

Practice Exam 2007

1. A special JAK-STAT signaling system (JAK5-STAT5) was recently identified in which a gene called TS5 becomes selectively transcribed and expressed in the liver upon induction by a novel cytokine IL-25. TS5 encodes an important tumor suppressor protein that keeps us from developing liver cancer. Patients with certain liver tumors are found to express no or very little TS5. However, treatment with IL-25 can prevent liver cancer in these “responder” patients but not in other liver cancer patients.
 - A. Briefly describe how IL-25 treatment might be able to help this “responder” subgroup of liver cancer patients.
 - B. Why aren't others or all liver cancer patients helped by IL-25 treatment? Describe 2 kinds of mutations that would lead to down regulated TS5. Briefly describe how specific small molecule drugs could reverse the effects of this mutation
 - C. The promoter and cis-regulatory sequence of the TS5 gene were cloned and sequenced. Which transcription factor binding sites would you expect to find? Explain your answer.
 - D. (What in vitro assay would you use to determine the precise binding sequence of your candidate transcription factor? Explain why you chose this technique, and how it works.
 - E. It was observed that treating your candidate transcription factor with a protein phosphatase (an enzyme that removes phosphate groups from proteins) obliterated the ability of your transcription to bind DNA. Explain why you would have expected this to happen?
2. The ability to generate gene knockout and transgenic organisms have had a profound impact both on basic research capabilities as well as drug discovery and development in the pharmaceutical industry.
 - A. (Briefly describe 4 of the technical or reagent “advances” that made it possible to routinely generate KO mouse lines carrying mutation specific genes.
 - 1.
 - 2.
 - 3.
 - 4.

- B. Today, one of the most important uses of KO mice involves drug development. Which 2 aspects of drug discovery and development have been most influenced by our ability to KO specific genes in mice and propagate such KO mouse lines?
- C. If you compare the procedures for generating KO versus transgenic animals, which of these do you think is more difficult to successfully carryout? Explain why.
- D. In constructing a specific vector for the tissue specific expression of a cloned gene in a transgenic animal – what cis-control elements must you build into such a vector? For example, let's say we want to over express human insulin in red blood cells of sheep.
- E. There is a great deal of debate as well as suspicion and mis-information about genetically modified organisms (GMO's) – especially agricultural products such as rice, corn, wheat, etc. Describe 2 cases in which GMO's would be or have already been generated that might make a big impact in the 3rd World where food supply is limited relative to the population. Given this potential for tremendous “good” of GMO's, why are certain governments and even some scientists reluctant to embrace GMO's?
- F. Although we have successfully generated KO mice, has this technology been easily translated to work for many other organisms? Explain why? How about transgenics? Have they been applied to many species?

3. One of the key steps in regulating differential gene expression in all organisms is the control of transcription. In prokaryotes, the transcriptional machinery is relatively simple consisting of RNA pol and Sigma factors plus a few activators and repressors. However, in eukaryotes and especially metazoans, the transcriptional machinery has evolved into a much more elaborate system of protein complexes that carry out multiple functions.

- A. Name and describe three of the critical and distinct stages of the transcription process for the synthesis of primary RNA products destined to become mRNA.
- B. What are 3 different classes of large multi-subunit protein assemblies that participate in the formation of an activated pre-initiation complex (PIC) and that promotes gene transcription in animal cells?
- C. The TATA binding protein (TBP) is part of a large transcription complex – what is it called and what are 3 functions attributed to the TBP-containing complex?

