

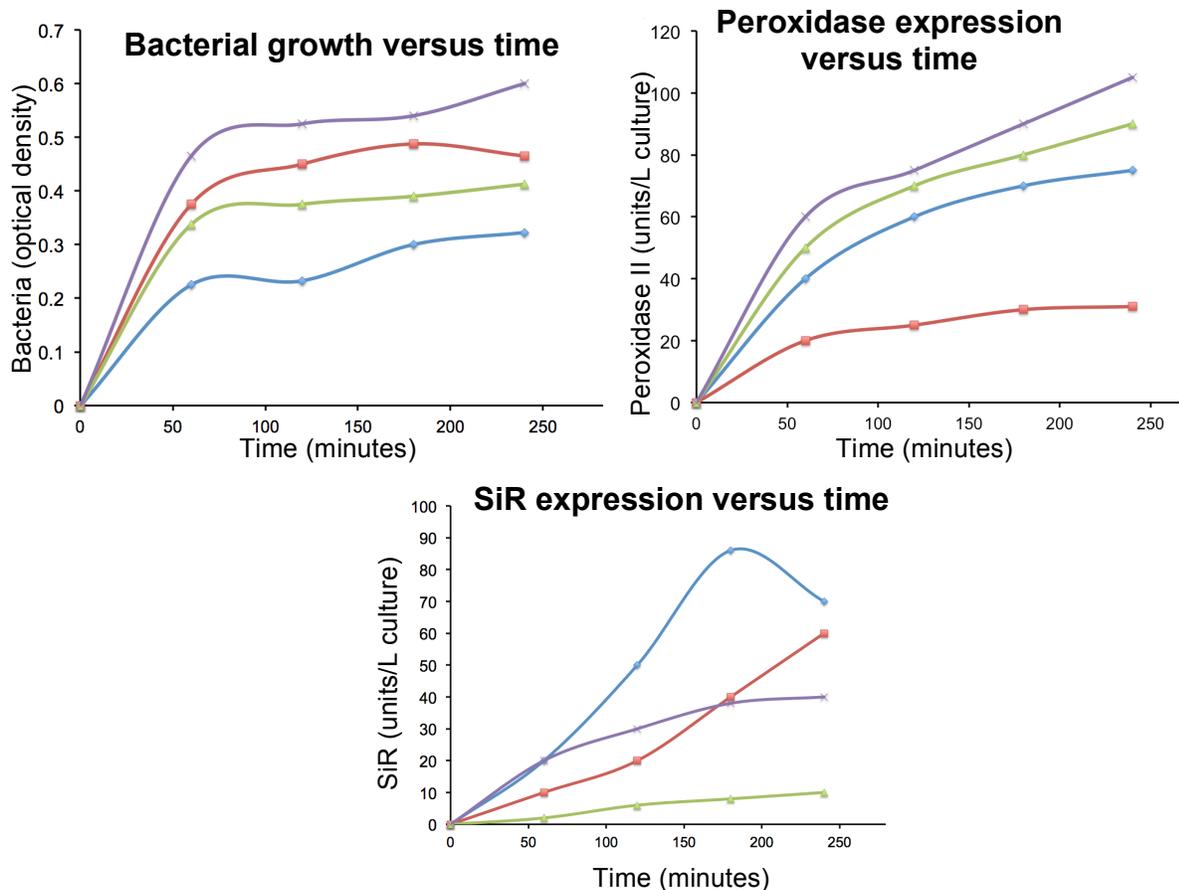
Argumentation activity: Gene expression regulation in bacteria

You work in a lab that is interested in understanding the enzymatic basis for sulfur metabolism in the environment. Your goal is to overexpress and purify a protein called sulfite reductase (SiR), a bacterial enzyme that is important for sulfur metabolism. You make the following observations while trying to purify SiR from bacteria:

Using a technique called denaturing gel electrophoresis to analyze the purity of your protein sample, you see two proteins at 60 and 64 kDa molecular weight that are the two polypeptides that comprise SiR. In addition to those proteins, you see a third, unexpected protein that is about 200 kDa molecular weight.

