an XY male, and undergo limited crossing-over at these terminal regions. However, the bulk of the Y chromosome cannot undergo crossing-over, and is destined to accumulate mutations. The article notes that the Y chromosome has large, repeated regions, and suggests that these regions represent extra material that is available for repairing mutations.
3.
 - a. In mammals, gene dosage of X chromosome genes is regulated by X-inactivation. During early development, both X chromosomes express a gene called *Xist* into Xist RNA. This RNA binds the X chromosome. Slight differences in the amount of Xist RNA cause one X to be condensed into an inactive Barr body; the other expresses its genes. In effect, female mammals have one functional X, just like males. Regulation is more complex than that, because having only one X chromosome, as in Turner syndrome, is not the same as having one inactivated and one expressed X.
 - b. In other organisms with two different sex chromosomes, like *Drosophila*, dosage compensation can occur by either increasing the expression of X-linked genes in males, or decreasing expression in females.

Activity 7: You Be the Judge

Based on video content

15 minutes

Setup

The video for this unit included a bit of the history of gender testing in the Olympics, and covered some of the issues in human gender determination. Now that you've had a chance to apply and reflect on this information, discuss the following questions in pairs or in a group.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. According to *The Journal of the American Medical Association*, at the 1992 Winter Olympics in Albertville, France, 8 of 3387 (1 per 423) women were found to have the Y-linked gene *SRY*. Seven of the eight had complete or partial androgen insensitivity syndrome, and the eighth probably had a defect in an enzyme in a testosterone biosynthesis pathway.
In your opinion, which, if any, of these people should be allowed to compete in women's events?
2. Do you think it would be fair for a true male who could "pass" as a female—and perhaps had the internal sense that he was more female than male—to compete in women's events in golf? swimming? soccer? basketball? boxing?
3. If you don't think this would be fair, should all athletes be tested for gender? How?
4. In your opinion, which is more important in determining gender: sex chromosome composition? appearance of external genitals? internal anatomy? self-identification? levels of sex-determining hormones?
5. In general, do you think biological or psychological criteria are more important in determining gender?
6. In biological terms, what do you think determines the critical features of maleness and femaleness in humans? What genes might be involved, and what might they encode?

Sources:

Simpson, J. et al. 2000. *JAMA* 284:1568–69.
Simpson, J.L. 1986. *JAMA* 256(14):1938.

Notes

Activity 1: Before and After

Based on video content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

We all appreciate both the beauty and the biological importance of areas with natural diversity. This unit explores the current techniques for measuring, investigating, and making hypotheses about this diversity. The issues go far beyond aesthetics and basic biology, and have an impact on human health, recovery of land after natural disasters, and the preservation of life on earth.

Before viewing the video for this unit, spend a few minutes thinking about some of the issues in this field, presented as questions on the handout. With another person, discuss or write a few thoughts or questions you have about each of these issues. After watching the video, go over them again as a group. Did your thoughts correspond to what you heard in the video? Were your questions answered?

Materials

- One copy of the list of Biodiversity Questions per person (master copy provided)

Biodiversity Questions

These questions represent some of the issues surrounding the science and politics of biodiversity. What are your thoughts on these issues?

1. How is biodiversity defined?
2. How is biodiversity measured?
3. What are some of the reasons to maintain biodiversity?
4. What is habitat fragmentation? What impact does it have on ecosystems and the community that includes humans?
5. How does the present rate of extinction compare to previous eras? What is the sixth mass extinction?
6. What factors contribute to the probability that a species will go extinct?

Activity 2: Quantifying Biodiversity

Based on video and online text content

45 minutes

Setup

One of the challenges confronting biologists who study biodiversity is how to measure and quantify diversity. In 1982, Terry Erwin used a “kill ‘em and count ‘em” method to estimate insect diversity in a tropical forest. (Read more in the Biodiversity online text, available at <http://www.learner.org/channel/courses/biology/>.) As shown in the video, the current method is to use field measurements combined with mathematical models; this is the technique used in this activity.

Working in pairs, follow the step-by-step instructions on calculating a diversity index for an example population. Then, test the effects of habitat fragmentation on populations you will create, using small, colored objects to represent individuals of different species. Share your results in the post-activity discussion.

Materials

- One copy of the Worksheet for Quantifying Biodiversity with Diversity Indices plus Discussion Questions per person (master copy provided)
- Approximately 125 small colored objects to represent individuals in an ecosystem. For every two people, have five small items (e.g., strips of paper, toothpicks, matchsticks, colored candies, etc.) in each of five different colors (five red, five green, five white, etc.), for a total of 25 per two people.
- Tips and Suggested Answers

Worksheet for Quantifying Biodiversity with Diversity Indices plus Discussion Questions

Background

Imagine a section of marsh that contains 43 species of plants. In terms of numbers of species, it is more diverse than a neighboring section that contains only 26 species. Suppose, however, that in the section with 26 species, the species were all roughly comparable in numbers of individuals; and in the section with 43 species, a few species were prevalent and the rest were rare. Which section would then be most diverse?

Ecologists have used various indices as a means to quantify biodiversity. One simple index is the Simpson index.

To calculate this Simpson index, we need to know the number of individuals for each particular species (n) and the sum of those numbers (N).

$$D = \text{SUM } (n/N)^2$$

For example, in a community of three species where one species has 6 members, a second has 12, and a third has 42:

$$D = (6/60)^2 + (12/60)^2 + (42/60)^2 = 0.54.$$

So when we are calculating D , we are summing the squares of the proportion each species makes to the total.

D actually is inversely related to diversity. When $D = 0$ (its theoretical minimum), there is infinite diversity. When $D = 1$ (its maximum), there is no diversity as all of the individuals are from just one species.

Ecologists will often use the reciprocal of D , $1/D$. This figure actually has meaning in that it is the effective number of species in the area.

In the case above, the effective number of species is $1/0.54$ or 1.852. Although there are three species in this area, because one species is common and the other two are relatively rare there is less diversity than there would be if there were two equally frequent species. Two equally numerous species would have a D of 0.5 and $1/D$ of 2. The maximum number of effective species is equal to the actual number of species and is achieved only when all species are equally abundant.

