Problem Set 5: Gene Regulation

Be sure to try these problems before you look at the answers!
(if you don't allow yourself that chance of searching, wondering, and reaching, you rob yourself of the moment when the best learning happens)

1. What is a common feature of Transcriptional Regulatory Proteins?

All have the ability of DNA sequence-specific binding; all have a DNA binding domain (or can interact with a protein that has one).

2. Two genes located on different chromosomes may encode very different proteins, and yet they may be coordinately regulated at the level of transcription. Explain what this statement means, and how this can be accomplished.

It means that the two genes can be turned on/off at the same time/in response to the same signal/in the same cell/etc. This is accomplished by the presence of a similar DNA sequence near the gene that serves as a recognition sequence for a transcriptional regulatory protein

3. We discussed HIF1 as an example of a Transcriptional Regulator.
   a. What is the signal that activates/inactivates this Regulator?

Normal levels of O_2 "inactivate" HIF1 because it gets hydroxylated and then degraded; low levels of O_2 (hypoxia) "activate" the protein by preventing its hydroxylation and degradation (either answer is correct).

   b. How can this regulator control 100 different genes?

Each of the 100 genes has a specific DNA sequence near it that serves as a binding site for HIF1 (sequence is called HRE).

   c. What do these 100 genes have in common?

The presence of the HRE nearby; the pattern or regulation (ie. their transcription is increased when cellular O_2 levels are low)

4. Look back at the β globin and α globin gene clusters (lecture 10-23 or 26); why do you think mutations in the α globin gene are more easily tolerated than β globin mutations?

There are two genes on each chromosome that can encode α globin; all in all each person has 4 copies of α globin compared to only two copies of β globin, so there are more "back up copies" of the α globin gene than β globin.

5. At right is a Figure from the Faustino et al '02 paper that we discussed in class. In this paper we heard about an individual who is homozygous for a mutation in the β globin gene called "β^o-Black 1,393-bp deletion." At left is shown the normal gene (transcript is indicated as a rectangle and exons are black) and the DNA that is deleted in this mutant chromosome is that which is between the dotted lines. This individual inherited the same mutant version of the gene from his two parents, so it was present in all of his cells.

   a. Consider his mother who carried one regular β globin gene and one mutant β globin gene (she was heterozygous). Because of the regular copy, she did not suffer as much as her poor homozygous son. But what if some of her somatic cells underwent a mutation in her "good" copy of the β globin gene. What would be the consequence to her and her cells if that cell was:
      i. a B lymphocyte?

Nothing because β globin is normally not expressed (nor needed) in a B lymphocyte

ii. A reticulocyte (precursor to RBC)?

The resulting RBC will not have any β globin and will have to get by with fetal globin…there will be no overall effect as this is just one of trillions of RBCs

iii. A hematopoetic stem cell?

All the RBC's derived from this stem cell will be defective (as described in ii), but there will be some normal RBCs that are derived from other stem cells…so the mother would have a mix of normal and defective RBCs; the proportion would depend upon when in her life this happened.

b. Look at the diagram of the deleted β globin gene. Below are described two other (hypothetical) types of chromosomal deletion mutants of the β globin gene. For each deletion mutant, discuss how you think the deletion would affect hemoglobin production (assume the individual carrying such a mutation is homozygous for this mutation):

i. Deletion that starts in the same place as that shown by the dotted line in the figure, but ends just before the transcription start site (transcript is indicated by the rectangle)

This is ironic! Although the coding part of the gene would be intact, this deletion would cause the same effect as the β^{o}-Black 1,393-bp deletion because the entire promoter region with all the regulatory DNA sequences is gone, thus regulatory proteins that need to bind and interact with the LCR are absent; in this case the fetal γ gene is likely to be highly expressed as is seen for the β^{o}-Black 1,393-bp deletion patient, because there is no competition from regulatory proteins bound in front of the β globin gene, so those bound at the γ gene can access the LCR and its regulatory proteins .

ii. Deletion that stars in the first intron and ends in the same place as that shown by the dotted line in the figure (in intron #2)

Again, a surprising prediction! Although much smaller a deletion, this one would probably be *worse*, and a homozygous person would die within a few months after birth. This gene could not generate a functional β globin product, but the regulatory proteins could still bind in front of the β globin gene and interact with the LCR regulators, thus generating a transcript and making it impossible for the fetal γ globin gene regulators to have access to the LCR regulators. Thus this individual could not be saved by expression of the fetal γ gene.

6. Describe four ways in which a Transcriptional Regulatory Protein could be inactivated?

- Inactivation by protein modification, such as phosphorylation which will affect its 3D conformation
- Inactivation by associating with another protein to form a heterodimer that is inactive (only the monomer was active – for example maybe the heterodimer masks the DNA binding domain of the monomer). ["heterodimer" means a dimmer composed of two different subunits in contrast to a "homodimer" which is composed of two identical subunits).
- Inactivation by cleavage or degradation
- Inactivation by losing a ligand that was previously abundant (a hormone, or other small molecule)

7. Cortisol, EPO, and O_2 are three very different "signals" that we talked about in class. What do these signals have in common and how do they differ?

They are all small molecules that can act as signals and elicit a change in gene transcription. They differ in terms of which subset of genes are regulated and how that regulation takes place. The only molecule of the three that interacts directly with the transcriptional regulatory protein is cortisol; the others have an indirect effect in that they interact with other proteins.