CELL BIOLOGY

Versatile clusters formed by light

As a tool for light-dependent protein clustering, cryptochrome offers many opportunities to manipulate and query processes in the cell.

Optogenetics researchers are engineering new uses for natural light-sensing molecules. Four years ago, Chandra Tucker and her colleagues at Duke University showed that the *Arabidopsis thaliana* photoreceptor cryptochrome 2 (CRY2), which associates with the protein CIB1 in the presence of blue light, can be used in mammalian cells to rapidly bring together any two proteins with controlled light pulses. Her group, now at the University of Colorado, recently expanded the CRY2 repertoire.

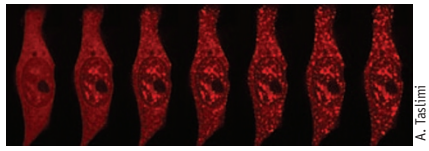
Inducing dimerization with light rather than chemicals has well-known benefits: the interaction can be rapid, reversible and precisely localized within a single cell. The CRY2-CIB system can also be broadly applied across cell types—and the versatility of CRY2 does not end there.

David Schaffer and his group at the University of California, Berkeley, showed in mammalian cells that CRY2 can form light-dependent homo-oligomers (Bugaj *et al.*, 2013). "There's a long list of signaling pathways where homo-oligomerization plays a role in activation," says Schaffer. CRY2 can be used to trigger cell receptor activation, cytoskeletal changes and focal-adhesion formation. The clustering approach does come with some conditions. Because oligomerization rate and cluster size depend on protein concentration, Schaffer says that it is critical to adjust CRY2 expression for each cell type. The approach has led to successes "from receptor tyrosine kinases to β-catenin to rho-GTPase signaling," he says.

Tucker has also developed CRY2 as a tool for clustering. "The wild-type cryptochrome does cluster, but it's very dependent on what you attach to it," she says. In particular, multivalent proteins appear to cluster more readily. In screening for CRY2 mutants with higher affinity for CIB1, her group isolated a variant with stronger oligomerization properties (Taslimi *et al.*, 2014). The new 'CRY2olig' protein is very robust. "We can see clustering every time in every cell," says Tucker. By fusing CRY2olig to actin adaptor proteins, they could nucleate actin clusters in response to light.

Oligomerization can also be used to inactivate processes in the cell. For example, the assembly of proteins into complex structures can be disrupted by clustering. The Tucker lab reversibly blocked endocytosis by fusing CRY2olig to the clathrin light chain. Won Do Heo and colleagues at the Korea Advanced Institute of Science and Technology also showed that a protein fused to CRY2 could be inactivated by sequestering it in giant clusters that recruit CIB1 fused to a large multimeric protein (Lee *et al.*, 2014). Although CRY2olig forms robust clusters, Tucker says that the sequestration mechanism will work in cases where protein localization is obviously disrupted by clustering.

The Tucker lab leveraged the fact that CRY2olig clusters are consistently large enough to be visualized by developing a light-induced coclustering (LINC) assay. In LINC, the CRY2olig-tagged 'bait' protein and a fluorescent-tagged 'prey' are imaged in the same cell before and after applying blue light to trigger clustering. Coclustering is thus a simple readout for protein interaction that does not need a specialized microscope or optimization. "I think of it as a poor man's FRET," says Tucker. Her team used LINC to study the dynamic interaction between calmodulin and CaMKII.



A. Taslimi

A cell expressing CRY2olig-mCherry responds to a pulse of light.

To study protein interactions in compact molecular structures such as the synapse or kinetochore, fluorescence recovery after photobleaching (FRAP) can be used. Rather than looking for a change in prey localization upon adding light, FRAP measures the exchange rate and mobile fraction of the prey.

Clustering is clearly a versatile strategy, and its application depends on the protein or pathway being studied. CRY2olig provides robust clusters but returns to monomers more slowly, whereas CRY2 clusters more dynamically (without necessarily forming visible puncta) but is more sensitive to fusion site and expression levels.

The possibilities for probing new biology are exciting. Tucker is interested in clustering enzymes within a metabolic pathway, and Schaffer is using CRY2 as a tool to understand how neural stem cells respond to signals to make fate decisions. Both look forward to seeing whether the dimerization and oligomerization properties of CRY2 can be separated as the toolbox expands and CRY2 finds even more uses.

Tal Nawy

RESEARCH PAPERS

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Taslimi, A. *et al.* An optimized optogenetic clustering tool for probing protein interaction and function. *Nat. Commun.* **5**, 4925 (2014).