“What Did You Say?”
How Cells Talk to Each Other

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Overview

This unit is designed to give students a concrete understanding of cell communication, using immunology as the source of illustrative examples. Topics covered will include innate and adaptive immune tactics, as well as how the various adaptive immune cells communicate with each other. This unit will introduce students to a common laboratory technique called a “pull down” assay that is used to determine if proteins interact with each other. The intention is for this unit to be used in an AP Biology course to teach the concepts of cellular communication and the basics of immune function.

Science Background

Teachers will need to cover cell communication topics to include cell to cell recognition, cell signaling, signal reception, transduction, and cellular response as a result of the signal transduction pathway. In addition, teachers will need to cover immunology basics including distinguishing self from non-self, characteristics of the innate immune system, characteristics of the adaptive immune system, and functions of T and B cells. A pull down assay is a technique that allows the isolation of a specific protein from a complex mixture, and also can pinpoint whether two proteins interact with each other. It uses antigen-antibody interactions in a manner similar to an ELISA assay. However, in this assay, an antibody (or other protein) is bound to beads in a column and either a complex mixture or a sample of pure protein is run through the column. If there is an interaction between the “bait” on the beads and the “prey” in the solution, it is possible to detect that relationship on an SDS-PAGE gel. Applications of such an assay in this context could include defining interactions between host and pathogen proteins.

Learning Objectives

As a result of this unit, students should be able to:

- Describe the ligand-receptor interaction and explain how such interactions lead to a signal transduction pathway
- Compare and contrast G-protein-linked receptors, tyrosine-kinase receptors, and ligand-gated ion channels
- Describe the three main stages of cell-signaling (reception, transduction, response)
- Describe how signal information is transduced into cellular responses depending on the type of ligand (steroid or non-steroid)
- Explain how the same signal can elicit multiple cellular responses
- Distinguish between innate and adaptive immune responses

- Distinguish between antigens and antibodies
- Define the roles of B cells and T cells
- Explain the process of antigen presentation
- Distinguish between the primary and secondary immune responses
- Distinguish between the various types of B cells and T cells
- Compare the functions of class I MHC and class II MHC molecules
- Distinguish between humoral and cell-mediated immunity
- Describe how a pull-down assay works and how it might be utilized in a laboratory situation

Standard Correlations (College Board)

Essential knowledge 2.D.3

Biological systems are affected by disruptions to their dynamic homeostasis.

- Disruptions at the molecular and cellular levels affect the health of the organism.

  LO 2.28 - The student is able to use representations or models to analyze quantitatively and qualitatively the effects of disruptions to dynamic homeostasis in biological systems.

Essential knowledge 2.D.4

Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis.

a) Plants, invertebrates and vertebrates have multiple, nonspecific immune responses. Vertebrate immune systems have nonspecific defense mechanisms against pathogens.

b) Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis.

   The mammalian immune system includes two types of specific responses: cell mediated and humoral.

   - In the cell-mediated response, cytotoxic T cells, a type of lymphocytic white blood cell, “target” intracellular pathogens when antigens are displayed on the outside of the cells.
   - In the humoral response, B cells, a type of lymphocytic white blood cell, produce antibodies against specific antigens.
   - Antigens are recognized by antibodies to the antigen.
   - Antibodies are proteins produced by B cells, and each antibody is specific to

a particular antigen. A second exposure to an antigen results in a more rapid and enhanced immune response.

LO 2.29 - The student can create representations and models to describe immune responses.

LO 2.30 - The student can create representations or models to describe nonspecific immune defenses in plants and animals.

LO 2.43 - The student is able to connect the concept of cell communication to the functioning of the immune system.

Essential knowledge 3.B.2

A variety of intercellular and intracellular signal transmissions mediate gene expression.

a) Signal transmission within and between cells mediates gene expression.
b) Signal transmission within and between cells mediates cell function.

LO 3.22 - The student is able to explain how signal pathways mediate gene expression, including how this process can affect protein production.

Materials and Equipment

Pull Down Assay supply list (covers up to 20 lab groups, 3-4 students each)

1. Egg albumin
   Carolina Biologicals, #84-2251, 500g, $15.50
2. Tris Buffered Saline packs
   Thermo Scientific (Fisher), #28379, 10 packs, $78.00
3. Pierce Strong Anion Exchange Columns
   Thermo Scientific (Fisher), #90010, 24 spin columns, $146.00
4. Anti-chicken Ovalbumin polyclonal antibody
   BioRad, #0220-1682, 500µL, $168.00
5. Invitrogen Novex 4-20% Tris-Glycine protein gels
   Thermo Fisher, #XV04200PK20, 20 gels, $169.00
6. Protein Standard, BLUeye Prestained Protein Ladder
   Amazon, PM 007-0500, $109.00

7. Protein gel loading dye, 2X
   Amazon, P-18100-5.0, $38.99
8. Equipment for protein electrophoresis is also needed for this lab

*School districts that have a purchasing contract with Fisher Science may get a discount when ordering from Thermo Fisher. You don't need a separate account to order from Thermo if your district has an account with any of the Fisher companies.

