Lecture 13

- In prokaryotic transcription, it is easy to recognize the length of the promoter and consensus sequences in the promoter
  - the holoenzyme, which is the core enzyme α2ββ' and σ are responsible for recognizing the consensus sequence at the -35 and -10. The -35 is for binding the DNA and -10 is where the helix has to melt
- In eukaryotic transcription, involves RNA polymerases. The polymerases still have to start transcription at the promoter. The promoter has to be asymmetric so that the binding is asymmetric so that it knows which is the template strand and which is the coding strand.
  - The RNA polymerase in eukaryotes do not directly recognize the promoter, they don't have affinity for DNA
  - have two subunits that are homologues, the β and β' subunits of prokaryotic polymerases that bind the nucleotides and are involved in catalysis.
    - in eukaryotic transcription, there are many more diverse genes, many more diverse promoter. The role of binding promoter is left to TFs.
      - These are proteins that are not subunits of RNA polymerase, but they are factors that are responsible for recognizing promoter elements.
      - the limit of the promoter usually involve empirical information, where by you take larger sequences surrounding the gene of interest and try shortening this fragment and see what will support transcription in a normal level
        - find that instead of having one consensus sequence, the promoters are built of many consensus sequence, each of which bind many factors.
          - These factors are called basal factors
            - basal factors - minimum required to initiate transcription in eukaryotes.
              - some of these factors directly recruit the RNA polymerase to the start point of transcription
              - If you have just basal factors, transcription would occur at such a low level that transcription would not be detected.
        - there are many other factors either called activators, these activators bind sequences of the promoter that are either upstream of the start point, or way up or downstream called enhancers
          - have many factors involved in recruiting the polymerase
  - sequences necessary for transcription in eukaryotes can take up to 30,000 bases or can even be a million bases away from where transcrip-

tion begins. Making it difficult to clone a gene and put it so an affected promoter adjacent to a transfected cell, because the sequences necessary for proper transcription include such a large amount of DNA and such a great distance from where transcription begins

- can have two types of genes that are transcribed: those that are not regulated and are constitutively expressed
  - constitutive - genes are on all the time, some times they are called house keeping genes regardless of their role in the cell because they are not regulated
    - those genes have these short consensus sequences that are bound by factors that would be found in all cells.
    - The cis acting consensus sequences to initiate the initiation of transcription require factors that are not regulated either. Together these factors always in cells and consensus sequences or house keeping genes require these factors
  - the other types of genes that are usually found in differentiated cells are regulated. Meaning that they are only expressed in certain cell types, certain periods of development, or response to certain environmental situations.
    - these genes have additional binding site, additional DNA consensus sequences, and their initiation is dependent on the recruitment of factors that binding them
      - It is not sufficient to have constitutive factors to get these genes expressed
- In Prokaryotes, if the promoter has a poor consensus to the -35 or -10 some prokaryotic promoters will fire ancillary factors
  - ancillary factors - are extra special factors to make initiation occur at that promoter
- Tissue specific promoters in eukaryotes are analogous, they get tissue specific initiation requiring binding of unique sequences that may only be found in specific genes. These sequences require unique developmentally regulated TFs.
  - the way you get tissue specific or developmentally regulated expression, is to have unique binding sites required for initiation that mean to have unique transcription factors.
  - If I have two genes, a pancreas gene and a liver gene. The reason the gene in the pancreas is only expressed in the pancreas is that gene that sponsors its initiation has a [several] unique binding sites. Those binding

sites have to be saturated by pancreatic specific transcription factors that are not produced in the liver.

- the reason the liver doesn't express the pancreatic gene is it doesn't produce the specific TF. Even though the gene is there, the promoter/consensus element is there it restricts the ability to synthesize these pancreatic transcription factors.
- Reciprocally, the liver specific genes to initiate transcription of pancreatic genes have unique binding factors for their own transcription factors; and the liver produces unique transcription factors.
    - Certain genes can be transcribed in the liver, whereas the liver and the pancreas may share 80% of gene expression
        - 80% of genes that are common to both don't require unique binding sites, and the constitutive factors that are present in all cells, are present in both of these organs, so the majority of genes require general factors.
- Transcription in eukaryotes is modulate with many TFs binding to short consensus sequences in DNA in order to recruit the RNA polymerase to the start point
- Function of TFs
    - Bind to RNA polymerase
    - Bind to short DNA sequences
    - bind to other TFs
- In prokaryotes there are different sets of genes that have different sets of sequences, so the majority of prokaryotic genes use -35 and -10 consensus sequences, and use the typical σ70 that normally is exposed to a holoenzymes, but when you have special sets genes such as heat shock genes, flagella genes, sporulation genes you get a unique consensus sequence upstream of the start of transcription of sequences of DNA with a different -35 and -10 and use a different σ.
    - even though σ is changed the core polymerase, $\alpha 2\beta\beta'$, is the same.
        - the change of σ is what allows for the recognition of different consensus sequences
- In eukaryotes, they have evolved three different polymerases to do different sets of genes
    - these different polymerases are made up of 12-14 subunits.
    - each require different TFs to initiate transcription
- majority of the RNA polymerase in the nucleus is found in the nucleolus, which is where ribosomal RNA is made.