Exercises

Some answers are in the Tips and Suggested Answers.

Exercise 1: Practice calculating diversity.

1. Calculate D and then the effective number of species in a community that consists of the following:
Species A – 35
Species B – 26
Species C – 13
Species D – 6
Species E – 4
2. How does D for this scenario compare to the numbers worked out in the example? How does this population compare to the example population with three species where one dominates, or the example of two equally frequent species?

Exercise 2: Make (and fragment) a habitat.

1. Make an ecosystem using the colored items (e.g., the strips of paper, matchsticks, or colored candies) to represent individuals of five different species. Each pair will put in 25 items—five each of the five different colors—until the ecosystem is complete. Each color will represent a different species; for example, if there are five groups, there should be 125 total items.
2. Calculate D and the effective number of species for the total population.
3. In pairs, take a random sample of 25 items from the total.
4. In pairs, calculate D and the effective number of species for its sample.

Post-Activity Discussion

Each pair will share its results with the group.

1. Were the consequences of habitat fragmentation more or less severe than you expected?
2. In the ecosystem you made, the different colors represented different species. Imagine three of the colors were plants and two were animals. What effects would you expect in the fragmented communities that you created randomly? Reconsider the scenario with one object being a bacterium, one a flying insect, one a nematode soil worm, one a plant, and one a mammal.
3. What kind of practical steps can we take to reduce the fragmentation of habitats? What incentives might motivate developers and civic leaders to consider habitat fragmentation in urban planning?

Quantifying Biodiversity Exercises

Exercise 1: Practice calculating diversity.

$D = 0.3$ and the effective number of species is $1/D = 3.3$. This population has a higher effective number of species than either of the previous examples.

Exercise 2: Make (and fragment) a habitat.

1. If your community has five different species, each equally represented, $D = 0.2$ and the effective number of species is 5.0.
4. Answers will vary, but D should be significantly higher and the effective number of species should be significantly lower than those values for the total. This demonstrates that sampling reduces diversity.

Activity 3: Extinction Risk

Based on video and online text content

25 minutes

Setup

As discussed in the text and seen in the video, many factors will influence the susceptibility of a species to becoming extinct. In this exercise, work in teams and discuss the likelihood of extinction for an imaginary species. Characteristics of the species and the online text will provide information about which of the species is more likely to go extinct.

Materials

- One copy of the Pairs of Species to Compare for Extinction Susceptibility plus Discussion Questions per person (master copy provided)
- One copy of the Biodiversity online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Pairs of Species to Compare for Extinction Susceptibility plus Discussion Questions

Answers are in the Tips and Suggested Answers.

Example 1:

Compare these species and decide which is more likely to go extinct. Consult the online text for more information on factors that determine extinction probability.

Ficolia nigra — The black bark beetle — Current population size is estimated to be 150,000 individuals. It is found in wooded areas over large sections of the Midwest and Central Plains, but is generally rare across its range. It can live in a variety of different species of trees.

Ficolia confusum — Resembling and often confused for *F. nigra*, this species is a specialist on birch trees. Its range, which is enclosed by that of *F. nigra*, is much smaller. *F. confusum* is usually found at higher altitudes than *F. nigra*. Where *F. confusum* is found, it is usually abundant. Estimated population size of 250,000 individuals.

Example 2:

Compare these species and decide which is more likely to go extinct. Consult the chapter text for more information on factors that determine extinction probability.

Silpera impratus is a small annual plant that occurs in and near marshland. This species is an obligate outcrosser: individual plants have either male or female flowers, but not both. Its population size is in the hundreds.

A related species, *Silpera stebbinsi*, occurs in similar habitat. Its population size is also in the hundreds. Unlike *Silpera impratus*, individuals of this species contain both male and female flowers. Selfing is common.

Discussion Questions

1. Are captive breeding programs useful in preserving endangered species? What are some advantages and disadvantages of these programs?
2. Climate changes can cause species to adapt to different environmental conditions. They may also lead them to be exposed to new sets of species as ecosystems shift and organisms migrate. What are some effects that might occur from reshuffling species assemblages?

Pairs of Species to Compare

Example 1: Both species are likely to persist given their large population sizes, but *F. confusum* may be at some risk because it has a more restricted range and a more restricted habitat.

Example 2: Both species face substantial probabilities of going extinct. *S. stebbinsi* is less likely to go extinct than *S. simpratus*, because the former's ability to self decreases the likelihood of going extinct via demographic stochasticity.

Activity 4: Concept Maps

Based on video and online text content

20 minutes

Setup

Biodiversity encompasses large issues in biology and social and political policy. Concept maps can be a useful tool for generating overviews and organizing thoughts on global topics like biodiversity. Work individually to draw concept maps using the suggested topics, and then discuss them with two or three other people. Add concepts to the maps as they come up in the discussion.

Materials

- One copy of the Suggested Concepts to Map per person (master copy provided)
- One copy of the Biodiversity online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

Suggested Concepts to Map

Build concept maps to show how the following groups of ideas might be related. Consult the online text for clarification of the concepts. Then, discuss the individual concept maps with two or three other people.

1. dilution effect, development, habitat destruction/fragmentation, zoonotic disease (has both human and animal hosts), species richness
 - How does Lyme disease illustrate the interactions in this concept map?

2. productivity (biomass), diversity, stability
 - Is a stable system one that doesn't change?

3. species inventory; extinction; sustainable practices; specimen collections, seed banks, and DNA banks
 - How might applying these concepts to animals or microorganisms differ from applying them to plants, as was shown in the video?

Activity 5: Wrap-Up Discussion

Based on video and online text content

15 minutes

Setup

In this brief discussion, review the reasons for preserving biodiversity, and consider the practical issues in creating policy and implementing preservation practices. As teachers, what issues will you bring to the classroom and in what context will you present them?