*BioRad offers an educator (EDU) discount and will apply this discount even if the catalog does not show it as an option on that product. The antibody lists for considerably more than $168.00, but they gladly offered me the discount when I contacted them.

*Aside from the antibody, the rest of these lab components will last for more than 1 school year.

Adaptive Immunity Activity

1. Hershey's kisses
2. Sixlets
3. Large puffballs (or cotton balls)
4. Googly eyes (to put on the puffballs)
5. Smaller sparkly puffballs
6. Poker chips
7. Pipe cleaners cut into 1" pieces
8. Ziploc sandwich bags

For each group of students (3-4 per group), put the following into a bag:

- 4 Hershey kisses
- 1 sleeve of Sixlets
- 2 large puffballs with eyes
- 2 blue poker chips
- 4 sparkly small puffballs
- 2 red poker chips
- Roughly 10-12 pipe cleaner pieces

Curriculum Outline

(based on 50 min. class periods)

Day #1-2: cell signaling lecture/introductory information and activities

- TED Talk: How Bacteria Talk
  https://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate
- Bozeman: Evolution of Cell Communication
  http://www.bozemanscience.com/036-evolutinary-significance-of-cell-communication
- Questions for Evolution of Cell Communication
  https://drive.google.com/open?id=0BzhPdt_laG2bOGotZGQzTmx6TDg

Day #3-4: immune system lecture/introductory activities

- HHMI: Cells of the Immune System
  https://www.hhmi.org/biointeractive/cells-of-the-immune-system
  (use with the Questions for HHMI Primer)
- Questions for HHMI Immune Primer
  https://drive.google.com/open?id=1e3vvX_SI_iPJIzDNyiAGMVvAfKutLMk7
- Bozeman: Cell Communication
  http://www.bozemanscience.com/037-cell-communication
- Adaptive Immunity activity
  https://drive.google.com/open?id=1_VTmIxTqfciJ5vmhK7vrv1oft3P83A_W

Day 5-7: Pull Down Assay

Teacher Information for Pull Down Assay

Lab Breakdown

- Day 1 - students will run protein solutions through the columns
- Day 2 - students will load samples from Day 1 and run on gels
- Day 3 - students will visualize gels and analyze results

Equipment

- Micropipettes and tips
- Protein electrophoresis rig(s) and power source
- Light box for gel visualization
- Hot bath/block
- Table top centrifuge

Materials per Lab Group (3-4 students)

- Ice bucket
- Micro pipettes and tips
- 1% albumin solution (bait)
- 1 mg/ml anti-albumin solution (prey)
- Tris buffer solution
- Pierce strong anion exchange column
- 6 empty microtubes
- 4-20% tris glycine protein gel
- 1x tris glycine SDS protein buffer
- Loading dye
- Protein reference ladder
- Float rack
- Sharpie

Solution Preparation

1. **Tris buffer solution -** comes in pre-measured packets. Mix one packet with 500 ml of deionized water.
2. **1X SDS buffer -** used for running protein gels. Depending on how many rigs you will be running at one time, you may need up to two liters. Comes in 10X concentration, so combine 100 ml of 10X SDS with 900 ml of deionized water to make one liter of 1X SDS buffer.
3. **1% albumin solution (bait) -** combine 0.2 g of albumin with 20 ml of deionized water. Keep refrigerated.

4. **1 mg/ml anti-albumin solution (prey) -** the anti-albumin comes from Bio Rad as 5 mg/ml. Add 80 µL of anti-albumin antibody to 320 µL Tris buffer to make your working stock. Keep frozen until use.

General Lab Prep Suggestions

1. Aliquot solutions so that you have enough for each lab group to have their own supply of materials.
2. Make sure all materials for day one are kept on ice.
3. If you are crunched for time, it is probably best for you to put the gels in the protein rigs yourself rather than teaching the kids to do it.
4. Students should have a working knowledge of how to pipette, balance a centrifuge, and load a gel.

Analysis Question Answers

1. Explain what is meant by the terms bait protein and prey protein.
   A bait protein is a protein used to attract another protein. It is added to the column first in a pull down assay. A prey protein is a protein that should bind to the bait protein when it is added to the column.
2. Explain the interactions between the beads, the bait protein, and the prey protein.
   There is a substance coated on the beads in the column that will bind the bait protein to the beads. The prey protein will then bind to the bait protein as it is run through the column, thereby "capturing" it.
3. What purpose did it serve to take samples of the buffer, the albumin, and the antibody ahead of time?
   The pre-lab samples will serve as a positive control for comparison on the gel.
4. Add a sketch or photo of your protein gel into your lab notebook.
   Results will vary
5. Based on your protein gel results, can you say whether or not the bait and prey proteins in this lab interacted? Explain your answer.
   Results will vary, but basically, if bands for both the bait and prey proteins show up together in the sample, or a band appears that is larger than both the bait and prey proteins separately in the sample that was taken when they were eluted off of the column, then that means the proteins interacted together.
6. Why is a protein ladder used?
   Since the size of each of the bands on the ladder is known, it can be used to estimate the size of unknown protein bands.

