Determining the Bacteriorhodopsin Network
Group-ANSWER KEY

Your goal as a group is to use the data provided to determine how the production of bacteriorhodopsin is regulated in halobacterium.

Each person in your group studied a different type of data that gave part of an answer to our question, but none of these were sufficient to answer the question alone. Now you will put the different types of information together to form a network and answer our question of how halo respond to light.

Putting together your data in the form of a network

1. Briefly share with the members of your group the type of data you analyzed and the conclusions you made based on the data. After sharing, each person will follow the steps below to build and interpret the network regulating BR.
   *see individual answer keys for partial network (2-4 below)
2. Metabolic data: Copy the metabolic network onto the back of your introductory questions page.

3. Homology data: The edges in the metabolic network indicate enzymes that catalyze the reactions. Use the homology data to label the edges with their enzyme name.

4. Microarray data: Draw a circle as a node to represent the gene for bat. Draw arrows from bat to the enzymes that it influences. Indicate if the relationship between bat and the enzymes is positive (+) or negative (-).

Guiding question:
How do halobacterium cells control the amount of BR expressed in response to light? What is the gene and protein network that regulates the expression of BR?

Draw your completed network following the directions below: see next page

5. Draw light as a node in the network. If you hypothesize that light has a relationship with any of the other nodes in the network, draw an edge between light and any of these nodes. Indicate with an edge whether light has a positive (+) or negative (-) affect on any of the nodes.

6. Clarify your network diagram for it to make sense to another person.
   a. Edges: Does the arrow from GG-PP to phytoene mean the same thing as the arrow from bat to CrtB1? If the arrows have different meaning, identify them with different symbols. For example, you could use a dashed line for one type of edge and a solid line for another type of edge.
   b. Nodes: Is GG-PP the same type of node as bat? If the nodes have different meaning, identify them with different symbols. For example, you could use different shapes for different types of nodes.
   c. Make a key or legend to indicate what the different symbols in your network mean.

To review this with students, open the 'Purple Membrane Network' simulation. Use it to manipulate variables; OR revisit the ppt 'making lab connection to other data'.

_(students will likely have this rotated)

Analysis Questions

Use the bacteriorhodopsin network that you have drawn as a group to answer the following questions:

1. What effect should the amount of light (increase or decrease) have on the amount of bacteriorhodopsin? positive feedback (increase light = increased bR, decrease = decrease)

2. How does changing the expression of the enzymes in the metabolic pathway affect the amount of bacteriorhodopsin produced? crtB1, crtY, and brp are enzymes. They are all needed and are similar to light in affecting bR.

3. Which protein controls the expression of the enzymes in the metabolic pathway? bat

4. If bat is over-expressed, how is the network affected? How does this affect the amount of bacteriorhodopsin produced? Explain your reasoning. increased production of crtB1, crtY, brp and bop leads to increased bR (purple). Bat increases transcription of genes associated with the formation of bacteriorhodopsin (handout calls it a Transcription Regulator). When light is present, it is able to bind to the UAS region more efficiently (due to a shape change), increasing transcription of these genes. Due to the cascade of reactions, increasing bop and the enzymes leads to an increase in BR production.

5. Explain how the network you put together could allow halo to change the amount of BR in response to the amount of light. (Make sure you include metabolites and enzymes in your explanation.) students should point out that only in the presence of light is bat increased which is required to increase activation of other genes producing proteins needed to make bR

6. Your final constructed network and its explanation is a model of what we think is happening based on the data. In other words, this is a hypothesis for how the halobacterium regulates BR. How could you test your hypothesis? (include predictions) change any particular enzyme (turn off or on) and a change in the color of the Halo should occur.

7. Look back to your answer sheets from your original expert groups (homology, microarray, metabolism). Now that you have a full picture of the network discuss how your previous answers are supported. Data from the 2 other groups fit in and fill out the network

Big ideas

8. Why does the cell regulate the network and change the amount of BR in response to light? Why not make BR all the time? It is expensive (in terms of ATP and materials) to continuously make all of these proteins.

9. The cell regulates an entire network of genes, enzymes, other proteins, and metabolites. Why not have bop directly respond to light instead of using the relationship with bat? All the other proteins would be produced since bop cuts out at the end...wasteful.

10. Why did we need to analyze multiple types of data from different expert groups when figuring out how the network functions? Without understanding metabolism, which genes are active at any given time and which genes are related, we could not arrive at a complete network.

11. We integrated multiple types of data into a network. How did depicting the information in a network format allow us to make better sense of the different types of information in answering the question of how halobacterium responds to light? clearly shows relationships and allows for prediction.