Genetics Notes Chapter 12: Regulation of Gene Expression in Eukaryotes

12.1-Transcriptional Regulation in Eukaryotes

- Gene regulation takes place at many levels including the mRNA level through alternative splicing and mRNA stability (**transcriptional level**), and after translation through protein modification (**post-translational level**).
- DNA in eukaryotes is packaged into proteins that form **nucleosomes** and **chromatin**.
- In prokaryotes a gene's **ground state** is "on," in eukaryotes the ground state is "off."
- The ground state is off because genes are normally wrapped up in chromatin and not easily accessible.
- In prokaryotes genes are transcribed by one type of RNA polymerase in eukaryotes there are three types of RNA polymerase.
- Extensive processing of mRNA in eukaryotes.
- RNA polymerase 2 is much more complicated in eukaryotes than RNA polymerase in prokaryotes.
- Most genes in the eukaryotic genome are not expressed at a given time.
- To start transcription of mRNA RNA Pol 2 and other general transcription factors interacts with DNA sequences called **promoter-proximal elements**. These sequences are close to the promoter.
- The second set of transcription factors bind to regulatory DNA sequences called **enhancers**. These sequences may or may not be close to the promoter. These regulate a smaller subset of genes.
- The simple binding of RNA polymerase to DNA is not enough to produce enough transcript. The transcription factors must bind to promoter-proximal elements.
- These elements include the CCAAT box and a GC rich region further upstream.
- If these elements are mutated then transcription is generally decreased.
- Regulatory proteins have one or more of the following functional domains.
- A domain that recognizes a DNA regulatory sequence (the protein's binding site)
- A domain that interacts with transcriptional proteins (like RNA pol or a cofactor)
- A domain that interacts with proteins bound to regulatory sequences.
- A domain that influences chromatin condensation
- A domain that senses the physiological environment in the cell.
- **Yeast** is the primary eukaryotic model organism.

12.2 The Galactose System in Yeast

- Yeast imports **galactose** and uses a biochemical pathway of enzymes to convert galactose to usable glucose.
- GAL1, GAL2, GAL7, and GAL10 are genes that code for these enzymes and GAL3, GAL4, and GAL80 are genes that encode for additional proteins that regulate the expression of these genes.
- Like the lac operon it is the presence or absence of galactose that determines the on or off gene expression of the biochemical pathway.
- Because the enhancers of GAL4 are located upstream of the genes they regulate they are called **Upstream Activation Sequences**.

- The Gal4 protein has two distinct domain the **activation domain** and the **binding domain.**
- To measure how the Gal4 protein worked mutants were tested by looking at the expression of a **reporter gene** (a gene whose level of expression is easily measured). The expression of this gene reflects the functionality of the protein regulating it.
- In a Gal4 protein with a DNA binding domain but lacking an activation domain binding occurs but transcription does not start.
- However in a hybrid protein where an activation domain of one protein is grafted to the DNA binding domain of another protein transcription does occur and the protein is fully functional.
- **Gal80** comes in and blocks the activation domain of **Gal4**. Gal80 is continually synthesized, always trying to repress the transcription of GAL genes.
- In the presence of galactose **GAL3** is synthesized and binds to Gal 80 and knocks it off of gal4 so that the genes may be expressed.
- Gal 80 is an inhibitor and Gal3 is an activator.
- GAL 4 regulation are common in a broad array of eukaryotes demonstrating that all eukaryotes generally have the transcriptional machinery and mechanisms in common
- In eukaryotes activators generally work **indirectly** as opposed to prokaryotes that stimulate transcription by directly interacting with DNA and RNA polymerase
- In eukaryotes activators can interact with protein subunits to play a role in transcription initiation
- Activators can recruit proteins that modify chromatin structure; this gives rna POL and other proteins access to DNA

- TATA box
    - Ch 8: transcription factor IID binds to the TATA box of the Eukaryotic promoters
        - **TATA binding protein**
    - GAL 4 activates gene expression by binding TATA binding protein at activation
        - Recruits the TFIID complex, then RNA pol to promoter
    - 2nd Way GAL4
        - Interaction with mediator complex:
            - Recruits RNA pol to gene promoters
            - Is a **coactivator:** a protein complex that facilitates gene activation by transcription factor but is not part of transcription nor is a DNA binding protein
    - Yeast mating types
        - 3 mating types
            - **Mating Type A:**
                - Haploid
                - Mates only with Alpha
                - Secretes A factor
                    - Arrests alpha cells' cycle
                    - Necessary for successful mating
            - **Mating Type Alpha:**
                - Haploid
                - Mates only with A
                - Secretes pheromone "alpha factor"

            - Arrests A cells in cell cycle
            - Necessary for successful mating
    - Heterozygote: A/alpha:
        - Diploid
        - Don't mate
        - Don't respond to mating hormones
    - Cell type controlled by **MAT locus**
        - MAT – A allele
        - MAT – Alpha allele
        - Diploids have both alleles
    - Certain strains switch mating type
        - Later revisit
    - Both cell types have certain structural genes, only expressed in that cell type
        - MAT locus controls which set of these structural genes expressed in each cell type
        - Alpha or A
- A cell type
    - MAT A locus codes for reg protein A-1
        - However, A-1 is only activated in diploid cells
    - Haploid A cell: reg protein MCM1 turns on A structural genes by binding to promoter sequences for A specific genes
- Alpha cell type
    - MCM1 protein must be prevented from activating A1 specific gene
    - MAT1 alpha allele encodes 2 specific genes
        - Alpha 1 and alpha 2 genes
            - Alpha 1: activates alpha specific genes by binding in concert with MCM1 (binds with MCM1 to turn on alpha-specific genes)
            - Alpha 2: binds to another MCM1 to turn off A-specific genes
            - Alpha 1 activator of Alpha
            - Alpha 2 repressor of A
- Diploid Cells
    - Structural cell-mating genes shut down (All)
    - A1 used: encoded by MAT1: forms complex with alpha 2 causing repression of the haploid specific genes.
    - This A1-alpha 2 complex formation also causes alpha 2 to lose affinity for the alpha specific genes so those are no longer expressed.
    - Alpha 2 also binds MCM1 to repress the transcription of a specific structural genes.
    - Binding partners determine which DNA seq are bound
    - Important for different gene expression patterns