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
secondary structure
tertiary structure
quaternary structure
active site/ligand binding site
alpha-helix
beta sheet (or beta pleated sheet)
turn-between beta sheets
random coiled region

Discussion Questions

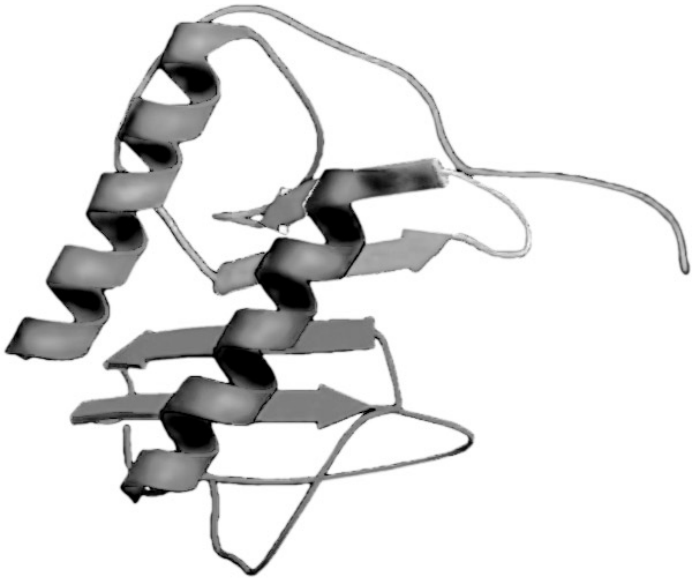
1. Which of the diagrams is best suited for showing secondary, tertiary, and quaternary structure?

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

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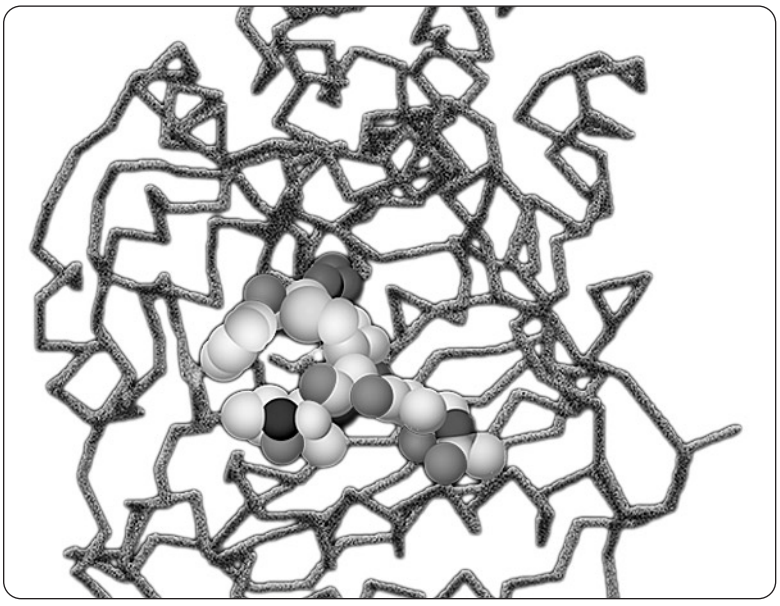
2. penicillin-binding protein with the drug cephalosporin in the middle



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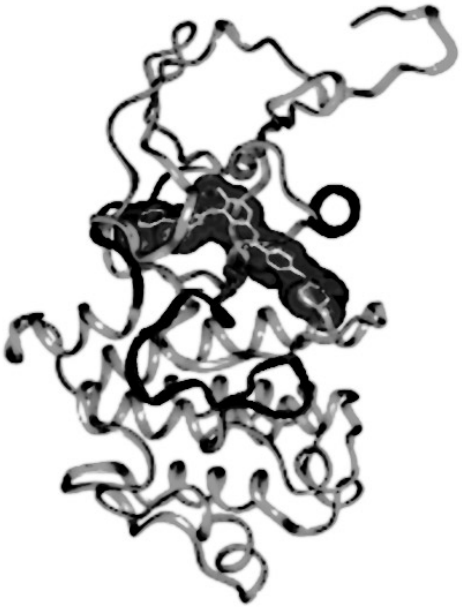
3. Abl kinase with the drug Gleevec in the middle



