Regulatory Mechanisms of Dynamic Scaffolding in Drosophila Phototransduction

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Drosophila phototransduction converts information about light contrast into an electrical signal through G protein signaling. We recently identified that the visual scaffold InaD undergoes a light-driven conformational change which is predicted to affect binding of the activator molecule phospholipase C and the latency and efficiency of the visual response. This conformational switch is regulated by higher-order interactions within InaD as well as by phosphorylation of the scaffold.

I. Background

Drosophila phototransduction converts light contrast information into an analog electrical signal. A single photon of light activates one rhodopsin receptor molecule, which then activates a few heterotrimeric G_Q proteins. These activated G proteins in turn activate a few phospholipase C (PLC) molecules, which break down phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol triphosphate (IP_3). This reaction results in the opening of 15-25 cation channels of the transient receptor potential (TRP) family. Calcium then enters the cell, initially reinforcing the opening of channels but then subsequently inhibiting channel opening through multiple mechanisms, including phosphorylation of multiple targets by protein kinase C (PKC). Together these signaling reactions generate a stochastic, transient opening and closing of ion channels known as a "quantum bump" in response to a single photon of light.

Phototransduction takes place in a specialized light-sensing organelle of the photoreceptor cell known as the rhabdomere, which is composed of 30,000 microvilli. Each individual microvillus is thought to act as a single photon detector whose sensitivity and kinetics are coordinated primarily by the intracellular concentration of calcium.

Drosophila phototransduction is one of the fastest known signaling systems—the entire quantum bump is finished within 100 ms. A scaffolding protein, InaD, which binds to PLC, TRP, and PKC, among other signaling molecules, has been shown to be critical for ensuring fast, coordinated visual signaling. [1]

Recently we showed that InaD switches between two conformational states *in vivo* as a disulfide bond forms in its fifth PDZ domain (PDZ5) in response to light in a PKC-dependent manner. The disulfide-bonded scaffold is predicted not to bind PLC. Mutant flies which are unable to form this disulfide bond lack a refractory period following quantum bump generation and display slow inactivation at higher light intensities. [2] Stochastic modeling of the quantum bump [3] predicts that PLC activity directly controls the latency and efficiency of the visual response.

In order to understand how this physiologically important remodeling of InaD is regulated, we measured the energetics of PDZ5 disulfide bond formation in various fragments of InaD in the absence or presence of phosphomimic mutations at a site shown to be phosphorylated in vivo. We also studied the physiology of flies expressing InaD with a point mutation to mimic or prevent phosphorylation.

II. Results and Conclusions

Using a maleimide labeling agent in a gel-based assay [2], we showed that purified PDZ5 by itself readily forms a disulfide bond and measured the free energy change. Incorporating additional regions of InaD strongly destabilized the disulfide bond.

How is disulfide bond formation regulated? We detected phosphorylation at a site in PDZ5. A phosphomimic mutation at this site does not affect the stability of the disulfide bond in PDZ5 alone, but could alter the effect of other regions of InaD on the disulfide bond. Flies expressing InaD with mutations at this site have a phenotype that is similar to flies which cannot form a disulfide bond but also has novel features, suggesting phosphorylation at this site is important for proper regulation of the disulfide bond.

These results indicate that this switch which controls the dynamics of the visual response is regulated by higher-order interactions within the InaD scaffolding protein. Additional work is needed to understand the interplay between these mechanisms.

References

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