BCHM 421/422 Project: 2025-26

Project Outline:

Transcription regulation is critical for normal growth and survival. Consistently, transcription dysregulation if often seen in diseases states including cancer. In eukaryotes, transcription is performed by several RNA polymerases (RNAPs), of which RNAPII transcribes all protein-coding genes and many types of non-coding transcripts. Several factors contribute to transcription regulation, a complex process that remains poorly understood. The Mediator complex is a key transcriptional regulator, composed of several subunits organized into modules. The Cdk8 kinase module, which reversibly associates with the rest of the Mediator complex, is composed of the cyclin-dependent kinase Cdk8, its cyclin partner CycC, Med12, and Med13. Cdk8 acts by phosphorylating several transcription factors and transcription-associated factors, resulting in transcription activation or repression under context-specific conditions. For example, Cdk8 phosphorylates Gcn4, Ste12, and Phd1 in response to low nutrient conditions, resulting in targeted proteasomal degradation of these transcription factors and decreased expression of their target loci. In contrast, Cdk8 phosphorylates Gal4 in response to the presence of galactose in the growth media, an activity that results in the activation of Gal4 and the expression of its target loci. Despite our understanding of Cdk8 function, gene expression defects observed upon deletion, degradation, or nuclear depletion of Cdk8 cannot be explained by its known substrates, making it unclear exactly how it regulates transcription.

Leveraging existing Cdk8 deletion, degradation, and nuclear depletion gene expression datasets, we have identified six putative Cdk8 transcription factor substrates. These transcription factors **1)** regulate the expression of genes with altered mRNA expression when Cdk8 activity is absent or disrupted, **2)** contain phosphate acceptor residues within putative Cdk8 target motifs, and **3)** those phosphate acceptor residues have been found to be phosphorylated in screen of protein phosphorylation. To improve our understanding of Cdk8 function, the student will leverage the ease of genetic manipulation of the *Saccharomyces cerevisiae* model system to determine if Cdk8 phosphorylates any of the proposed substrates. This will be done by using a combination of *in vitro* and *in vivo* approaches, including immunoprecipitation followed by mass spectrometry (IP-MS) to isolate the proteins and identify specific residue that are phosphorylated by Cdk8. In parallel, the student will perform *in vitro* kinase reactions using wild-type and kinase dead Cdk8. Transcription factors found to be phosphorylated by Cdk8 *in vitro* will be examined for the effect of Cdk8-dependent phosphorylation *in vivo*. This will include the generation of phospho-mutant and phospho-mimic (phosphorylated residues mutated to aspartic or glutamic acid) versions and examining their effects on growth and mRNA levels of representative target genes. This analysis will help establish these transcription factors as functional substrates of Cdk8. Ultimately, this work will improve our understanding of transcription regulation as well as provide insight into the mechanisms by which upregulated Cdk8 is linked to cancer.

Supervisor: Maria Aristizabal

Project Title: Function and regulation of Cdk8

Project Goals: Identify novel Cdk8 substrates and determine the impact of phosphorylation on substrate function

Experimental Approaches: cloning, western blotting, RT-qPCR, Mass spectrometry, in vitro kinase reactions, and others.

References:

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