BCHM 421/422 Project – 2023-24

Project Outline:

The development of several cell types, including cardiac muscle, is a tightly regulated process that occurs primarily at the transcriptional level. The KLF family of transcription factors plays a prominent role in these processes and typically involves interactions between their intrinsically disordered transactivation domains and the multi-domain CBP/300 transcriptional coactivator. Dysregulation at the transcriptional level can result in the development of several disease states, including heart failure, metabolic and skeletal diseases, and cancer. The underlying biochemical mechanism associated with normal cell development and associated diseases involves transcription factor:ligand interactions, including those between KLF members, CBP/p300, and other transcription factors such as myocardin. These interactions are often further regulated by posttranslational modifications.

We are interested in characterizing the structural and functional properties of these transcription factors, and their interactions with other transcriptional regulators. We would also like to better understand how post-translational modifications might alter these interactions in health and disease.

Supervisor: Steven Smith / Matt Fishman / Holly Spencer

Project Title:

Structural and functional analyses of KLF-associated transcriptional networks associated with cardiac development and disease.

Project Goals:

1. Assess the structural properties of transactivation domains of KLF family members, including KLF2 and 15, myocardiin, and their interaction with the coactivator CBP/p300.

2. Identify the sites within the KLF activation domains that undergo post-translational modifications (i.e., acetylation, phosphorylation, and ubiquitination) and characterize their structural and functional impacts.

Experimental Approaches:

Towards pursuing our research goals, you will perform heterologous expression of various KLF, myocardin, and CBP/p300 protein constructs in *E. coli* and purify them using standard chromatographic methods. You will use bioinformatics to identify putative posttranslational sites within the activation domains. You will also receive training in the use of biophysical (e.g., circular dichroism, isothermal titration calorimetry), biochemical assays, and cell-based assays to assess transcription factor-ligand interactions.

References:

1. Prosdocimo, D.A., Sabeh, M.K., Jain, M.K. (2015) Kruppel-like factors in muscle health and disease. *Trends Cardiovasc Med* 25: 278-87.
2. Zhao, Y., Song, W., Wang, L., Rane, M.J., Han, F., Cai, L. (2019) Multiple roles of KLF15 in the heart: Underlying mechanisms and therapeutic implications. *J Mol Cell Cardiol* 129: 193-196.
2. Conroy, B.S., et al. (2017) Backbone 1H, 13C, and 15N NMR resonance assignments of the Krupple-like factor 4 activation domain. *Biomol NMR Assign* 11: 95-98.
3. Dyson H.J. & Wright, P.E. (2016) Role of intrinsic protein disorder in the function and interactions of the transcriptional coactivators CREB-binding protein (CBP) and p300. *J. Biol Chem* 291: 6714-6722.
4. Denis, C.M., et al. (2012) Structural basis of CBP/p300 recruitment in leukemia induction by E2A-PBX1. *Blood*. 120: 3968-3977.