Collect ideas from the students and write them on the board. Then ask the following question:

- Given the answers to the previous questions, what types of control might exist in cells to deal with the longer-term versus shorter-term responses?

Doing this exercise before assigning Activity 19.1 serves two purposes:

- It teaches students the types of questions they should be asking themselves as they study.
- It helps students understand how to put what they learn into a logical context that makes learning (and remembering what they learn) easier.

Activity 18.4

Many students are unaware of how complex the cell cycle controls are. It is often counterintuitive to many that some of the controls actually function to disable or kill cells with damaged control systems.

Answers

Activity 18.1 How is gene expression controlled in bacteria?

Fill in the chart to organize what we know about the *lac* and *trp* operons.

Operon:	*lac*		*trp*	
Is the metabolic pathway anabolic or catabolic?	Catabolic Hydrolyzes or breaks down lactose(a disaccharide) into glucose and galactose (two six-carbon surgars)		Anabolic Synthesizes tryptophan from precursors	
What regulatory genes are associated with the operon and what functions does each serve?	Genes: *lacI* promotor, CRP binding site operator	Functions: *lacI* produces an active repressor protein that binds to the operator in the absence of lactose. The promotor is the site where RNA polymerase binds to the DNA. Binding of RNA polymerase is enhanced when cAMP interacts with	Genes: *trpR* promoter, operator	Functions: *trpR* produces an inactive repressor protein that binds to the operator only when complexed with excess trypto-phan. RNA poly-merase binds at the promotor site and transcribes the genes for making trypto-phan as long as trypto-phan is not is excess.

(*Continued on next page*)

Operon:		*lac*		*trp*
		CRP and the complex bind at the CRP site on the promotor region. As a result, the enzymes for lactose digestion are produced only when needed and only when glucose is not present.		
What structural genes are included in each operon and what does each produce?	Genes: *lacZ* *lacy* *lacA*	Products: *LacZ* codes for the β-galactosidase enzyme, which digest lactose into glucose and galactose. *LacY* codes for a permease to allow lactose to enter the cell. *LacA* codes for transacetylase. Its function is unclear.	Genes: *tryA* *tryB* *tryC* *tryD* *tryE*	Products: These five genes code for the five enzymes required to convert a precursor molecule to tryptophan (one of the amino acids required for protein synthesis)
Is the operon inducible or repressible?	The *lac* operon is inducible.		The *trp* operon is repressible.	
Is the repressor protein produced in active or inactive from?	The repressor protein is produced in its active form. (The active form binds to the operator and stops transcription of the structural genes)		The repressor protein is produced in its inactive form.	
The repressor protein becomes active when it interacts with:	The repressor is active until it complexes with lactose (or allolactose). Then it becomes inactive. lactose = inducer		The repressor is inactive until it complexes with excess tryptophan in the cell. Tryptophan changes the configuration of the repressor, and it is capable to binding to the operator and stopping transcription of the enzymes that synthesize or make tryptophan.	

tryptophan = corepressor.

Activity 18.2 Modeling the *lac* and *trp* operon systems: How can gene expression be controlled in prokaryotes?

Using the information in Activity 18.1 and in Chapter 18 of *Biology*, 8th edition, construct a model or diagram of the normal operation of both the *lac* and *trp* operon systems.

In your models or diagrams, be sure to include these considerations:

regulatory and structural genes

inducible versus repressible control

anabolic versus catabolic enzyme activity

negative versus positive controls

Use your model to answer the questions.

1. Under what circumstances would the *lac* operon be "on" versus "off"? The *trp* operon?

   The *lac* operon would be off when there is no lactose in the cell. The *lac* operon would be on when lactose is present and there is little or no glucose in the cell. However, the *lac* operon would be off (or operating at very low levels) even when lactose is present if sufficient glucose is simultaneously present.

   The *trp* operon would be off when excess tryptophan is readily available to the cell. It would be on at all other times.

2. How are the *lac* and *trp* operons similar (in structure, function, or both)?

   Both have regulatory genes that produce repressor proteins that can interact with the operator and shut down transcription of the structural genes.

3. What are the key differences between the *lac* and *trp* operons?

   The *lac* operon is inducible; the presence of lactose induces production of the enzymes needed for lactose digestion. The *trp* operon is repressible; it is ordinarily on, producing tryptophan, which is needed for protein production by the cells. It is turned off or repressed only when an excess of tryptophan is available to the cell.

   The *lac* operon is controlled by both a regulatory protein, which interacts with the operator and blocks RNA polymerase action, and a CRP site. RNA polymerase does not attach effectively to the operator unless CRP (complexed with cAMP) is attached at the CRP site. Once attached, it enhances the interaction of RNA polymerase with the promoter region. cAMP levels in cells tend to be low when glucose is present. As a result, even if lactose is present at relatively high levels, this second control keeps production of the enzymes for digesting lactose at very low levels if glucose is also present in the cells.

4. What advantages are gained by having genes organized into operons?

   As they are needed, both systems are set up to simultaneously turn on (or off) all of the genes required in a metabolic pathway. This is much more efficient than having each gene under independent control.

5. Strain X of *E. coli* contains a mutated *lac* regulatory gene on its bacterial genome. As a result, the gene produces a nonfunctional *lac* repressor protein. You add a plasmid (an extra circular piece of double-stranded DNA) to these cells. The plasmid contains a normal regulatory gene and a normal *lac* operon.

   Build a model or diagram of what one of these modified *E. coli* cells would look like. Then answer the questions and use your model or diagram to explain your answers.

   a. Before the addition of the plasmid, would the *E. coli* strain X cells be able to produce the enzymes for lactose digestion? Explain.

      Yes. The *lacI* gene ordinarily produces an active repressor protein that inhibits production of the genes for lactose digestion. In this case, this gene is mutated so that it cannot produce the repressor protein.

   b. After the addition of the plasmid, would the plasmid's *lac* operon produce the enzymes for lactose digestion constitutively (all the time) or only when lactose was the available sugar source? Explain.

      The plasmid contains a normal regulatory gene and a normal *lac* operon. As a result, the plasmid's *lac* operon should produce the genes for lactose digestion only when lactose was the available sugar source.

   c. After the addition of the plasmid, would the bacterial genome's *lac* operon produce the enzymes for lactose digestion constitutively or only when lactose was the available energy source? Explain.

      The regulatory gene on the plasmid could produce enough repressor protein molecules to affect both the plasmid operon and the bacterial chromosome's operon. So, after addition of the plasmid, the bacterial genome's lac operon would produce enzymes for lactose digestion only when lactose was the available energy source.

   d. If equal amounts of lactose and glucose were present in the cell, would the *lac* operon in the bacterial DNA be off or on? Would the *lac* operon on the introduced plasmid be off or on? Explain.

      In combination with cAMP, CAP (catabolic activator protein) is an activator of transcription for the *lac* operon. When glucose is present, cAMP levels in the cell tend to be low. As a result, cAMP does not interact with CAP and CAP is unable to bind to the DNA. Therefore, even if lactose is present, little *lac* mRNA will be synthesized from either the *E. coli* or plasmid DNA.