Solutions to Practice Problems for Molecular Biology, Session 5: Gene Regulation and the Lac Operon

Question 1

a) How does lactose (allolactose) promote transcription of LacZ?

1) Lactose binds to the polymerase and increases efficiency.
2) Lactose binds to a repressor protein, and alters its conformation to prevent it from binding to the DNA and interfering with the binding of RNA polymerase.
3) Lactose binds to an activator protein, which can then help the RNA polymerase bind to the promoter and begin transcription.
4) Lactose prevents premature termination of transcription by directly binding to and bending the DNA.

Solution: 2) Lactose binds to a repressor protein, and alters its conformation to prevent it from binding to the DNA and interfering with the binding of RNA polymerase.

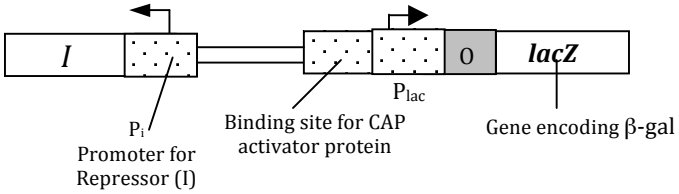
b) What molecule is used to signal low glucose levels to the Lac operon regulatory system?

1) Cyclic AMP
2) Calcium
3) Lactose
4) Pyruvate

Solution: 1) Cyclic AMP.

Question 2

You design a summer class where you recreate experiments studying the lac operon in *E. coli* (see schematic below). In your experiments, the activity of the enzyme b-galactosidase (β -gal) is measured by including X-gal and IPTG in the growth media. X-gal is a lactose analog that turns blue when metabolisize by b-gal, but it does not induce the lac operon. IPTG is an inducer of the lac operon but is not metabolized by b-gal.



a) Which of the following would you expect to bind to β-galactosidase? Circle all that apply.

Lactose (or allolactose) X-gal IPTG

b) Which of the following would you expect to bind to the lac repressor? Circle all that apply.

Lactose (or allolactose) X-gal IPTG

Question 2, continued

After mutagenesis you find 7 mutants that never turn blue as shown in the table below. *Note each mutant has a single loss-of-function mutation.

Cell Type	Media			
	+ glucose No lactose + X-gal	+ glucose + lactose + X-gal	No glucose No lactose + X-gal	No glucose + lactose + X-gal
Wild type	White colonies	White colonies	White colonies	Dark blue colonies
Mutants 1-7	White colonies	White colonies	White colonies	White colonies

c) A single loss-of-function mutation in which component or components (I, Pi, CAP binding site, Plac, O, lacZ) could produce the unidicible phenotype seen in these mutants? List all that apply.
CAP binding site, Plac, lacZ

You also find three mutants with the following phenotype. *Note that each mutant has a single loss-of-function mutation.

Cell Type	Media			
	+ glucose No lactose + X-ga	+ glucose + lactose + X-gal	No glucose No lactose + X-gal	No glucose + lactose + X-gal
Wild type	White colonies	White colonies	White colonies	Dark blue colonies
Mutants 8-10	White colonies	White colonies	Dark blue colonies	Dark blue colonies

d) A loss-of-function mutation in which component or components (I, Pi, CAP binding site, Plac, O, lacZ) could produce the constitutive phenotype seen in these mutants?
I, Pi, O

e) You introduce a piece of DNA into the constitutive mutant 8 bacterial cells that contains the following: LacI and its promoter, LacZ, LacA, and LacY and their promoter. (Introducing additional DNA is a common laboratory technique in bacteria, and all added genes and regulatory regions can be expected to act as if they were a part of the genome.) This does not rescue the mutant phenotype observed in mutant 8; that is, these bacteria are still constitutive. Does this additional information allow you to narrow your options as to possible causative mutations? Explain your answer.
Yes. Because this extra piece of DNA does not rescue the constitutive mutant phenotype we know the mutation in mutant 8 is in the O region. Mutations in I or P_i would be complemented "in trans" because having a single functioning repressor protein could regulate expression from the genomic lac operon and the inserted lac operon sequences. If the O region on the genomic lac operon is mutated such that it can not bind to the repressor protein, then the added DNA can not stop the transcription of the genes adjacent to the mutated O region.

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