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. List as many reasons as you can think of for preserving biodiversity. Which would you classify as utilitarian, and which would you classify as non-utilitarian? How would you define the difference between these classifications?
2. What factors, utilitarian or non-utilitarian, should be used to determine if an area warrants protection? How should the importance of those factors be determined and weighted in making decisions about developing or preserving an area?
3. Some argue that richer countries have a responsibility to help poorer countries preserve biodiversity. What are some ethical and economical reasons to support this position? Which are most convincing to you? Which are the least convincing? Why?
4. With the others in your group consider the concepts in this unit, and discuss how they relate to and inform concepts you currently teach. How might these concepts be used in a team-taught class with a civics or political science teacher?

Notes

Activity 1: Coming Attractions

Based on video content

15 minutes (10 minutes before and 5 minutes after the video)

Setup

Before watching the video, answer a few quick questions about genetically modified organisms. Don't think too long about the questions—just write down your first response. You might already know the answers to some of them; otherwise, just take an educated guess. During the video, listen for the topics addressed by the questions. After the video, review the questions and the answers as a group.

Materials

- Transparency of the Coming Attractions Questions (master copy provided)
- Transparency of the Coming Attractions Answers (master copy provided)

Coming Attractions Questions

1. What two U.S. crops are the most heavily genetically modified?
2. What fraction of U.S. corn is genetically engineered?
3. What percent of U.S. processed food contains some genetically modified corn or soybean product?
4. All known food allergies are caused by one type of molecule. Is it proteins, sugars, or fats?
5. What is the substance called "bt" used for?
 - a. Bonus: What does "bt" stand for?
 - b. Bonus: When was "bt" first registered with the USDA?
6. What does "totipotent" mean?
7. What nutrient is produced by "golden rice"?
8. According to the World Health Organization, how many children go blind each year from vitamin A deficiency?
9. What is the normal function of "anti-thrombin"?
10. For what was "Dolly the sheep" famous?
11. What's the difference between a "transgenic" animal and a "cloned" animal?
12. Yes or no: Are all "transgenic" animals also "cloned"?
13. Which are easier to clone: animals or plants?
14. What do "restriction enzymes" do?
15. What is the function of "microinjection" in genetic engineering?

Coming Attractions Answers

1. What two U.S. crops are the most heavily genetically modified?
Corn and soybeans
2. What fraction of U.S. corn is genetically engineered?
Almost one-third
3. What percent of U.S. processed food contains some genetically modified corn or soybean product?
70%
4. All known food allergies are caused by one type of molecule. Is it proteins, sugars, or fats?
Proteins
5. What is the substance called “bt” used for?
It’s an insecticide.
 - a. Bonus: What does “bt” stand for?
Bacillus thuringiensis
 - b. Bonus: When was “bt” first registered with the USDA?
1961
6. What does “totipotent” mean?
A cell or cells with the ability to develop into the whole organism
7. What nutrient is produced by “golden rice”?
Beta-carotene, a vitamin A precursor
8. According to the World Health Organization, how many children go blind each year from vitamin A deficiency?
Up to half a million
9. What is the normal function of “anti-thrombin”?
It’s a blood protein, involved in anti-coagulation/clotting and anti-inflammation.
10. For what was “Dolly the sheep” famous?
She was the first cloned mammal.
11. What’s the difference between a “transgenic” animal and a “cloned” animal?
A transgenic contains a foreign gene; a cloned animal contains an entire nucleus of genetic material from another individual.
12. Yes or no: Are all “transgenic” animals also “cloned”?
No
13. Which are easier to clone: animals or plants?
Plants. Many plants can be cloned with cuttings or by grafting. Animals require nuclear transfer into an egg, followed by development in a surrogate mother.
14. What do “restriction enzymes” do?
Cut DNA at specific sequences into small pieces
15. What is the function of “microinjection” in genetic engineering?
It introduces DNA into cells like eggs or embryonic cells.

Activity 2: What's the Difference?

Based on video and online text content

50 minutes

Setup

Many of the terms used in genetic engineering have multiple, alternate definitions. Many sound similar to other terms that have a very different meaning. This exercise asks “What’s the difference?” between two or more closely related genetic engineering terms. Work in pairs, with each pair taking at least one of the questions to work on. Use the Genetically Modified Organisms online text chapter as a reference. After five minutes to come up with a concise explanation for the difference between the terms, go over the questions as a group. First, spend a few seconds letting each person think of how he or she might answer the question. Then the pair who was working on this question will give the answer they came up with. Others can ask for clarification or add to the answer. Explanations of similarities are welcome, too!

Materials

- One copy of What’s the Difference? Questions per person (master copy provided)
- Transparency of What’s the Difference? Questions (master copy provided)
- Tips and Suggested Answers
- One copy of the Genetically Modified Organisms online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

What's the Difference? Questions

1. What's the difference between a "cloned" gene, a "cloned" bacterium, and a "cloned" sheep?
2. What's the difference between traditional, selectively bred animals and transgenic animals?
3. What's the difference between "cloning" a plant and "cloning" an animal?
4. What's the difference between the term "vector" (when applied to genetic engineering) and the term "plasmid"?
5. What's the difference between an intron and an exon?
6. What's the difference between DNA, cDNA, and T-DNA?
7. What's the difference between Dolly (a cloned sheep) and Polly (a transgenic sheep)?
8. What's the difference between microinjection and a gene gun?
9. What's the difference between a cell that contains a transferred nucleus (from somatic cell nuclear transfer) and a cell that contains recombinant DNA?
10. What's the difference in technique, objective, and outcome in reproductive human cloning and human cloning for therapeutic or research purposes?
11. What's the difference between cloned human cells and human embryonic stem cells? (What's the connection between these cell types?)
12. What's the difference between the laws regarding human cloning in the U.S. and in other countries?

Note: For possible ways to answer these questions, see the Tips and Suggested Answers that follow.