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

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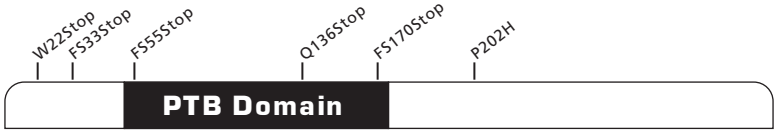
4. LDL receptor adaptor with PTB (phosphotyrosine binding domain) and amino acid changes caused by several inherited mutations (e.g., P202H means proline number 202 is changed to histidine).



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

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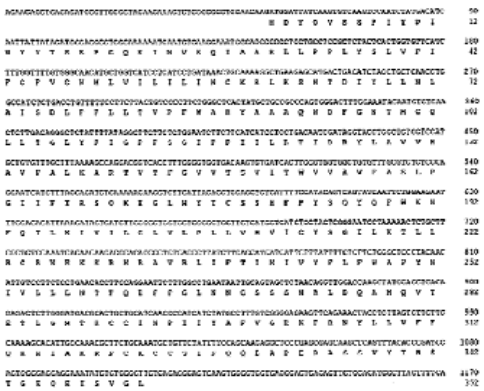
5. CCR5 cytokine receptor, DNA, and amino acid sequence



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

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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.

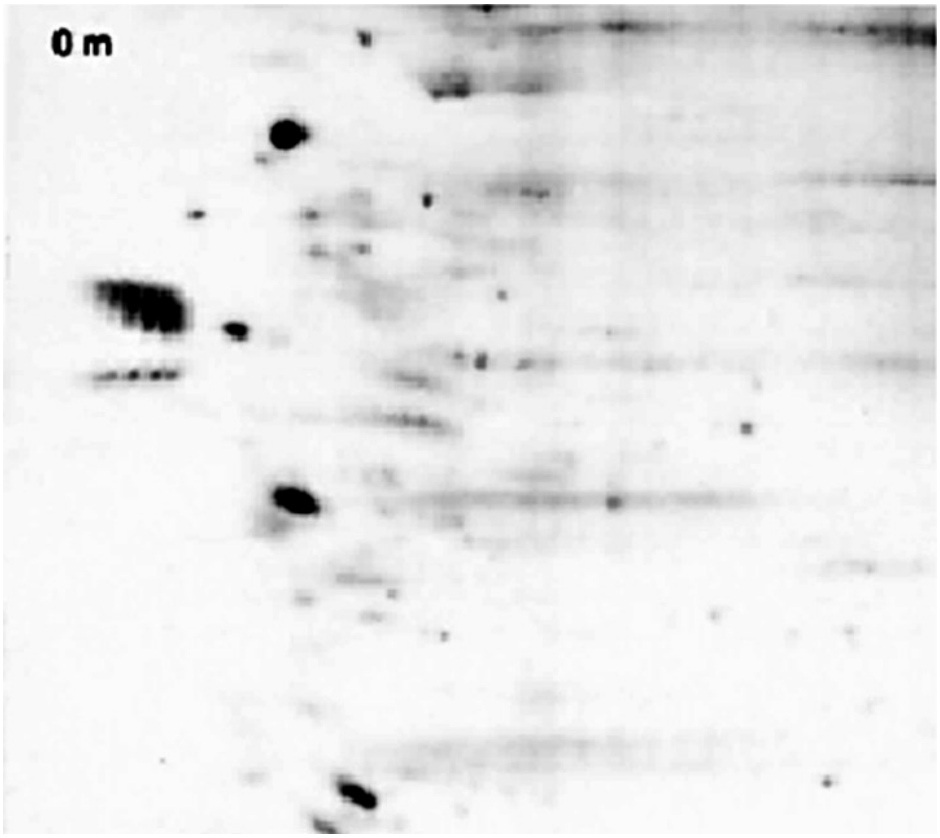
First, view the transparency of the 0 minute 2D gel. Then overlay the transparency of the next time point, which is 3 minutes after the ligand is added, directly onto the 0 minute transparency. Then overlay the next time point and the next, comparing changes to the proteomic profile each time. As a group,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

