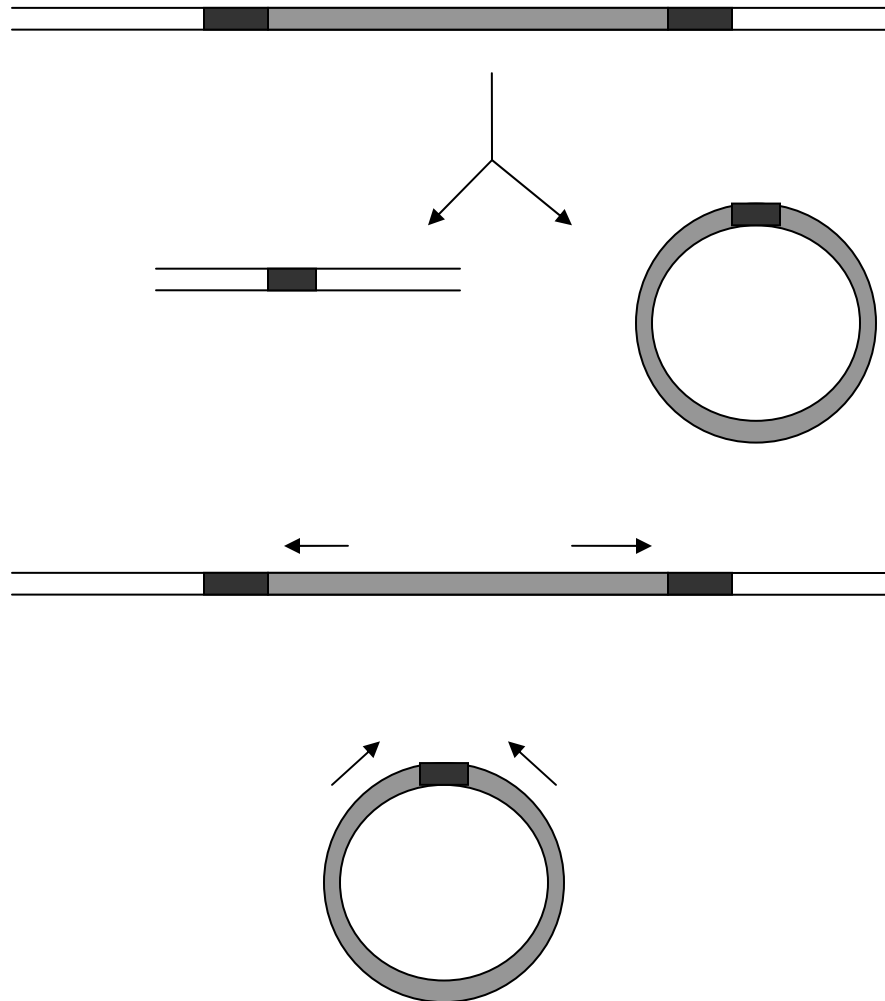


Discussion Handout 5/1/07 ANSWERS

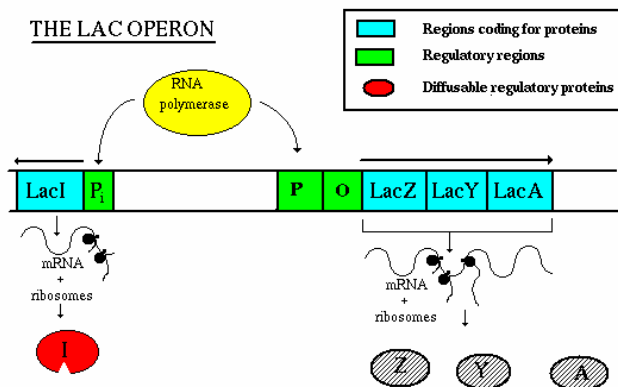
1. In a species of ciliated protist, a segment of gDNA is sometimes deleted. The deletion is a genetically programmed reaction associated with cellular mating. A researcher proposes that the DNA is deleted in a type of recombination called site specific recombination, with DNA on either end of the segment is joined together with the deleted DNA ending up as a circular DNA reaction product. Suggest how the researcher might use PCR to detect the presence of the circular form of the deleted DNA in an extract of protist.



2. *E. coli* cells are placed in a growth medium containing lactose. Indicate how the following circumstances would affect the expression of the lactose operon (increase/decrease/no change).