What's the Difference? Suggested Answers

1. What's the difference between a "cloned" gene, a "cloned" bacterium, and a "cloned" sheep?
"Cloned" in this case means genetically identical. When a gene is cloned, it means it has been genetically engineered into a bacterial cloning vector, usually a plasmid, so many identical copies of it can be generated. Bacteria that grow asexually naturally make genetically identical, cloned populations. A cloned animal has been generated by transferring the nucleus of an adult animal into an enucleated egg. If the implanted egg develops, the result is a genetically identical copy of the adult who donated the nucleus to the enucleated egg.
2. What's the difference between traditional, selectively bred animals and transgenic animals?
Traditional selective breeding relies on sexual reproduction to mix genes of organisms. Then humans select individuals with the desired traits. Over many generations, populations with suitable traits are obtained. Transgenic animals contain introduced genes that may be from another organism. These genes introduce desired traits directly, without relying on sexual reproduction or mutation to generate these traits randomly.
3. What's the difference between "cloning" a plant and "cloning" an animal?
Cloning a plant can often be done by grafting, or taking a cutting and getting the stem of the cutting to develop roots. Individual cells can be cultured into fully developed plants by plant tissue culture methods. Plants are commonly totipotent, so their cells maintain the ability to develop into almost any specialized plant cell.

Cloning an animal requires replacing the nucleus of an enucleated egg with the nucleus of the individual to be cloned and stimulating division into a few embryonic cells. At that point, the embryo must be implanted into a surrogate mother for development into the cloned animal.
4. What's the difference between the term "vector" (when applied to genetic engineering) and the term "plasmid"?
A vector is a more general term that means any piece of DNA that can be used to introduce recombinant DNA into a cell. Some are engineered viral chromosomes and some are engineered plasmids. A plasmid is a small, extrachromosomal piece of naturally occurring DNA, commonly found in bacteria, and easily isolated from and re-introduced into bacterial cells. They are used to clone genes and introduce them into cells.
5. What's the difference between an intron and an exon?
Both are parts of genes, but the DNA of exons contains "coding information" or instructions for amino acid sequences of the protein encoded by the gene. Introns are intervening sequences that are transcribed into RNA, but spliced out before translation.
6. What's the difference between DNA, cDNA, and T-DNA?
All are "DNA" (deoxyribonucleic acid). cDNA has been made as a double-stranded DNA copy of a messenger RNA (mRNA), using the retroviral enzyme reverse transcriptase. T-DNA is a plasmid carried by the plant pathogen *Agrobacterium tumefaciens*. It is used to introduce genes into genetically modified plants.

7. What's the difference between Dolly (a cloned sheep) and Polly (a transgenic sheep)?

A cloned sheep like Dolly is a genetically identical copy of an existing adult sheep. The sheep from which Dolly was cloned donated a nucleus to an egg, which was taken from a different female sheep and "enucleated" so the chromosomes were removed. Dolly contained no non-sheep genes. A transgenic sheep like Polly contains an introduced gene, which may be from another organism (for example, the gene for production of human anti-thrombin III). A transgenic animal is not necessarily a clone or an identical copy of another, although it is possible to make a clone of a transgenic animal.
8. What's the difference between microinjection and a gene gun?

Microinjection uses a microscopic needle to introduce DNA directly into the nucleus of a cell or egg. A gene gun shoots pellets coated with DNA into cells—especially cells with an otherwise impenetrable cell wall, like those of plants. Some of the DNA may get shot into the nucleus and might be incorporated into the chromosomes.
9. What's the difference between a cell that contains a transferred nucleus (from somatic cell nuclear transfer) and a cell that contains recombinant DNA?

Like the difference between a cloned animal and a transgenic animal, the transferred nucleus contains ALL the genetic information of a cell. If the transferred nucleus is put into an enucleated egg, and the egg is successfully implanted and develops, a cloned individual will result. A cell that contains recombinant DNA contains only one or a few introduced genes.
10. What's the difference in technique, objective, and outcome in reproductive human cloning and human cloning for therapeutic or research purposes?

Both reproductive and therapeutic cloning starts with an egg. The nuclear DNA is removed, usually using microinjection technology. The "cloning" part is the introduction of a nucleus from a different cell, with subsequent cell division. The next step is the difference in technique and objective between reproductive and therapeutic cloning. In reproductive cloning, the embryonic group of cells would be implanted into a "surrogate mother" for the purpose of generating an entire new individual, a clone of the nucleus donor. Therapeutic cloning does not implant the embryonic cells, but uses them for research or for generating cells for transplants. Cloned cells that are genetically identical to a transplant recipient are less likely to be rejected.
11. What is difference between cloned human cells and human embryonic stem cells? (What's the connection between these cell types?)

These are two different types of cells but they are often discussed in the same context.

Cloned human cells would be generated by the technique described in the answer for "What's the difference in technique, objective, and outcome in reproductive human cloning..." They are genetically identical to the nucleus donor.

Embryonic stem cells are the pluripotent cells from an embryo, which have the potential to become any cell type. Embryonic stem cells can come from an embryo generated by fusing a sperm and an egg. In that case, they are genetically unique. Embryonic stem cells could, in theory, come from an embryo generated by therapeutic cloning techniques. In that case, they would be clonal, or genetically identical, to the nucleus donor. Differentiated cells generated from them could be used for a relatively rejection-free transplant.
12. What's the difference between the laws regarding human cloning in the U.S. and in other countries?

The answer will depend on changing referenda and laws.

Activity 3: Troubleshooter

Based on video and online text content

50 minutes

Setup

Amazing techniques have been developed for making genetically engineered or cloned organisms, but the process is not always easy. Here are several cases of a failed attempt at a genetic engineering experiment. You will be the troubleshooter trying to figure out what went wrong.

Working in teams of two or three, read about one of the failed experiments. Each case comes with several possible explanations. Evaluate each possible explanation as the source of the failure, and think of an experiment that would test it, with possible results and conclusions. (The Genetically Modified Organisms online text might help in understanding the different techniques.) After about five or ten minutes of consideration and discussion, give the handout explaining the case to a neighboring team and receive a new one from an adjacent team.

After all teams have had a chance to work on all the cases, go over them quickly as a group and see if there is discussion about the possible causes. What kinds of experiments did the teams come up with and which would the group most like to try?