Materials

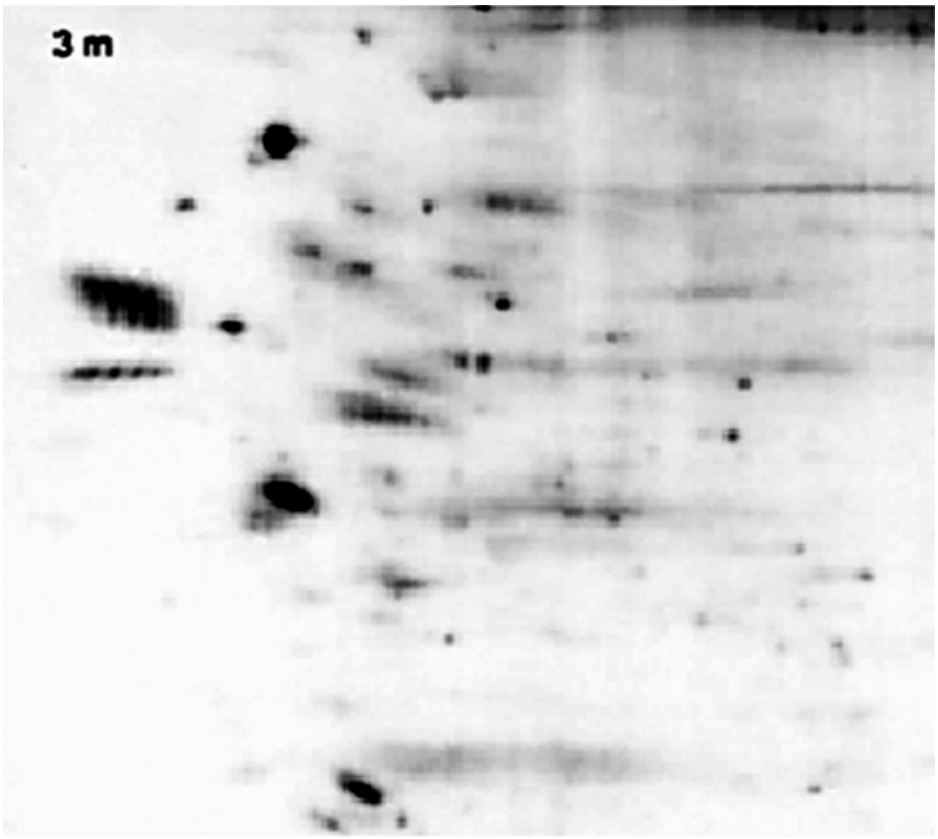
- Transparencies of 2D Gels, each on a separate transparency (master copies provided)
- Pens to write on transparency
- One copy of the Discussion Questions per person (master copy provided)
- Tips and Suggested Answers

