

PRACTICE EXAM ANSWERS 2007

1.

A. Essentially, we want the tumor suppressor TS5 to be expressed at normal level. You should know that the Jak Stat pathway regulates this tumor suppressor, and that TS5 induction is good. IL25 is a cytokine that will cause the upregulation of TS5. IL25 binds to the receptor on the outside of the cell activating a signal transduction pathway that ultimately leads to expression of TS5 which is needed for normal liver function. Since treatment of these patients with IL25 can prevent liver cancer then we can surmise that the receptor and corresponding pathway is intact otherwise no amount of IL25 would mitigate the disease. Therefore, this responder sub-group has liver cancer because of a decreased amount of IL25 production for some reason.

B. This question is divided into two parts—the first part would include potential mutations in the Stat proteins, or mutations in the Jak proteins. All liver cancer patients are not helped by IL25 treatment because the problem lies somewhere in the pathway that IL25 induces, not in the amount of IL25 present in the body.

Possible mutations and how a small molecule drug could help:

1. in the receptor – use a small molecule that binds and activates (causes dimerization) the receptor even though it does not recognize IL25.

2. a mutation at one of the residues that is phosphorylated in the pathway – you could use a small molecule that binds to the protein that should be phosphorylated that mimics phosphorylation and allows the pathway to be activated

3. a mutation in the phosphorylation recognition module of JAK or STAT – use a small molecule that activates the protein anyway, even though it does not recognize its normal phosphorylated partner

C. The simplest answer is that these are Stat binding sites. STAT binding sites are typically inverted repeats of a specific DNA sequence – this is because STAT's are obligate dimers.

D. The best in vitro assay would be footprinting but you could, with some effort, also use gel shift. Additionally, a type of SELEX experiment could be used. DNase footprinting is best because it most accurately maps the sequence of the STAT5 recognition element. You could confirm this by using point mutations of the mapped binding site to be absolutely sure.

E. In the STAT pathway, there must be a Jak mediated Phosphorylation of the transcription factor STAT5. This happens because phosphorylation is required for STAT dimerization, which in turn is required for DNA binding and activation of trxn. Treating STAT5 with a phosphatase would prevent dimerization and the subsequent formation of an active STAT5.

2.

- A. The following represent major advances that allowed KO technology
 1. ES cell discovery, isolation and cell culture
 2. Targeted homologous recombination in mammals
 3. Development of blastocyst injection techniques
 4. Mammalian cell culture transfection and vector developments
 5. “Plus-minus” dominant selection of transfected ES cells

- B.
 1. Creating animal models of human diseases
 2. Testing the consequences of inhibiting a specific target gene / Proof of Concept for potential drugs
 3. Drug testing / looking for side effects and/or drug specificity
- C. KO animals are more technically demanding. The procedure is more complicated, and success relies on achieving and detecting a very rare event – targeted homologous recombination as well as germ line transmission.
- D. The overexpression vector needs to contain regulatory sequence that will be recognized by factors in the tissue you would like to express your gene in. In this case, you require a sheep (or mammalian or at least eukaryotic) basal promoter and enhancer elements from sheep red blood cells—probably also useful to have a good ribosome binding site, splicing donor/acceptor sites, poly-A signal etc.
- E. Several possible examples although we specifically talked about in 2 class : sheep that produce clotting factor and golden rice. Hopefully some people will describe other examples and were able to list two genetically modified food organisms, since that is what the question is about. Social resistance to change, potential metabolic side effects of the transgene, and potential danger to the environment all issues that would be accepted in the second part. Since the question asks for reasons why both governments AND scientists sometimes rebuff accepting GMOs, only listing social issues will get only partial credit. Also, they can't just say “because people think GMOs might be bad.”

- F. While transgenics have been used across different species, knockout's have not been as successful. The problem is mostly with homologous recombination frequencies although establishing good ES cells can also be problematic.

3

A. Pre-initiation / initiation; promoter clearance/elongation; termination/ processing.

Pre-initiation: involves the formation of stable protein/DNA complexes between promoter DNA, the RNA pol II complex and various auxiliary factors (TFIIA, B, D,E,F,H).

Promoter clearance/ elongation: involves the ATP (energy) dependent disengagement of the RNA pol II complex from the core promoter, the opening up or strand separation for laying down the 1st few RNA products; the elongation step requires the processive movement of RNA pol II aided by various elongation factors to synthesize long primary transcripts.

Termination/processing: involves the release of the elongating RNA pol II complex accompanied by 3' cleavage and poly A addition of the RNA transcript.

B.

- Activator/enhancer complexes (enhancesomes)
- RNA polymerase II and the basal transcription complex including TFIIA, B,D, E, F, H.
- Co-activator or co-repressor (co-regulatory) complexes (TBP/TAFs; SAGA; CRSP/mediator; CBP etc.)
- Chromatin remodeling and modification complexes: BAF, SWI/SNF/ Brahma, HDAC, HAT

C. TBP is part of the TBP/TAF complex also called TFIID. This multi-subunit complex has the following documented functions:

- Binding to specific seq elements of the core promoter (ie TATA, INR, DPE)
- Targeted interaction with activation domains of enhancer binding activators (ie. the TAFs can serve as co-activators linking activators to the pre-initiation complex.)
- Some of the TAF's have the ability to catalyze various enzymatic reactions including kinase, acetyl transferase, Ub-ligase.
- At least one TAF subunit is also involved in a chromatin recognition function via bromodomains that bind to acetylated lysines on the N-terminal tails of H3 and H4.

D. Catalytic Activities:

RNA pol II large subunit – catalyzes RNA synthesis

TFIIH subunits – catalyze ATP hydrolysis

TFIID large subunit – catalyzes protein Kinase, acetyl transferase, Ub-ligase

E. A major class of co-regulators that works in concert with the basic transcriptional apparatus include: chromatin remodeling cofactors (BAF, ISWI, etc.) and chromatin modifying complexes: HAT's and HDAC's

4.

