

## Homework for Regulation of Gene Expression - I

Due Friday, September 22 before class

1. Review - what is the definition of an operon?

An operon is the genetic sequences expressed on the same RNA, being expressed from a common promoter. The cis-active regulatory sequences are sometimes included in the operon.

2. What is the difference between an inducer and a corepressor?

An inducer acts by binding to either an activator or repressor protein to stimulate gene expression, while a corepressor acts by binding to a repressor to decrease gene expression.

3. Which is easier to construct by random mutation - a constitutive *lac* operon or a constitutive *ara* operon? Why?

It is much easier to construct a constitutive *lac* operon because all you have to do is inactivate the LacI protein. To make a constitutive *ara* operon, you would have to make a point mutation that makes AraC assume the activator configuration in the absence of binding arabinose. These would be very rare mutations. (We all know from experience that it's easier to break something than improve up on it!)

4. Give an example of a *cis* active versus *trans* active constitutive *lac* mutation.

There are many possibilities here. Constitutive *cis* would be a mutation in the operator so that LacI cannot bind anymore. Constitutive *trans* would be a nonsense mutation that eliminates production of the LacI protein.

5. Why is it better to use IPTG to induce the *lac* operon as opposed to lactose, when you are attempting expression of a gene cloned behind a *lac* promoter.

The IPTG is not metabolized, in contrast to the lactose, which will be broken down and no longer available for induction.

6. If the *lac* and *trp* operons are each negatively regulated, how is it that adding lactose to a culture induces the *lac* operon, but adding Trp represses the *trp* operon?

Essentially, lactose is an inducer acting to prevent the binding of the repressor to the operator, while Trp is a corepressor that enables the repressor to bind. (I just wanted you to appreciate the dichotomy in action here.)

7. To what other form of genetic regulation is attenuation of the *trp* operon most similar?

OK, I meant genetic event, or something like that. The 3-4 stem loop of the *trp* mRNA is essentially a factor (Rho)-independent terminator.

8. What is the P1 form of AraC and how does it affect expression from the pBAD promoter (both in terms of up or down regulation and the mechanism of this regulation)?

The P1 form is AraC that does not contain arabinose. It binds to the operator region in a way that prevents the P2 (arabinose-containing) form from binding and acting as an activator. This is supposedly not true repression. It binds to the *araO2* and *araI1* sequences forming a loop or bend that makes the promoter inaccessible.

9. How is the transport of glucose directly linked to the regulation of the catabolite repression system?

When the glucose transport-phosphorylation enzyme ( $\text{IIA}^{\text{glc}}$ ) has transported and phosphorylated a glucose molecule, it is temporarily unphosphorylated. Hence, the unphosphorylated enzyme is a signal for the presence of glucose. However, if there is no glucose, the protein will phosphorylate (signaling lack of high energy carbon) and then can enable adenylate cyclase to be active, make cAMP, which then acts with CAP/CRP to activate (usually) gene expression.

10. How is the heat shock alternative sigma factor, sigma-32 encoded by the *rpoH* gene, post-transcriptionally regulated? How does this regulation tie into the function of the heat shock regulon itself?

The DnaK protein binds to denatured proteins and signals them for degradation. If there are no denatured proteins in the cell (no heat shock) DnaK is available to bind to RpoH and cause its post-translational degradation. If DnaK becomes tied up with other denatured proteins (heat shock situation), it does not cause degradation of RpoH, hence RpoH levels rise and the alternative sigma factor stimulates the heat shock response.

Extra credit - What is the phenotype of a *lac<sup>F</sup>* mutation at the level of gene expression?

This is a promoter-up mutation for the *lacI* gene.

Another extra credit - As we pondered in class on Friday, how is the LexA protein related to the action of RecA on the CI repressor of lambda?

LexA is a repressor protein for the SOS repair system genes. When bound by single stranded (damaged) DNA, RecA can bind to LexA, causing it to cleave itself and become useless. So if there is no damaged DNA, RecA leaves LexA alone to repress the SOS response. However, if damaged DNA is present, RecA causes LexA self-cleavage and enables the induction of SOS genes. If there is damaged DNA, RecA will bind to CI causing it to cleave itself (note that in class I might have said that RecA acts as the protease, but the book disagrees - and none of you pointed this out!), thereby shutting down repression of lytic phase (maintenance of lysogeny). So LexA does not directly interact with lambda regulation of lysogeny or lytic infection. It is acted upon by RecA in a manner that is similar to CI.