- D.** Several of the different large multi-subunit complexes of the PIC also contain polypeptides that carry out various enzymatic activities. Name and briefly describe 3 such enzymes and the nature of their catalytic function.
- E.** In addition to the integral components of the transcriptional pre-initiation complex that must be assembled at eukaryotic promoters, what other class of co-regulators play an important function in activating gene transcription? (HINT: recall what the nature of the DNA template is for transcription in eukaryotes). How does this class of co-regulators work to help activate specific genes?
- 4.** The DNA of Eukaryotic organisms is wrapped up into chromatin which is a repeating unit of protein and nucleic acids also called nucleosomes.
- A.** Briefly describe an experiment that would allow you to determine the length of DNA that is typically wrapped around and protected by a mononucleosome. Do you expect the length of DNA that is wrapped around single nucleosomes to be different from the inter-nucleosome DNA length? Explain why.
- B.** Once you have a test tube full of purified mononucleosomes, what experiments could you do to analyze the size and charge of the protein components of mononucleosomes?
- C.** If all or most of the DNA in the eukaryotic nucleus is wound up into nucleosomes, how can sequence specific transcription factors like SP1 bind to cis-regulatory DNA? Describe at least two different mechanisms that would allow specific binding of transcription factors to chromatin templates.
- D.** Since cis-regulatory elements of metazoan genes can be anywhere from -50 to -50,000 bp upstream of the start site of transcription, what experiment could you perform to roughly map potential recognition sites for a given transcription factor (let's say the well characterized Estrogen Receptor) at a gene when you only have the start site but not the cis-regulatory sequences identified. To refine this mapping what complementary assay could you use?
- E.** Once you have roughly mapped a putative ER control region (let's say it's between position -3250 and -3000) what assay would you use to show that ER actually can occupy (ie be bound to) this putative ERE in a living cell (ie in vivo). Briefly describe how your assay works.
- F.** Given that ER is a hormone inducible, ligand gated transcription factor that is only supposed to bind its recognition sites when estrogen is present

– what experiment could you do to show that occupancy of ER at the (-3250 to -3000) region in cells is hormone dependent.

5.

A. In addition to switching transcription up or down to regulate gene expression, what are 5 other steps in the flow of biological information from

DNA--->Protein that can be regulated in eukaryotic organisms?

B. What process during gene expression was discovered in eukaryotes that was completely unexpected and initially made no sense? Briefly describe how this unusual molecular “processing” event works.

C. In the translation of mRNA into proteins, there are several key steps that require energy input in the form of nucleotide triphosphates. What are three of these steps and what is the purpose of each one of these reactions?

D. Briefly explain how auto catalytic RNA works and which of the two major gene expression stages utilizes such RNA enzymes?

E. In bacteria, transcription and protein synthesis are temporally and physically coupled. What processes in eukaryotes are “coupled” to transcription? What key molecular components within the RNA polymerase II complex is thought to be a molecular integrator of this coupling system?

6. The bacteria *B.subtilis* is unusual in being able to live as a normal free swimming “vegetative” bug when nutrient conditions are good (rich) but when starved for carbon (glucose) it can go into a dormant state called a spore. This ability to sporulate requires the expression of “sporulation genes” that are regulated by specialized sigma factors.

A. Briefly describe the mechanism for transcriptional control involving sporulation specific sigma factors and how it would induce spore formation? (HINT: how do sigma factors work?)

B. If you were to sequence the -10; -35 promoter regions of vegetative genes would you expect the sequence to be different or the same as the -10; -35 sequences of sporulation specific genes? Explain why?

C. After purifying the RNA Pol holoenzyme from vegetative and sporulating *B.subtilis* -- you compare the subunit composition of these 2 enzymes by SDS Gel electrophoresis. What would you expect to see (i.e. Which subunits of RNA Pol would you expect to be the same and which ones

different?) Explain the reason for your answer. Extra credit: show pattern on bands with relative migration.

- D.** Let's assume that the *B.subtilis* genome encodes a total of 7300 genes and 5125 are expressed in the vegetative state while 3532 are expressed during sporulation. What modern technique would you use to quickly identify the 5125 vegetative and 3532 sporulation genes? Briefly describe the steps needed to determine pattern of gene expression. Notice that these 2 numbers don't add up to 7300 – how might you explain this? (Assume the entire *B.subtilis* genome has been sequenced and all gene coding sequences determined.)
- E.** A mutant of *B.subtilis* was isolated that could only grow vegetatively and had lost the ability to sporulate – Describe 3 different targets in the transcription machinery that when mutated, would give you such a phenotype.