A. The length of DNA that is wound around a mononucleosome can be found by using the micrococcal nuclease assay and then running the DNA out on an agarose gel. The micrococcal nuclease assay is performed by digesting chromatin with the enzyme micrococcal nuclease, thus cleaving the DNA into integral multiples of unit length. (The nucleosomes could be run on a sucrose gradient to first resolve fractions of monomers, dimers, etc.) The DNA should then be extracted from the protein using high salt washes and then run on a gel with appropriate size markers.

Internucleosome length (50) should be different from the length of DNA wrapped around the histone – which is ≈ 146 bp. The center to center length of DNA between native chromatin/nucleosome units would thus be ≈ 200 bp.

B. To find the size and charge of the protein components there are a variety of techniques that you could use. SDS page gel, 2 D isoelectrifying gel electrophoresis, ion exchange chromatography, size exclusion chromatography etc..

- C. We know that there are sequence specific transcription factors that can bind to nucleosomal DNA by either interacting with the “outside” surface of the DNA wrapped around nucleosomes or by actually competing with nucleosomes for binding to DNA (i.e. some factors, with the help of chromatin remodelling factors can mobilize nucleosomes and move them around to allow transcription factor access to DNA.)
- D. The best way to roughly map potential transcription factor sites is to use the DNASE hyper-sensitive assay which allows you to find “nucleosome” free DNA sequences within 10-100Kd of a start site. By treating chromatin after salt extraction, you remove transcription factors from the chromatin but not the nucleosomes thus exposing “hyper sensitive” sites which were created by transcript factor binding to DNA.
- E. You would need to do a ChIP (Chromatin Immunoprecipitation) experiment to map more accurately ($\approx 200\text{-}500\text{bp}$) the *in vivo* occupancy of a candidate transcription factor by using a specific antibody to the factor and pulling out its associated DNA binding fragment after wither UV or formaldehyde cross linking of protein to DNA in the nucleus of your candidate cells. You will identify the associated DNA sequence by PCR.
- F. This goes back to the ChIP experiments. Essentially cross link cells in the presence and absence of the hormone and at varying concentrations.

5.

- A. Multiple steps in the control of gene expression other than transcription:
 - RNA processing that includes: exon splicing, 5' capping and 3' poly A addition
 - RNA transport in and out of nucleus and stability of RNA products
 - Control of protein synthesis (initiation, elongation, termination)
 - Control of protein secretion, sorting and stability
 - Regulating the intra cellular localization of gene products (nuclear, cytoplasmic, membrane embedded, etc.)
 - Covalent modification of proteins by phosphorylation, acetylation, ubiquitination, methylation, sumolation, etc.
- B. One of the most unusual and unexpected findings regarding the control of gene expression has been the discovery of RNA splicing –the highly controlled and accurate process of removing introns and splicing multiple exons to stitch together a complete gene coding sequence with correct uninterrupted open reading frames. RNA splicing fundamentally involves 2 consecutive trans-esterification reactions catalyzed by RNA itself with the help of a large complex of RNP's (RNA/protein complexes) called the spliceosome. Another equally unusual but less dramatic finding related to splicing was the discovery of catalytic RNA or ribozymes as well as the double stranded RNA triggered siRNA system.
- C. GTP hydrolysis is used at several stages during protein synthesis including:
 - formation of the initiation complex
 - First step in elongation : binding of the second amino-acetyl-tRNA
 - 3rd step in elongation – translocation

- the other step that requires NTP hydrolysis is charging of t-RNA's—the attachment of aa's to tRNA using hydrolysis of ATP as energy.

D. Auto catalytic RNA is involved in RNA splicing and in protein synthesis. These processes generally involve the folding of RNA into very specific structures that juxtapose various key “catalytic” residues such that multiple transesterification reactions can occur. Usually either a 3' or 2' OH participates in the esterification reaction with a phosphate moiety at the 5' end of the RNA.

E. In eukaryotes, the processes of RNA processing that include 5' capping, splicing and 3' end cleavage and poly A addition appears to be “coupled” to transcription. One of the key molecular “handles” that seems to help coordinate the coupling is the CTD of RNA pol II, a long repeating unit of 7aa rich in ser, thr etc.

6.

- A.** Upon glucose starvation, sporulation specific sigma factors would become active, replacing vegetative sigma factors and forming a new holo-RNA polymerase which would begin transcribing a set of sporulation specific genes giving rise to gene products (i.e. proteins) necessary to direct spore formation.
- B.** The promoter sequence on the -10 and -35 regions of the sporulation genes will have sequence differences when compared to vegetative genes which would correspond to different sigma factors needed to initiate new programs of gene expression during spore formation.
- C.** You would expect the RNA polymerase to have β , β' , α , and ω subunits which are all part of core RNA polymerase to be the same. However, the σ subunits would be different. Note for the extra credit: The patterns would look like this:
- D.** You need to use an expression microarray in order to answer this question. To set up this experiment you will need to have two populations of RNA molecules. The first comes from sporulating *B. Subtilis*, the second comes from vegetative. Once cells are lysed, RNA from each of the two populations will be isolated and used in the microarray experiments. The reason that the numbers of active genes do not add up is that there are likely to be housekeeping genes that must be active in both stages in order for basic cell processes to occur.
- E.** There are several possible answers for this. For example, the sigma subunit could be mutated. One kind of mutation might prevent binding of σ factor to DNA, while another mutation might prevent binding to core RNA polymerase. Similarly, there might be problems with the other subunits of RNA pol so that SPO σ can't bind and form a functional sporulation competent holo-enzyme.