Materials

- One copy of each of The Genetic Engineering Experiment Cases (master copy provided)
- Tips and Suggested Answers
- One copy of the Genetically Modified Organisms online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)

Troubleshooter: The Genetic Engineering Experiment Cases

Case 1

Background

Someone in your lab tried to clone a gene from *Salmonella* bacterium into a plasmid in *E. coli* bacterium. He cut the gene out of the *Salmonella* chromosome with a restriction enzyme, cut the plasmid with the same restriction enzyme, and used ligase to combine the *Salmonella* gene into the plasmid. He used electroporation to introduce the plasmid into *E. coli*, and then plated onto plates containing ampicillin. The next day, instead of a few colonies of cells that had acquired the plasmid, the plate was covered with a “lawn” of cells, as though every single cell had grown.

What could have gone wrong? Which of the reasons below is a possible explanation for the problem? Think of an experiment that would test if this is the explanation. What results would be expected?

Possible Explanations

1. Electroporation cannot be used on bacterial cells; it works only on plant cells.
2. He left out the selecting antibiotic when making the medium, so all the cells grew whether they had the plasmid or not.
3. The *E. coli* he used were already resistant to the antibiotic.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 2

Background

Someone in your lab tried to clone a gene from *Salmonella* bacterium into a plasmid in *E. coli* bacterium. He cut the gene out of the *Salmonella* chromosome with a restriction enzyme, cut the plasmid with the same restriction enzyme, and used ligase to combine the *Salmonella* gene into the plasmid. He used calcium chloride to introduce the plasmid into *E. coli*, and then plated onto plates containing ampicillin. The next day, instead of a few colonies of cells that had acquired the plasmid, there were no colonies.

What could have gone wrong? Check off those that are a possible explanation for the problem. Then, think of an experiment that would test if this is the explanation. What results are expected?

Possible Explanations

1. The restriction enzyme cut the plasmid in the middle of the gene for ampicillin resistance, disrupting the gene and making antibiotic resistance impossible.
2. He neglected to add antibiotic to the medium.
3. The treatment to make them calcium-competent killed the cells.
4. The mixture of cut gene and cut plasmid requires an enzyme (ligase) to covalently connect them and he forgot to add this enzyme. Neither the cut *Salmonella* gene alone nor the cut plasmid can confer antibiotic resistance.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 3

Background

Your lab has two sibling mice, one male and one female, that seem to be living longer than other mice. So far, they have lived 25% longer than the normal mouse lifespan. Thinking they might be good models for human aging, you would like to perpetuate them but are not sure if they will be able to reproduce.

You direct your technician to try cloning the mice. She obtains 10 eggs from the long-lived female and 10 eggs from another, unrelated female. After removing the nucleus from all 20 eggs, she introduces a nucleus into each one. Ten eggs receive a nucleus from the long-lived male and ten receive a nucleus from the long-lived female. All are implanted into different surrogate mothers. Two mice are born. When they are tested, one is genetically identical to the long-lived female in every way. The other is nearly genetically identical to the male but has a few differences. Your technician is disappointed in the results.

What could have gone wrong? Check off those that are a possible explanation for the problem. Then think of an experiment that would test if this is the explanation. What results are expected?

Possible Explanations

1. Nothing went wrong; these are actually good numbers. Animal cloning has a very low success rate.
2. The small genetic differences between the long-lived male mouse and his clone are because of mitochondrial DNA, present in the egg and not removed by the microinjection technique.
3. The small genetic differences between the long-lived male mouse and his clone are because not all the chromosomal DNA of the egg was removed by the microinjection technique.
4. It isn't possible to get an exact clone of the male because the surrogate mother mouse will always contribute some DNA.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 4

Background

Your lab is trying to produce a human protein that acts as a fat-regulating hormone for therapeutic purposes. Normally the protein is secreted by adipose tissue. The most efficient way to produce a large amount of protein seems to be to clone the human gene into a plasmid that can be introduced into bacteria, grow the bacteria in culture, and have the bacteria produce large amounts of the protein.

You clone the gene into a bacterial plasmid and successfully introduce the plasmid into lab strains of *E. coli*. When you harvest some of the bacteria after they have grown in culture, they still have the correct plasmid but have produced no protein.

What could have gone wrong? Which of the reasons below is a possible explanation for the problem? Think of an experiment that would test if this is the explanation. What results would be expected?

Possible Explanations

1. Bacterial cells and human cells are too far apart, evolutionarily, to produce each other's proteins. For example, bacteria and humans do not use the same codons to encode the amino acids methionine, tryptophan, and histidine.
2. The human gene contains introns, and the bacterial cell cannot splice them out of the mRNA.
3. The human protein might require modifications that the bacterial cell cannot perform, such as glycosylation (addition of sugars) or post-translational processing. Without these modifications, the protein might be unstable so it can't be detected.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter: The Genetic Engineering Experiment Cases

Case 5

Background

Your lab is attempting to introduce the gene for a special enzyme into corn plants. This enzyme will change the color of the corn endosperm, making it pink. A large food processing company is interested in this plant because they think pink corn chips will be a big marketing hit.

DNA containing the gene for the enzyme is coated onto gene gun pellets and shot into totipotent plant cells. From these cells, transformed plants are cultivated. Two plants are obtained that make the special enzyme but they do not express it in the endosperm.

What could have gone wrong? Which of the reasons below is a possible explanation for the problem? Think of an experiment that would test if this is the explanation. What results would be expected?

Possible Explanations

1. The cloned gene did not contain the proper regulatory sequences required to produce the protein in the endosperm.
2. When the DNA was shot into the cells, the only cells that acquired it and integrated it into the chromosomes were non-endosome cells like the cells of roots or leaves.
3. The gene gun technique works on only certain kinds of plants. The introduction of DNA should have been done with *Agrobacterium tumefaciens*, which introduces DNA into any kind of plant.

Note: Answers can be found in the Tips and Suggested Answers that follow.

Troubleshooter Answers

Case 1 Answers

1. Electroporation cannot be used on bacterial cells; it works only on plant cells.
This is not a possible explanation.
2. He left out the selecting antibiotic when making the medium, so all the cells grew whether they had the plasmid or not.
This is a possible explanation that could be tested by putting antibiotic-sensitive bacteria on the plate. If they grow, the plates do not contain antibiotics.
3. The *E. coli* he used were already resistant to the antibiotic.
This is a possible explanation that could be tested by putting the non-recombinant *E. coli* onto a plate with antibiotics without electroporation to add a plasmid. If they grow, they were already antibiotic resistant.

