Structural and functional analysis of human DICER1

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DICER1 is a multidomain protein that contains two RNase III domains (RNase IIIa and IIIb) that catalyze the final cleavage of a pre-microRNA to form the 3p (3' prime end) and 5p (5' prime end) strands of a mature microRNA (miRNA), respectively. While some initial characterization has been done to show how DICER1 binds to a pre-miRNA substrate, the exact mechanism by which the RNase III domains interact with the pre-miRNA during pre-miRNA cleavage is still largely unclear. One of the major causes behind this gap in knowledge is that no high-resolution (sub-3.5Å) structures of DICER1 are available for building a molecular model detailing how the protein engages and processes pre-miRNA substrates.

Recent biochemical studies have shown that point mutations at key metal-binding residues in the RNase IIIb domain, which are common in many DICER1 Syndrome patients, cause defective miRNA processing. Thus, these mutations offer a unique strategy to structurally understand how pre-miRNAs bind to the RNase III domains without interference of the full cleavage reaction. Additionally, recent work from the Garner lab has led to the discovery of distinct classes of natural products that can be used to inhibit DICER1-mediated pre-miRNA maturation. Thus, these newly discovered compounds can also be used as chemical tools to modulate DICER1 activity, and as an additional strategy by which to structurally and functionally characterize DICER1 interactions with a pre-miRNA.

This research proposal aims to characterize the structure-function relationship of human DICER1 bound to pre-miRNA substrates during miRNA biogenesis. The specific objective of this proposal is to determine how alterations due to mutations in the RNase III domains can be used to lock DICER1 in different conformational states, as well as to utilize natural products to modulate pre-miRNA interactions. This work is based on the hypothesis that DICER1 must undergo key conformational changes in the RNase III domains during miRNA cleavage in order to properly orient the pre-miRNA substrate towards a favorable interaction with the critical metal binding residues found in the catalytic active site.

To evaluate my hypothesis, I will combine cryo-EM structural studies as well as pre-miRNA cleavage and binding assays to study the underlying mechanism that dictates miRNA biogenesis. Furthermore, this work will shed new light on key binding pockets within DICER1 that can be targeted using small molecules and natural products as an alternative approach to therapeutically treating miRNA linked diseases.