2D Gels

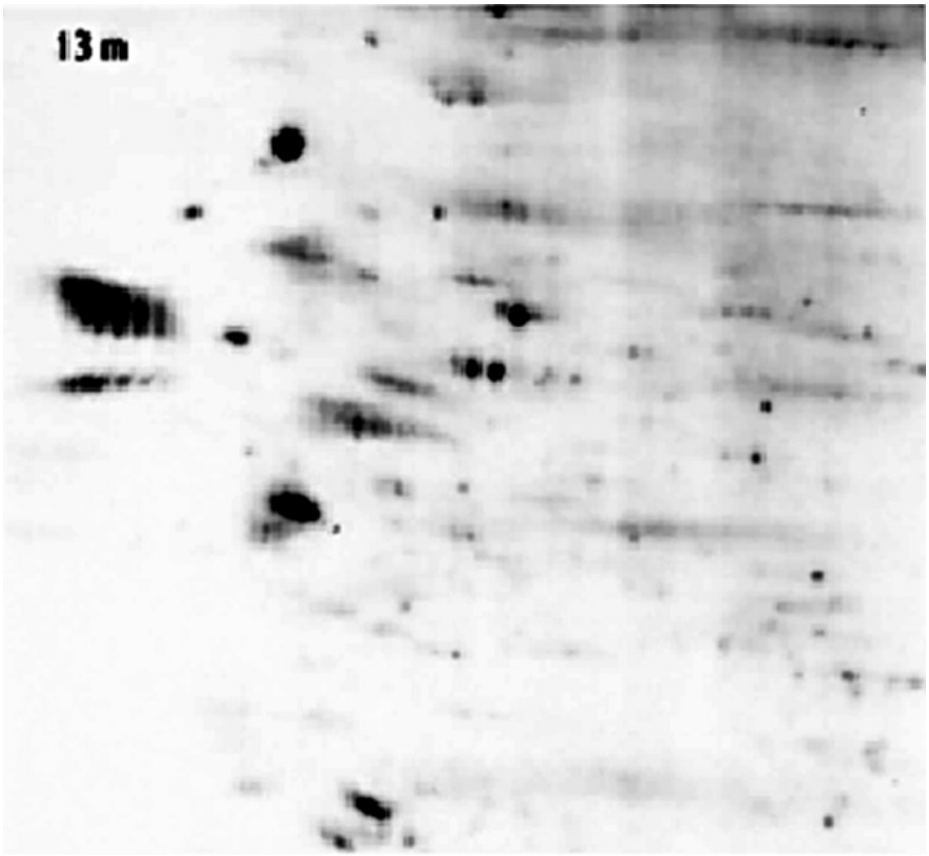
(Copy each onto a separate transparency.)



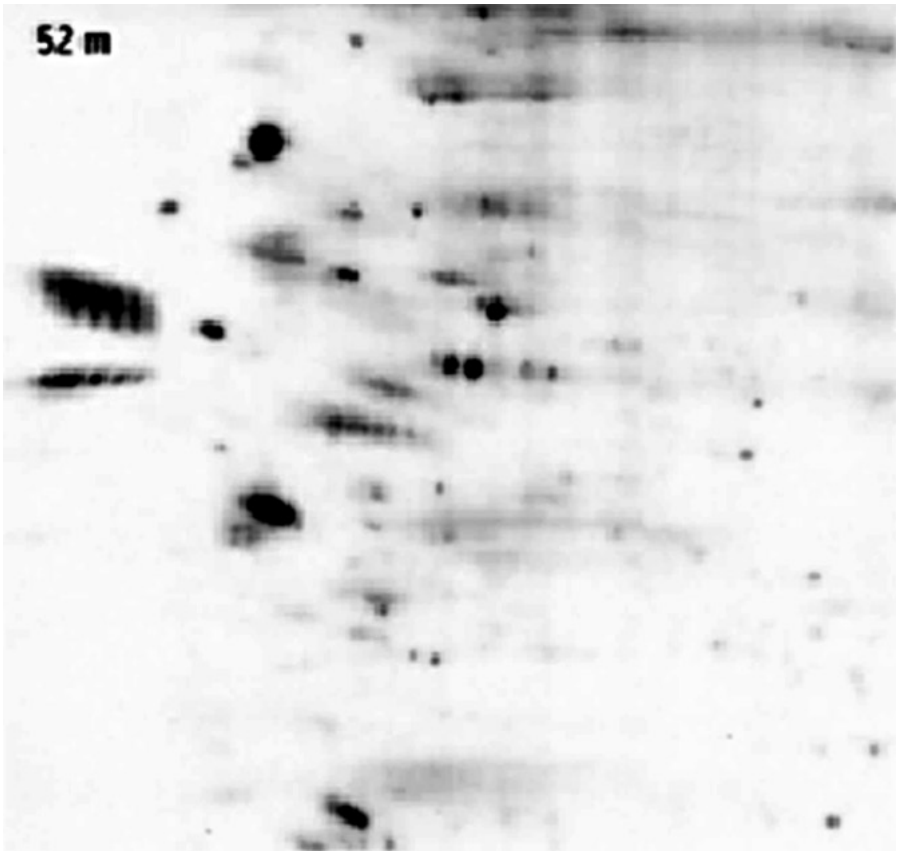
Source: http://www-lecb.ncifcrf.gov/phosphoDB/