Case 2 Answers

1. The restriction enzyme cut the plasmid in the middle of the gene for ampicillin resistance, disrupting the gene and making antibiotic resistance impossible.
This is a possible explanation, which could be tested by transforming with the plasmid, without cutting with the restriction enzyme; or checking the “map” or the sequence of the plasmid to see if the restriction enzyme has a recognition site within the amp resistance gene.
2. He neglected to add antibiotic to the medium.
This is not a possible explanation. In that case, everything would grow.
3. The treatment to make them calcium-competent killed the cells.
Probably not, but it could be tested by putting the calcium-treated cells directly onto medium with no antibiotic. They should all grow.
4. The mixture of cut gene and cut plasmid requires an enzyme (ligase) to covalently connect them and he forgot to add this enzyme. Neither the cut *Salmonella* gene alone nor the cut plasmid can confer antibiotic resistance.
This is a possible explanation that could be tested by starting over from the step combining the gene and the cut plasmid, this time adding ligase.

Case 3 Answers

1. Nothing went wrong; these are actually good numbers. Animal cloning has a very low success rate.
This is a possible explanation. Hundreds of eggs were enucleated and received nuclei, and 13 were implanted in order to get one cloned sheep, Dolly.
2. The small genetic differences between the long-lived male mouse and his clone are because of mitochondrial DNA, present in the egg and not removed by the microinjection technique.
This is a possible explanation. Most “cloned” animals are not exactly genetically identical to the nucleus-donating cell because the cloned animals cells contain the mitochondrial DNA from the egg donor. To test this, a Southern blot or other “DNA fingerprinting” type test could be performed, probing specifically for mitochondrial DNA, and comparing the egg-donating mouse’s mitochondrial DNA with the mitochondrial DNA from the long-lived male and the clone. If the difference is in mitochondrial DNA only, the clone will have the mitochondrial DNA of the egg donor, but all other DNA fingerprint markers will match the long-lived male who donated the nucleus.

3. The small genetic differences between the long-lived male mouse and his clone are because not all the chromosomal DNA of the egg was removed by the microinjection technique.
This is a possible, but unlikely, explanation. It could be tested with a Southern blot or other "DNA fingerprinting" test. A test like this might be able to detect egg DNA that remained and incorporated into the chromosomes of the developing embryo.
4. It isn't possible to get an exact clone of the male, because the surrogate mother mouse will always contribute some DNA.
This is not a possible explanation. The surrogate mother will not contribute DNA to the embryo.

Case 4 Answers

1. Bacterial cells and human cells are too far apart, evolutionarily, to produce each other's proteins. For example, bacteria and humans do not use the same codons to encode the amino acids methionine, tryptophan, and histidine.
This is not a possible explanation. Although there are a few examples of non-standard codon usage, the codon table is otherwise universal. *E. coli* cells and human cells use the same codons to encode the same amino acids.
2. The human gene contains introns, and the bacterial cell cannot splice them out of the mRNA.
This is a possible explanation. There are many ways to test this, including determining the base sequence of the entire gene. In fact, this would almost certainly be done before the cloning step. One way to cleanly remove the intron so the gene could be expressed in bacteria would be to generate a cDNA copy from mRNA of the gene extracted from eukaryotic cells. The mRNA would have the intron "spliced out," so the cDNA copy made from it would not contain the intron and bacteria would be able to produce the protein properly.
3. The human protein might require modifications that the bacterial cell cannot perform, such as glycosylation addition of sugars or post-translational processing. Without these modifications, the protein might be unstable so it can't be detected.
This is a possible explanation that is frequently a difficulty in producing eukaryotic proteins in bacteria. There are many possible tests for this, but one possibility is to have a eukaryotic cell, like yeast, produce the protein and see if it is modified properly.

Case 5 Answers

1. The cloned gene did not contain the proper regulatory sequences required to produce the protein in the endosperm.
This is a possible explanation, although difficult to test precisely. A version of the gene with known endosperm-expressing regulatory sequences should be made and introduced into the plants.
2. When the DNA was shot into the cells, the only cells that acquired it and integrated it into the chromosomes were non-endosome cells like the cells of roots or leaves.
This is not a possible explanation. When DNA is introduced into totipotent cells, they are not yet differentiated into roots and leaves. They have the ability to develop into any kind of cell. If a totipotent cell that acquires recombinant DNA is grown into an entire plant, all its cells will contain the introduced DNA although they may not express it into proteins.
3. The gene gun technique works on only certain kinds of plants. The introduction of DNA should have been done with *Agrobacterium tumefaciens*, which introduces DNA into any kind of plant.
This is not a possible explanation. The gene gun technique is actually more universal than introduction of DNA by infection with an *Agrobacterium*.

Activity 4: Two Thumbs Up?

Based on video content

5 minutes

Setup

Now that you've seen a video addressing some of the issues surrounding genetically modified organisms, discuss the following questions in teams of three or four.

Materials

- One copy of the Discussion Questions per person (master copy provided)

Discussion Questions

1. What information in the video or subsequent activities surprised you the most?
2. Was there anything that you hadn't heard of before? If so, do you think this would be completely new information for high school students as well?
3. Compared to the average adult, do you think your high school students would be more or less accepting of the use of genetically modified organisms in food and medical production? Why?

Notes

Credits

Project Team

Advisors

In addition to determining the content of the units, our advisors and consultants have been actively involved in reviewing the material for all 13 units throughout its development. Videos, animations, case studies, and text chapters have all been reviewed several times during their production for accuracy and to ensure that these materials are as useful as possible to the intended audience.

Our primary advisors and consultants consisted of a team of eight scientists involved in teaching, curriculum development, and research.

