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Structural Studies of Dicer-2 Complexes

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The RNA cleavage enzyme Dicer-2 is essential for antiviral activity in invertebrates. Biochemical studies show that Dicer-2 requires ATP to process viral-like dsRNAs through its helicase domain (Fig. 1A). This is unlike self dsRNAs, which bind to the Platform·PAZ domains in the absence of ATP (Fig. 1B). Both types of dsRNA are cleaved by the RNase III domains in the middle of Dicer-2 into siRNA substrates [1-3]. These siRNA substrates are then loaded onto Argonaute-2 with the help of Dicer-2 and an accessory co-factor R2D2. The co-factor R2D2 is a small dsRNA binding protein and interacts with the Dicer-2 helicase domain [4]. Argonaute-2 and one strand of the siRNA make up the active RNA-induced silencing complex (RISC), which can target RNA transcripts for silencing or degradation. The precise mechanism of how Dicer-2 and R2D2 help move one strand of the siRNA onto Argonaute-2 remains unclear. But the process is dependent on R2D2 as its knockout leads to inactive RNAi [5-6].

Current structures of *Drosophila* Dicer-2 reveal an "L" shaped structure of the enzyme in the absence of dsRNA, consistent with structures of other Dicers (Fig. 2); but in the presence of blunt dsRNA and ATPγS, only the helicase domain bound to the dsRNA was resolved [3]. In my unpublished work, I show a moderate-resolution structure of Dicer-2 bound to R2D2 (Fig. 2). My structure reveals that R2D2 binds to the Hel2i subdomain of the Dicer-2 helicase domain (Fig. 2, orange domain). When we compare my Dicer-2•R2D2 structure to a human Dicer structure bound to its accessory co-factor, TRBP [7], both TRBP and R2D2 interact with their respective Dicers at the Hel2i subdomain. This result potentially indicates that these co-factors may regulate their respective Dicers in a similar manner.

Part of my thesis research involves solving a high-resolution cryo-EM structure of the entire Dicer-2 protein in complex with accessory co-factors. To solve additional Dicer-2 structures, I will use an alternative approach by adding a recombinantly expressed Dicer-2 to *Drosophila melanogaster* embryo extracts, allowing near-native Dicer-2 complexes to form, and then eluting the tagged Dicer-2 and bound proteins from the resin. Obtaining Dicer-2 in a near-native complex may reduce the overall dynamics of the Dicer-2 protein, as accessory co-factors may force Dicer-2 to adopt less flexible conformations, as suggested by transient kinetic data [8]. These near-native complexes may allow me to solve high-resolution cryo-EM structures. Using embryo extracts, I will be able to solve numerous Dicer-2-related complexes from just one sample, potentially eliminating the complexities of expressing each protein of interest individually. A higher resolution structure will provide more precise locations for mutagenesis studies in both *in vitro* and *in vivo* experiments. I can then obtain a more detailed mechanistic understanding of how accessory proteins modulate Dicer-2 and how Dicer-2 binds, unwinds, and cleaves dsRNA substrates.

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