CANCER PREVENTION & RESEARCH INSTITUTE OF TEXAS

Award ID:
R1221

Project Title:
Recruitment of First-Time, Tenure-Track Faculty Members

Award Mechanism:
Recruitment of First-Time, Tenure-Track Faculty Members

Principal Investigator:
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Entity:
The University of Texas Southwestern Medical Center

Lay Summary:

I have always had a fascination with detailed structures and mechanisms of action in biology. This has, in many ways, driven my interestsand research goals throughout my training. After obtaining my A.B. in Biochemical Sciences at Harvard College, I became interested in gene regulation as a graduate student in the laboratory of Stephen C. Blacklow. There I studied Notch receptors, which mediate intercellular signaling to affect many essential cell fate decisions throughout development. Activation of Notch leads to release of the intracellular portion that can mediate transcriptional activation in the nucleus. Constitutively active Notch is linked to various cancers including T cell acute lymphoblastic leukemias. In collaboration with the laboratory of Jon C. Aster, I established that intracellular Notch forms a transcription complex with a DNA-binding factor (CSL) and a co-activator (MAML) in a cooperative manner, an event required for survival of cancer cells dependent on Notch signaling. I determined crystal structures of Notch/CSL/MAML/DNA quaternary complexes, revealing an atomic view of the human Notch transcription complex. Unexpected observation of potential dimerization contacts allowed me to explain why CSL recognition sites often exist as inverted pairs in nature. These studies have led to a detailed understanding of why activated Notch with mutations in the dimerization interface can no longer cause tumors or mediate normal T cell development.

The major focus of my postdoctoral research in the laboratory of Piotr Sliz has been understanding how the Lin28 protein regulates let-7 family microRNA biogenesis. MicroRNAs are small regulatory RNA molecules that are processed portions of endogenous transcripts. The canonical pathway for miR maturation involves the RNAse III molecules Drosha and Dicer, but many details are still unknown. Lin28 and let-7 play a role in major biological phenomena such as pluripotency, tumorigenesis, metabolism, inflammation, and developmental timing. Lin28 expression is high in ~15% of human cancers while low in most adult tissue, making it an attractive target for cancer therapy. Lin28 specifically inhibits the let-7 family of miRNAs during biogenesis in three different ways; Lin28 blocks cleavage of pri-let-7s by Drosha and processing of pre-let-7s by Dicer, and also recruits a terminal uridyl transferase (TUTase) that modifies and promotes degradation of let-7 precursors. Poor primary sequence conservation of let-7 precursors and a lack of detailed understanding of the three processing enzymes had limited our understanding of Lin28's mechanistic roles. I first established that Lin28 binds primarily to the pre-element (preE), or "terminal loop region", and determined that both the sequence and secondary structure of this RNA fragment are important for Lin28

recognition. Lin28 has two predicted folded regions, containing a cold-shock domain (CSD) and two tandem CCHC-type zinc binding motifs (CCHCx2). I used NMR spectroscopy to probe the Lin28/let-7 complex and obtained a preliminary solution structure of the protein domains, which showed a clearly disordered interdomain linker. With the insight gained from my biochemical and NMR studies, I was then able to produce high- quality crystals and determine the structure of Lin28 bound to three different let-7 preE sequences. The structural models explain several conserved features of preE-let-7s, and provides a framework for predicting Lin28 binding sites on other let-7s, as well as other RNA targets.

I have also worked on biochemical and structural studies of two other important molecular machines as part of my postdoctoral research. O-linked ß-N-acetylglucosamine (GlcNAc) transferase (OGT) is an essential enzyme that modifies many intracellular factors involved in signal transduction, cell division, and transcription, and is homologous to super sex combs, a Drosophila Polycomb group gene. The role of GlcNAc signaling in gene regulation is still being uncovered. My other project was in the laboratory of Tom A. Rapoport, on the post-translational protein translocation machinery that includes SecA, a DEAD-box motor protein, in complex with heterotrimeric SecYEG protein-conducting transmembrane channel. By having performed rigorous biochemistry using both soluble and membrane-bound proteins, I have the appropriate training to tackle challenging questions regardless of the nature of the molecules involved.

As a new investigator at UT Southwestern Medical Center, I will continue working on mechanisms underlying gene regulation, mediated by non-coding RNAs, particularly microRNAs. I will focus on how microRNA biogenesis is regulated by various signaling pathways important for tumorigenesis. By using both biochemical and structural approaches, I will uncover how tertiary structures of RNAs contribute to their function.