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?

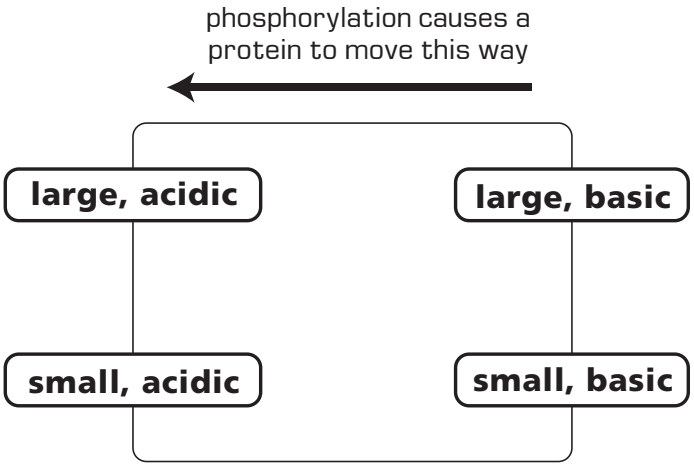
3. The first dimension was separated by isoelectric focusing point, or pI; this is the pH at which the protein has a net neutral charge. For example, this gel might be oriented so that the low-pH end of the isoelectric focusing gradient is to the left, and the high-pH end is to the right. The second 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.

