Regulatory Mechanisms of a Conformational Switch in a Scaffolding Protein

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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 dynamics 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.

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 with and without ligand bound. In addition, we investigated the kinetics of phosphorylation under different physiological states. Finally, we analyzed the behavior of the switch using stochastic simulations.

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 and the ligand PLC strongly destabilized the disulfide bond. We solved the structure of this larger fragment of InaD and found that it formed an extensive interface with PDZ5.

How is disulfide bond formation regulated? We phosphorylated InaD in vitro with PKC and detected phosphorylation at two sites in PDZ5. Neither site is directly contacting the redox-active cysteines, indicating that if these are the physiological triggers for redox switching, they act allosterically. Intriguingly, the rate of phosphorylation was coupled to the redox environment and binding. The effect of phosphorylation on the disulfide bond is currently under investigation.

The refractory period is very short but stochastic, averaging 175 ms in duration but ranging from 50-1000ms. This suggests that the dynamics of the reactions controlling this switch are likely to be very important for its physiological function. Therefore we are also using stochastic simulations to reveal unexpected features of the non-equilibrium nature of this switch and identify the constraints of its design.

References

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