Next, you use a technique called N-terminal sequencing to determine the sequence of the amino acids at the beginning of the 200 kDa protein so you can identify the unknown contaminant. The contaminant is an enzyme called peroxidase, an enzyme that helps to remove toxic compounds from the bacterium by turning them into nontoxic molecules.

To reduce the amount of the contaminant, you add glucose, lactose, or glycerol to the growth media. You measure cell growth, peroxidase production, and SiR production over time (Graph 1-3). **What is the best condition to maximize SiR expression but limit peroxidase expression? What hypothesis about peroxidase expression could explain these trends?**



You will work through this worksheet to develop an argument that answers this question and generate a hypothesis to explain the result. You will present your answer as a poster on the poster board provided with the following layout:

Question: What is the best condition to maximize SiR expression but limit peroxidase II expression?

Claim:

Evidence:

Rationale:

Hypothesis:

Once everyone finishes, we will have 15 minutes of “round robin” discussion to compare and contrast each group’s ideas. Then, you will return to your original group and make any revisions you wish based on your discussions.

Finally, you will write on your own a two-paragraph argument to persuade another scientist that your claim is valid (1st paragraph) and your hypothesis explicatory, testable, and reasonable (2nd paragraph). Turn this in on your way out; if you need more time you may bring it to class on Tuesday.

Remember to do the following for all arguments, written or discussed:

- 1) State the claim you are trying to support (The sky is blue ...)
- 2) Include evidence and rationale (...because as white light passes through the atmosphere the blue wavelengths are scattered to earth.)
- 3) Organize your paper in a way that enhances readability (topic sentence, supporting details, conclusion)
- 4) Use good grammar, punctuation, and spelling.

(This is a good guide for answering essay questions on exams, too!)

To answer this question, you need to think about how the environment of a cell determines the activities inside the cell. The general mechanism through which this occurs is conserved from bacteria (single cell prokaryotes without nuclei) to eukaryotes (single to multi cell organisms with a nucleus and other membrane-enclosed organelles).

A molecule in the environment (in the media, in the case of a single celled organism or in the extracellular matrix, in the case of a multicelled organism) finds its way to the surface of the cell's plasma membrane. There are several different ways it can then interact with the inside of the cell. First, it could bind to a protein receptor on the outside of a cell that then causes a change inside the cell. Second, it could be internalized into the cell by diffusion directly through the membrane or indirectly via a protein channel. Third, it could be actively internalized into the cell through a protein receptor.

Q1: Which of these is happening in this case?

Once the presence of the molecule registers inside the cell, the molecule is able to transduce the signal. In the simplest case, the signaling molecule binds to a protein that directly affects the DNA upstream of a region of the genome that codes for an important protein. For example, in the case of the *lac* operon found in *Escherichia coli* (a natural component of the human gut flora), the small molecule lactose (a sugar found in milk) controls expression of the genes whose protein products are responsible for metabolizing lactose (turning the lactose into energy).

Q2: What protein product(s) is (are) being is being made in this case?

Without lactose, the bacterium does not need to metabolize lactose. In this case, a protein molecule called a repressor is bound to a part of DNA that blocks access to the protein coding part of the gene (and repressing its transcription into RNA). In the presence of lactose (say, if you drink milk), there would be both lactose and allolactose (an isomer of lactose) present in your gut. The molecules enter the bacterium through active transport and then find their way through diffusion to the repressor protein that is bound to the DNA. When allolactose binds to the repressor, it falls off the DNA and then the protein coding part of the gene is available to be transcribed into RNA (and then translated into protein).

This is one example of a general mechanism repeated in different variations under myriad conditions whereby a small molecule (allolactose) interacts with other factors to regulate the molecules that are responsible for transcription. Cellular stresses, for example a shift in pH, temperature, salt concentration, metabolite concentration, or the presence of UV-light, can all induce changes in bacterial expression patterns.

Q3: What might be stressing the bacterium in the case of SiR expression?

Q4: How might the bacterium be responding?