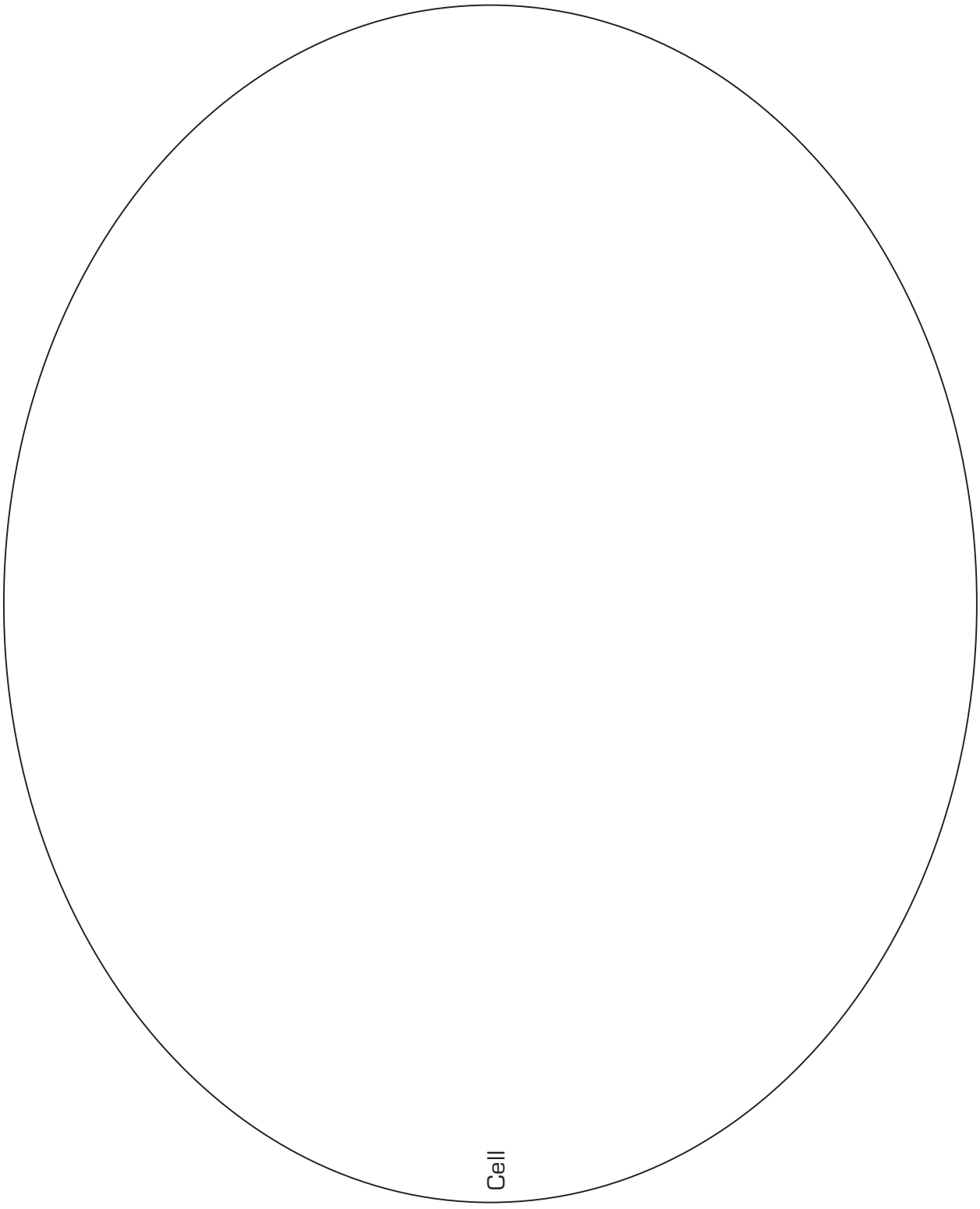
Work in teams of four. Each team will take one set of the Yeast Two-Hybrid Diagram Parts and Instructions. Take a few minutes to put the diagram parts together with the "positive control" parts and then with the "negative control" parts, according to the instructions.

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



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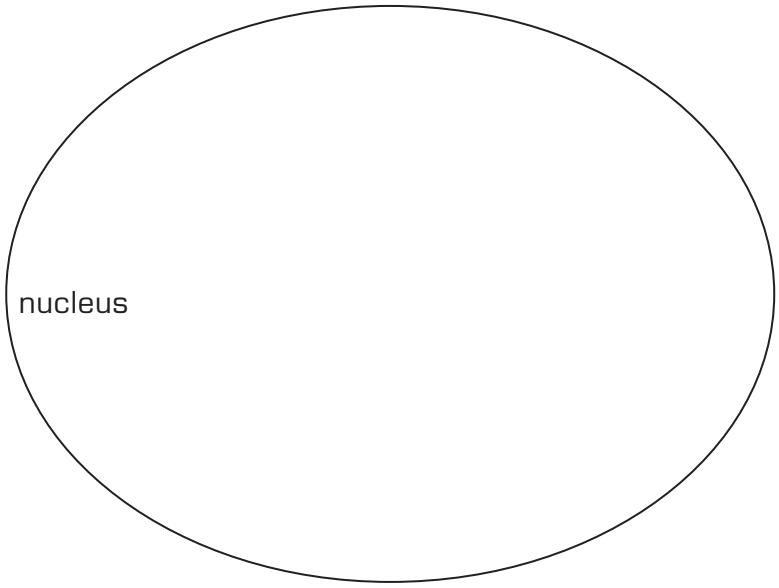