- Addition of high levels of glucose
- A Lac repressor mutation that prevents dissociation of Lac repressor from the operator
- A mutation that inactivates β -galactosidase
- A mutation that inactivates galactoside permease
- A mutation that prevents binding of CRP to its binding site near the *lac* promoter

Ans: (a) decrease; (b) decrease; (c) decrease (this enzyme converts lactose into allolactose, the inducer); (d) decrease (because external lactose would not enter the cell, and allolactose would not be present to induce the operon); (e) decrease

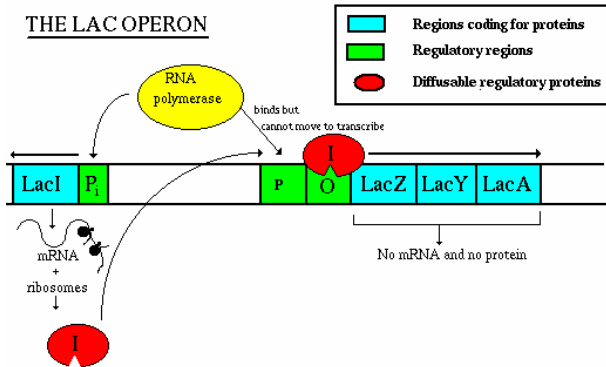


• **Three functional genes:**

- *lacZ* : B-galactosidase. hydrolyzes the bond between the two sugars, glucose and galactose
- *lacY* produces permease: spans cell membrane and brings lactose into the cell from the outside environment.
- *lacA* produces B-galactosidase transacetylase. The function of this enzyme is still not known.

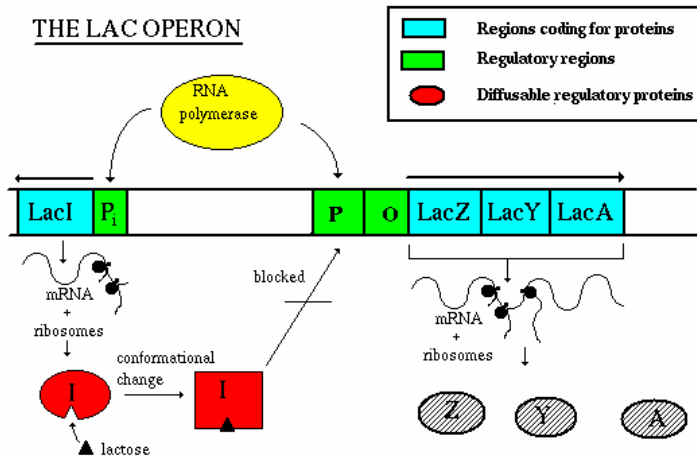
- **Promoter (P)** - aids in RNA polymerase binding
- **Operator (O)** - "on/off" switch - binding site for the repressor protein
- **Repressor (*lacI*) gene**
 - Repressor gene (*lacI*) - produces repressor protein w/ two binding sites, one for the operator and one for lactose
 - The repressor protein is under allosteric control - when not bound to lactose, the repressor protein can bind to the operator
 - When lactose is present, an isomer of lactose, allolactose, will also be present in small amounts. Allolactose binds to the allosteric site and changes the conformation of the repressor protein so that it is no longer capable of binding to the operator

If lactose is not present: the repressor gene produces repressor, which binds to the operator. This blocks the action of RNA polymerase, thereby preventing transcription.



If lactose is present:

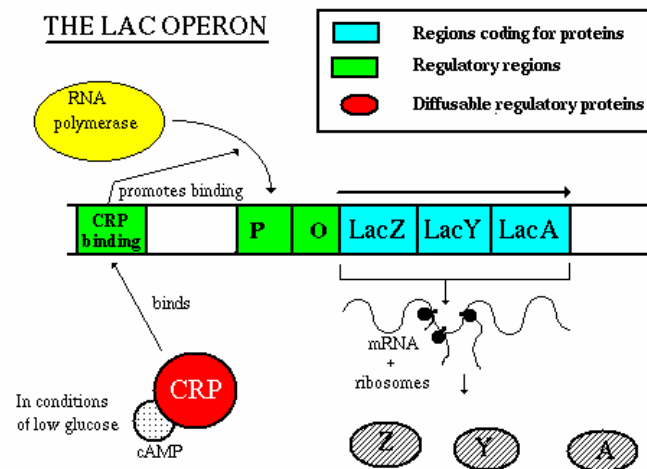
- the repressor gene produces repressor, which has a site for binding with allolactose.
- The allolactose/repressor compound is incapable of binding w/ the operator, so the RNA polymerase is uninhibited
- once the concentration of lactose decreases, the repressor-allolactose complex falls apart and transcription is again inhibited