7. Can you think of a situation in immunology where a pull-down assay would be useful?
   Answers will vary, but situations such as looking for an antibody to a specific antigen in patient blood/tissue samples would work.

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Student Lab Protocol

Fishing for Protein Interactions

Scientists frequently have a need to see if proteins “talk to” or interact with each other. A relatively simple test to see if this is the case is called a “pull down assay”. This technique uses “bait” proteins and “prey” proteins, a column that has a membrane (or resin beads) in it that will attract the “bait” protein and ends with running a protein gel to see if the two proteins leave the column separately (no interaction) or together (interaction). In a nutshell, it goes something like this:

- A buffer is run through the bead column
- Your “bait” protein is run through the column next. The membrane (or beads) is designed to be attractive to the bait protein so it will stick to them.
- The “prey” protein is run through next, and the column is incubated for a short time to give the two proteins time to interact (if they will).
- A solution is run through the column lastly to remove the bait protein (and hopefully the prey too) from the membrane.
- A sample of what comes off the column is taken each time something is run through. These samples are then run out on a protein gel. If the bait and prey proteins show up in the same sample, then they interacted and came off the column together. If they show up in separate samples only, then there was no interaction between them.

So let’s use some “bait” and go fishing!

Materials

- Ice bucket
- Micro pipettes and tips
- 1% albumin solution (bait)
- 1 mg/ml anti-albumin solution (prey)
- Tris buffer solution
- Pierce strong anion exchange column
- 6 microtubes
- 4-20% tris glycine protein gel
- 1x tris glycine SDS protein buffer
- Loading dye
- Protein reference ladder
- Protein electrophoresis equipment
- Centrifuge
- 90℃ hot bath/block
- Float rack
- Sharpie

Procedure - Day 1

1. **Protein solutions and buffer must be kept on ice!!**
2. Label 3 empty microtubes: albumin, anti-albumin, and buffer
3. Add 10μL of albumin to the albumin tube, then 10μL of the appropriate samples to each of the other tubes
4. Add 400μL of cold Tris buffer to the column and spin at 2000 rpm for 5 minutes.
5. Discard flow through
6. Add 100μL of 1% albumin solution (bait protein) to column and incubate on ice for 10 minutes
7. Spin column at 2000 rpm for 5 minutes
8. Discard flow through
9. Add 150μL of cold Tris buffer to the tube and spin down at 2000 rpm, **take a 10μL sample of the flow through and place it in a clean microtube labeled bait,** and then discard the flow through
10. Repeat step 9 two more times (without taking a sample)
11. Add 100μL of anti-albumin solution (prey protein) to column and incubate on ice for 10 minutes
12. Spin column at 2000 rpm for 5 minutes
13. Discard flow through
14. Add 150μL of cold Tris buffer to the tube and spin down at 2000 rpm, **take a 10μL sample of the flow through and place it in a clean microtube labeled prey,** and then discard the flow through

15. Repeat step 14 two more times (without taking a sample)
16. Add 400μL of elution buffer to the column and incubate at room temperature for 10 minutes
17. Spin tube down at 2000 rpm for 5 minutes
18. Take a 10μL sample of the flow through and place it in the last clean microtube labeled elution
19. Discard the column and flow through
20. Place samples in the refrigerator until the next class

Procedure - Day 2

1. Retrieve your samples from the refrigerator
2. Place samples in the hot bath for 10 minutes
3. Set up your electrophoresis equipment (if the teacher hasn't done so)
4. Load 10μL of protein ladder in lane 1
5. Load 15μL of each sample in a separate lane in the gel in the following order: albumin, anti-albumin, buffer, bait, prey, and elution
6. Run gel at 125V (35mA) for 1-1.5 hours
7. Place gel in Coomassie stain for 1 hour
8. Remove stain and cover gel with DI water overnight

Procedure - Day 3

1. Place your gel on the light box
2. Either take a photograph or make a quick sketch in your lab notebook of the band pattern on your gel
3. Discard your gel as directed by your teacher
4. Answer the analysis questions

Analysis

1. Explain what is meant by the terms bait protein and prey protein.
2. Explain the interactions between the membrane, the bait protein, and the prey protein.
3. What purpose did it serve to take samples of the buffer, the albumin, and the antibody ahead of time?
4. Add a sketch or photo of your protein gel into your lab notebook.
5. Based on your protein gel results, can you say whether or not the bait and prey proteins in this lab interacted? Explain your answer.
6. Why is a protein ladder used?
7. Can you think of a situation in immunology where a pull-down assay would be useful?