Mark Bloom, Ph.D., is a science educator at Biological Sciences Curriculum Study (BSCS). He has developed print and Web-based curriculum materials for students in middle school, high school, and college. Previously, he was the assistant director of the Dolan DNA Learning Center, where he ran workshop programs for high school and college teachers. He developed the first educational kits using the polymerase chain reaction and coauthored the college lab manual *Laboratory DNA Science*. Mark was lead advisor for the Genomics, Proteins and Proteomics, Cell Biology and Cancer, and Biology of Sex and Gender units.

Steve Boyarsky is the coordinator of curriculum improvement at Staff Development at Southern Oregon Education Service District. Steve coordinates professional development in a three-county region in southern Oregon. He taught high school biology and human anatomy/physiology for 18 years. Steve has been involved with state and national level biology education through the National Science Teachers Association, a congressional fellowship, grants, and curriculum projects. Steve commented on appropriateness of content, level, and style of all project components.

Alan Dickman, Ph.D., is the biology curriculum director and an associate professor of biology at the University of Oregon. He has organized summer outreach programs in science for middle school, high school, and community college teachers, and has been involved in nationally funded programs to improve college-level biology education. Alan teaches introductory biology courses and an upperdivision forest biology course. As lead scholar, Alan was responsible for final scholarly quality of all content of all project components.

Marion Field Fass, Sc.D., is an associate professor of biology at Beloit College. She has been involved in curriculum reform efforts in biology through the BioQUEST Curriculum Consortium and the SENCER (Science Education for New Civic Engagements and Responsibilities) project of AAC&U (Association of American Colleges and Universities). In 2002 she traveled to Kenya and Tanzania to work with professors who were developing undergraduate courses about the epidemic of HIV/AIDS and about its impact in their communities. Marion was lead advisor for the Microbial Diversity, Emerging Infectious Diseases, HIV and AIDS, and Genetically Modified Organisms units.

Writers

Chris Tachibana, Ph.D., has taught undergraduate biology since 1992 at Salt Lake Community College, Penn State University, and the University of Washington. She is a research scientist at the University of Washington Biochemistry Department and the Carlsberg Research Labs in Denmark. Chris developed two case studies: The Genetics of Resistance to HIV and Designing an Anti-Cancer Drug. She also authored the learning activities for the Genomics, Proteins and Proteomics, Emerging Infectious Diseases, HIV and AIDS, Cell Biology and Cancer, Biology of Sex and Gender, and Genetically Modified Organisms units. In addition, she produced the course guide for all 13 units, and edited all 13 units.

Andrea (Andi) White, Ph.D., is a postdoctoral research associate at the University of California, Berkeley. As a graduate student at the University of New Hampshire she was a teaching assistant for marine ecology, honors biology, economic botany, and a lab coordinator for plant biology. Her current research interests focus on algal stress physiology and biochemistry, and the generation of environmentally friendly, alternative fuel sources from green algae. Andi developed two case studies: Evolution of Tungara Frog Mating Calls and Plant Genetic Modification. She also authored learning activities for the Evolution and Phylogenetics, Microbial Diversity, Genetics of Development, Human Evolution, Neurobiology, and Biodiversity units.

Norman A. Johnson, Ph.D., is an adjunct research assistant professor at the University of Massachusetts at Amherst. His research has focused on speciation and several other areas of evolutionary genetics. In addition to the University of Massachusetts, Norman has also taught at the University of Chicago and the University of Texas at Arlington. Norman contributed to the learning activities for the Evolution and

Credits, cont'd.

Phylogenetics, Microbial Diversity, Genetics of Development, Human Evolution, Neurobiology, and Biodiversity units. Norman also served as the style editor for all 13 online text chapters, and is the author of the Evolution and Phylogenetics, Genetics of Development, Human Evolution, Neurobiology, and Biodiversity chapters.

Producer

Oregon Public Broadcasting (OPB) is a highly experienced producer of educational content with expertise in both traditional and new media approaches to formal education, community outreach, and television production.

OPB has produced many series for Annenberg/CPB, including *Unseen Life on Earth: An Introduction to Microbiology*; *A World of Art: Works in Progress*, a series on contemporary artists; *American Passages: A Literary Survey*, a multimedia series for college students; and *Artifacts & Fiction*, a professional development workshop series for teachers on interdisciplinary approaches to American literature. OPB has also been the co-producer for video series and digital materials to accompany several McGraw-Hill textbook publications. OPB has a long history of producing Web sites, teachers' guides, and other curriculum materials to accompany educational and PBS broadcast series. Working in close concert with national advisory boards, OPB's staff has produced curriculum materials in the humanities and sciences for a variety of grade levels and teacher professional development.

OPB is also a major producer of PBS Primetime documentary series, and has created programming for *Nova*, *Frontline*, and other programs as well as numerous specials and limited series.

Research Staff

Rediscovering Biology would not be possible without the hard work of the research and production staff at Oregon Public Broadcasting. The research staff provided critical support for video producers, authors, and activity developers.

Cindy Lefton has a bachelor's degree in zoology and a master's degree in mass communication with an emphasis on science writing and editing. She has served as the editor of a medical news magazine, and has edited several medical textbooks and journal articles. Her interests in science and nature have lead to volunteer service as an education coordinator for a wildlife rehabilitation facility, a zoo guide, and a science fair coordinator.

Liza Nicoll earned a bachelor's degree in biology and a bachelor's degree in health science at the University of Massachusetts at Amherst. Since completing work on *Rediscovering Biology* she has continued to work in television production, researching for a world history documentary series.

Stephani Sutherland earned a doctorate in neuroscience from the Vollum Institute at Oregon Health & Science University, where she coordinated an outreach program in public junior high and high schools called Kids Interested in Discovering Science (KIDS). Since leaving the research laboratory in 2001, she has worked as a science news reporter for the Los Angeles Times and traveled around the world. She now works for the Journal of Neuroscience and writes freelance science news for various journals. Stephani was also a coauthor for the Neurobiology chapter of the online text.

Interviewees

We are grateful to so many of these people who were willing to find time for this project. The following people provided valuable information to the project through interviews.

Genomics

David Altshuler, M.D., Ph.D.; James Carrington, Ph.D.; Jonathan Eisen, Ph.D.; and Eric Lander, Ph.D.