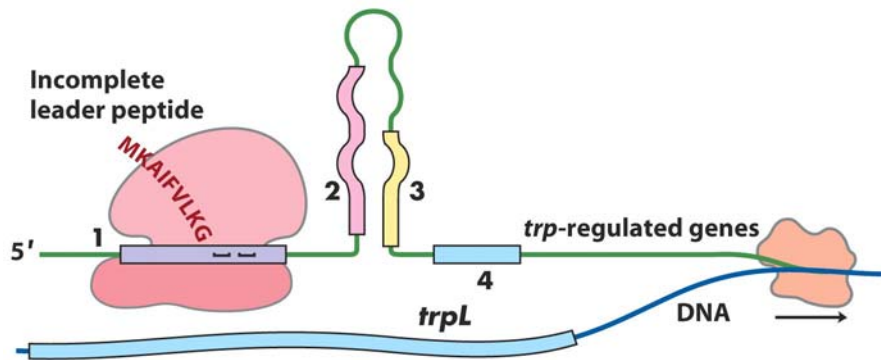
Glucose regulation:

It is not enough for lactose to be present to induce the *lac* operon

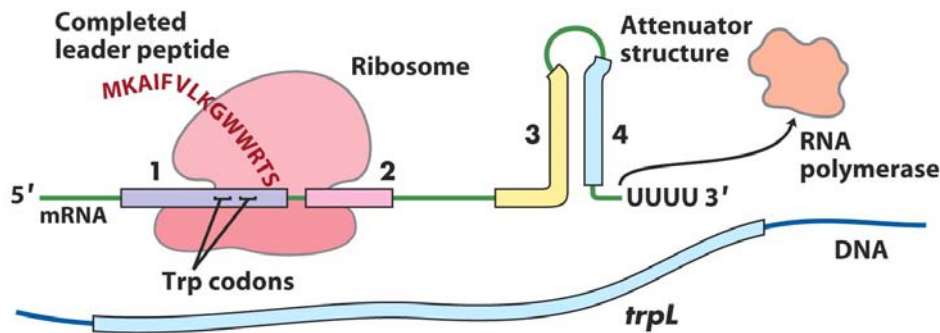
- Glucose is the sugar of choice of *E. coli* and if glucose is in supply, then the bacteria will preferentially break down glucose over lactose
- If glucose is present, the *lac* operon will be repressed - how does this happen you ask?
- RNA polymerase has a low affinity for the promoter of the *lac* operon unless helped by a regulatory protein - cAMP receptor protein (CRP)
- CRP only becomes activated if the concentration of cyclic AMP (cAMP) is high
- Glucose inhibits the formation of cAMP
 - If the concentration of glucose is high, the concentration of cAMP is low
 - If the concentration of glucose is low, the concentration of cAMP is high
- Therefore, if the concentrations of glucose and lactose are high, the concentration of cAMP will be low, CRP will not be activated, RNA polymerase will not be able to bind well to the promoter, and the operon will be operating at a very low level (i.e. almost off)
- However, if the concentrations of glucose is low and lactose is high, the concentration of cAMP will be high, CRP will be activated and bind to the DNA which will promote RNA polymerase binding and initiate transcription



3. What are the four major ways that prokaryotes control transcription?
 1. Alternate sigma factors that recognize specific promoter sequences
 2. DNA binding proteins that acts as either repressors or activators (lac operon)
 3. Coupling of transcription with translation which can sometimes allow RNA secondary structure to control transcription by premature termination of a leader transcript (Trp operon Fig 18-21)
 4. Invert DNA that contain promoters.



When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation, because sequence 3 is no longer available to form the attenuator structure with sequence 4. The 2:3 structure, unlike the 3:4 attenuator, does not prevent transcription.



When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like attenuator structure formed by sequences 3 and 4.