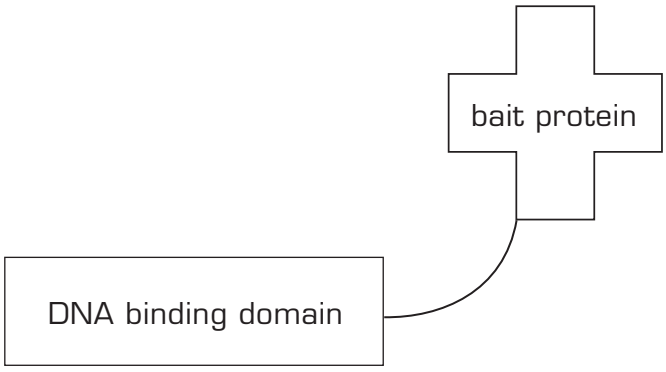

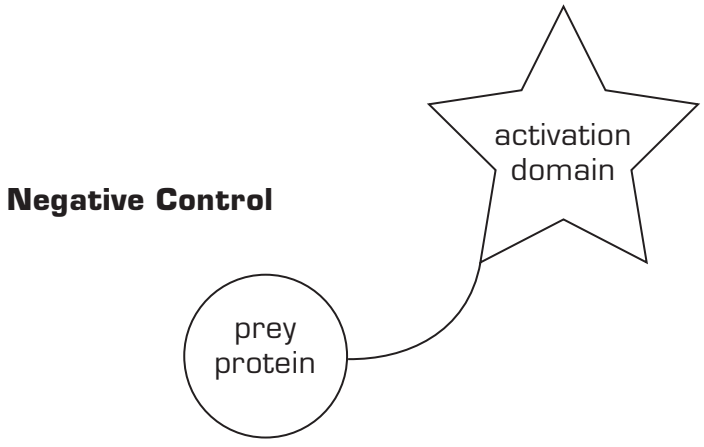

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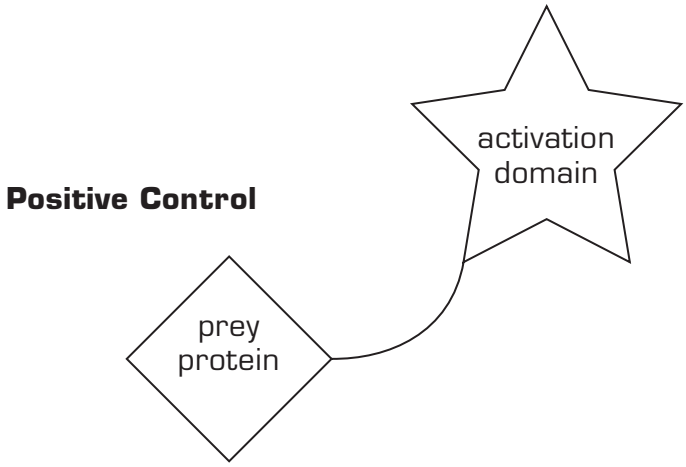
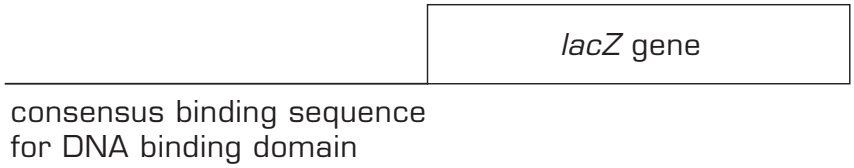
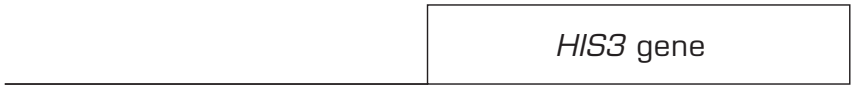




consensus binding sequence
for DNA binding domain



consensus binding sequence
for DNA binding domain

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 ß-galactosidase (ß-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 ß-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 ß-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 ß-galactosidase to detect, will this cell grow on a medium that lacks histidine? If we test with the ß-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 ß-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 ß-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