THE RHYTHMS OF FLIES

Evolution is an amazing tinkerer. Again and again biologists discover evidence that nature has taken an existing gene or protein and tweaked it to come up with a diverse new function. Changes that provide value are kept and may be improved upon through future meanderings of random mutation. Mother Nature's use of this flexible biological toolkit is evident in the cryptochrome/photolyase (CRY/PL) family of proteins that regulate functions as diverse as day-night circadian rhythms, magnetosensing, and DNA repair in response to ultraviolet or blue light in all the kingdoms of life. While these proteins share a similar overall structure and are all activated by light, they recognize diverse targets and fulfill diverse functions. Now, work by researchers using the 24-ID-E NE-CAT microbeamline at the APS has revealed the molecular basis for the functional differences between CRY and PL photoreceptor family members. Their solution of the three-dimensional (3-D) structure of a CRY protein from the fruit fly *Drosophila* provides important insight into the regulation of these fundamental light-driven processes that will affect how we understand our own circadian rhythms as well.

In flies, processes that are regulated by daily rhythms of day and night, such as hatching, are dependent upon the actions of proteins that can respond to light. The CRY photoreceptor in flies regulates circadian rhythms by binding to another protein, known as timeless, or TIM, in the presence of light. This causes both proteins to be destroyed and results in gene activation. Its protein "relative," the PL photoreceptor, performs a very different function by repairing damage to DNA caused by ultraviolet radiation and requires light to perform its function. The 3-D structure of PL shows that damaged DNA is aligned into a groove in the protein that recognizes the break and catalyzes a light-activated chemical reaction to fix it. A major question has been: How do these two proteins perform their very different functions using the same general protein fold and light activation mechanism? The solution of the 2.3-Å resolution structure of CRY provides some clues as to how this works.

The 3-D structure of CRY shows that it shares the same overall features as the fruit fly PL protein with some important exceptions. Most notably, the carboxy terminal tail (CTT) region of CRY, that is not present in PL and is important for targeting the TIM protein, appears to mimic the way damaged DNA binds to the PL protein. The CTT is positioned so that it juts directly into the catalytic groove of CRY where it causes the shift of an important phenylalaine-phenylalanine-tryptophan (FFW) motif to a location that mimics the way a damaged piece of DNA is arranged in the PL (Fig. 1). In both proteins, this interaction causes changes in the conformation of the protein that affects the light-sensitive cofactor. For PL, that means the light activates the DNA repair mechanism. For CRY, further experiments performed by the team on CRY mutants with changes to the amino acids in the CTT area showed that this is the area responsible for the light sensitivity of TIM targeting.

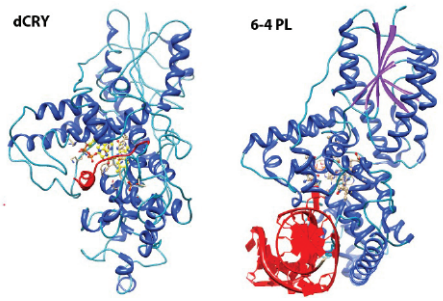

Fig. 1. Structural representations of the fruit fly cryptochrome and photolyase proteins (blue and purple ribbons) show that the C-terminal tail and damaged DNA (red in both panels) orient within a similar groove in the protein to interact with active site amino acids in light-activated reactions (yellow and beige).

These clear differences between CRY and PL were accompanied by some provocative clues about how CRY proteins could make use of subtle changes in the chemical interactions between amino acid side chains and the photo-activable cofactor used by PL proteins to perform DNA repair. These differences in the way electrons are transferred between cofactor and protein in CRY suggest that shifting the FFW motif into the active site may also facilitate photosensing or magnetosensing, providing insight into how these functions work as well. Now that the team has figured out how this subtle shift in structure has provided a new array of functions for the CRY/PL family, they plan to determine structures of CRY in light-activated states and in complex with its downstream targets.

— Sandy Field

See: Brian D. Zoltowski[1], Anand T. Vaidya[1], Deniz Top[2], Joanne Widom[1], Michael W. Young[2], and Brian R. Crane[1], "Structure of full-length *Drosophila* cryptochrome," Nature **480**, 396 (15 December 2011). DOI:10.1038/nature10618

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