

S1. Researchers have identified mutations in the promoter region of the *lacI* gene that make it more difficult for the *lac* operon to be induced. These are called *lacI<sup>Q</sup>* mutants, because a greater Quantity of *lac* repressor protein is made. Explain why an increased transcription of the *lacI* gene makes it more difficult to induce the *lac* operon.

**Answer:** An increase in the amount of *lac* repressor protein makes it easier to repress the *lac* operon. When the cells become exposed to lactose, allolactose levels slowly rise. Some of the allolactose binds to the *lac* repressor protein and causes it to be released from the operator. If there are many more *lac* repressor proteins found within the cell, it takes more allolactose to ensure that there are no unoccupied repressor proteins that can repress the operon.

S2. Explain how the pausing of the ribosome in the presence or absence of tryptophan affects the formation of a terminator stem-loop.

**Answer:** The key issue is the location where the ribosome stalls. In the absence of tryptophan, it stalls over the *trp* codons in the *trpL* mRNA. Stalling at this site shields region 1 in the attenuator region. Since region 1 is unavailable to hydrogen bond with region 2, region 2 hydrogen bonds with region 3. Therefore, region 3 cannot form a terminator stem-loop with region 4. Alternatively, if there is sufficient tryptophan in the cell, the ribosome pauses over the stop codon in *trpL*. In this case, the ribosome shields region 2. Therefore, region 3 and 4 hydrogen bond with each other to form a terminator stem-loop. This abruptly halts the continued transcription of the *trp* operon.

S3. With regard to the key proteins that affect the choice between the lytic and lysogenic cycles, which one(s) may be degraded by cellular proteases?

**Answer:** After infection, the key protein affected by cellular proteases is cII. If protease levels are high, as under good growth conditions, cII is degraded. This promotes the lytic cycle. Under starvation conditions, the protease levels are low. This prevents the degradation of cII and thereby promotes the lysogenic cycle. After lysogeny has been established, the key protein affected by cellular proteases is the  $\lambda$  repressor. Agents such as UV light activate cellular proteases that digest the  $\lambda$  repressor. This permits induction of the lytic cycle.

S4. In bacteria, it is common for two or more structural genes to be arranged together in an operon. Discuss the arrangement of genetic sequences within an operon. What is the biological advantage of an operon organization?

**Answer:** An operon contains several different DNA sequences that play specific roles. For transcription to take place, an operon is flanked by a promoter to signal the beginning of transcription and a terminator to signal the end of transcription. In between these two sequences are found structural genes that encode different proteins. An operon has two or more structural genes. A key feature of an operon is that the expression of the structural genes occurs as a unit. When transcription takes place, a polycistronic mRNA is made that encodes all of the structural genes.

In order to control the ability of RNA polymerase to transcribe an operon, an additional DNA sequence, known as the operator site, is usually present. The base sequence within the operator site can serve as a binding site for genetic regulatory proteins such as activators or repressors. The advantage of an operon organization is that it allows a bacterium to coordinately regulate a group of genes whose encoded proteins have a common function. For example, an operon may contain a group of genes involved in lactose breakdown, or a group of genes involved in tryptophan synthesis, etc. The genes within an operon usually encode proteins within a common metabolic pathway or cellular function.

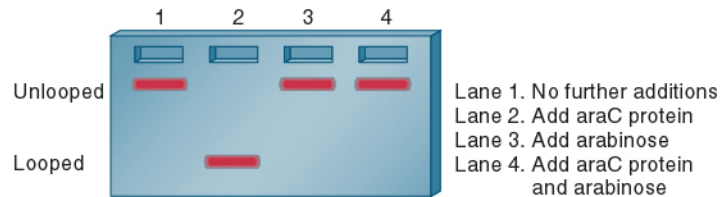
S5. The sequential use of two sugars by a bacterium is known as diauxic growth. It is a common phenomenon among many bacterial species. When glucose is one of the two sugars available, it is typical that the bacterium metabolizes glucose first, and then a second sugar after the glucose has been used up. Among *E. coli* and related species, diauxic growth is regulated by intracellular cAMP levels and the catabolite activator protein. Summarize the effects of glucose and lactose on the ability of the *lac* repressor and the cAMP-CAP complex to regulate the *lac* operon.

**Answer:** In the absence of lactose, the *lac* repressor has the dominating effect of shutting off the *lac* operon. Even when glucose is also absent and the cAMP-CAP complex is formed, the presence of the bound *lac* repressor prevents the expression of the *lac* operon. The effects of the cAMP-CAP complex are exerted only in the presence of lactose. When lactose is present and glucose is absent, the cAMP-CAP complex acts to enhance the rate of transcription. However, when both lactose and glucose are present, the inability of CAP to bind to the *lac* operon decreases the rate of transcription. The table shown here summarizes these effects.

### *lac* Operon Regulation

Sugar Present	Transcription of the <i>lac</i> Operon
None	The operon is turned off due to the dominating effect of the <i>lac</i> repressor protein.
Lactose	The operon is maximally turned on. The repressor protein is removed from the operator site and the cAMP-CAP complex is bound to the CAP site.
Glucose	The operon is turned off due to the dominating effect of the <i>lac</i> repressor protein.
Lactose and glucose	The expression of the <i>lac</i> operon is greatly decreased. The <i>lac</i> repressor is removed from the operator site and the catabolite activator protein is not bound to the CAP site. The absence of CAP at the CAP site makes it difficult for RNA polymerase to begin transcription. However, there is more transcription under these conditions than in the absence of lactose.

S6. The ability of DNA-binding proteins to promote a loop in DNA structure is an interesting phenomenon that is important in the structure and function of DNA. Besides the regulation of genes and operons, DNA looping is required in the compaction of DNA within the nucleoid of a bacterium and the nucleus of a eukaryotic cell (see chapter 10). In addition, DNA looping is frequently involved in the expression of eukaryotic genes (see chapter 15). In this solved problem, we will examine an experimental approach that made it possible for Robert Schleif and his colleagues to determine that the AraC protein causes a loop to form in the DNA. This work relied on the mobility of DNA in an acrylamide gel. A segment of DNA that contains a loop is more compact than the same DNA segment without a loop. Therefore, when these two alternative structures (looped versus unlooped) are run through a gel, the looped structure migrates more quickly to the bottom of the gel, since it can more easily penetrate the gel matrix. As a starting material, Schleif had a sample of DNA that contained a portion of the *ara* operon including both the *araI* and *araO<sub>2</sub>* sites. In the gel shown here, this segment of DNA was exposed to the following conditions before it was run on the gel:



Explain these results.

**Answer:** In lane 2, AraC was added and arabinose was not. As expected from the DNA looping hypothesis, a DNA loop was formed as evidenced from the faster mobility on the acrylamide gel. In lane 1, no AraC protein was added, so that no DNA loop was able to form. These results confirm the idea that it is the AraC protein that is causing the loop to form in the sample loaded into lane 2. In lane 3, only the sugar arabinose is present but not the AraC protein. Since the DNA remains unlooped, the sugar by itself has no effect. In the sample loaded into lane 4, AraC protein was added and arabinose was added as well. In this case, no DNA loop is observed. These results are consistent with the idea that arabinose binds to the AraC protein and breaks the DNA loop that is promoted by the AraC protein.