Proteins and Proteomics

Hamid Bolouri, Ph.D.; Ned David, Ph.D.; Stanley Fields, Ph.D.; Hunter Fraser; Aaron Hirsh; and Leroy Hood, Ph.D.

Evolution and Phylogenetics

Phillip Gingerich, Ph.D.; Timothy Read, Ph.D.; and Carl Woese, Ph.D.

Microbial Diversity

Anne Camper, Ph.D.; Bill Costerton, Ph.D.; Dan Kotansky, Ph.D.; Anna-Louise Reysenbach, Ph.D.; Frank F. Roberto, Ph.D.; Phil Stewart, Ph.D.; and Paul Sturman.

Emerging Infectious Diseases

Capt. Daniel Carucci, M.D., Ph.D.; Rita Colwell, Ph.D.; Laurie Garrett; Stuart B. Levy, M.D.; Judith M. Martin, M.D.; and Lukas K. Tamm, Ph.D.

HIV and AIDS

Edward Berger, Ph.D.; Laurie Garrett; Jay Levy, M.D.; Rob Roy MacGregor, M.D.; Erik Vonmuller; and David Weiner, Ph.D.

Credits, cont'd.

Genetics of Development

Judith Eisen, Ph.D.; Markus Grompe, M.D.; John Incardona, Ph.D.; Nipam Patel, Ph.D.; and John Postlethwait, Ph.D.

Cell Biology and Cancer

Elizabeth Blackburn, Ph.D.; Brian Druker, M.D.; Leland Hartwell, Ph.D.; Mary-Claire King, Ph.D.; and Robert Weinberg, Ph.D.

Human Evolution

Kari Stefansson, M.D.; Ian Tattersall, Ph.D.; Ajit Varki, M.D.; and Christopher Wills, Ph.D.

Neurobiology

Wolfhard Almers, Ph.D.; Fred Gage, Ph.D.; Richard Huganir, Ph.D.; and John Williams, Ph.D.

Biology of Sex and Gender

Holly Ingraham, Ph.D.; David Page, M.D.; and Eric Vilain, M.D., Ph.D.

Biodiversity

James Miller, Ph.D.; Richard Ostfeld, Ph.D.; Peter H. Raven, Ph.D.; Eleanor Sterling, Ph.D.; and G. David Tilman, Ph.D.

Genetically Modified Organisms

Leon Corzine; David L. Dornbos, Jr., Ph.D.; Rebecca J. Goldberg, Ph.D.; Marion Nestle, Ph.D., MPH; Thomas E. Newberry; and Gary H. Toenniessen, Ph.D.

Additional Acknowledgements

We would like to thank the following people at Oregon Public Broadcasting in addition to the researchers who made this project possible.

Executive Producer Meighan Maloney; Production Manager Doug Brazil; Production Media Manager Catherine Stimac; Production Assignment Manager Joshua Wolfe; Web Developer John Kin; Assistant Production Manager Mary Hager; Database Administrator Heather Chambers; and Copyeditor Jennifer Ingraham.

The *Rediscovering Biology* video series was produced by Oregon Public Broadcasting's Educational Media Production Department. The creative team consisted of the following:

Executives in Charge of Production David Davis and Jack Galmiche; Executive Producer Meighan Maloney; Producer/Writers Melissa Gerr, Nadine Jesling, Amanda

Lowthian, and Eric Slade; Writer Andrew Holtz; Series Host Lew Frederick; Academic Director Alan Dickman; Production Assignment Manager Joshua Wolfe; Production Manager Doug Brazil; Production Media Manager Catherine Stimac; Researchers Cindy Lefton, Liza Nicoll, and Stephani Sutherland; Director of Production Services Milt Ritter; Manager of Production Scheduling Bill Dubey; Director of Engineering Information Dave Fulton; Assistant Director Sean Hutchinson; Assistant Production Manager Mary Hager; Pre-Production Coordinator Thea Bergeron; Videographers Art Adams, Karel Bauer, S.O.C., David Dennison, Paul Jacobson, Lisa Suinn Kallem, Jim Langley, Michael McNamara, Corky Miller, Ben Nieves, John Patzer, Todd Sonflieth, Dave Spangler, and Wally Szczubialk; Editors Tom Babich, Bruce Barrow, Sarah Marcus, Chris Nolan, John Patzer, and Kate Schoningher; Field Audio Michael A. Bidese, Chad Birmingham, Darren Brower, Kevin Brown, Chris Callus, Francis X. Coakley, Tony D'Annunzio, Thom Dentler, Jay Farrington, Dave Foreman, Thomas Forliti, Gerry Formicola, G. John Garrett, Joel Groeblichhoff, Cindy Hogan, Chip Lake, Randy Layton, Gordon Masters, Casey Quinlan, C.A.S., Todd Schmidt, Brandt Sennhenn, Mike Tyrey, Ted Ver Valen, Bill Ward, and Matt Yeasley; Creative Director Tim Bergmann; Production Artists Dora Papay, Corrina Reff, and Jefferson P. Vowell; 3-D Animations Hot Pepper Studios, Animation Dynamics, Inc., and Kevin Washington; Rights Assistant Morgan Currie; Theme Music Cal Scott; Production Intern Larry Johnson; Production Art Interns Soumalay Douangmala, Kim Harshberger, and Kevin Jaquette; Production Assistants Michael Aaris, David Banyan, Emily Chapman, Mike Forest, Kenyatta Gomez, Madeleine Pappas, Michelle Pridemore, Anastasia Savko, Alex Selkowitz, and Jonathan Zintel.

The *Rediscovering Biology* Web Site was produced by the following creative team: Oregon Public Broadcasting Web Developer/Producer John Kin; Web Assistant Ryan Servatius; Database Administrator Heather Chambers; Project Coordination, Flash Interactive Development and Project Design AMAZING! Online Marketing, LLC in association with Moshofsky/Plant Creative Services and Bergmann Graphics.

For Annenberg/CPB

Michele McLeod, Project Officer
Libby Riker, Editor