characterization of even better small molecule inhibitors of HIV-1 capsid assembly will undoubtedly be aided by our improved understanding of the different protein-protein interactions within the CA lattice.

Despite this progress, a full understanding of HIV-1 capsid structure and function will require further advances, including: (1) learning how the flat hexagonal CA lattice adjusts to form the gradually curving body of the conical capsid; (2) visualizing the capsid lattice structure at a resolution sufficient to define sidechain interactions and guide inhibitor development; (3) characterizing the pentameric CA assemblies that are required to close the conical capsid; (4) establishing how sequential proteolytic processing of the Gag precursor protein drives capsid assembly during viral maturation; and (5) most importantly, learning the detailed fates of capsids as they enter the cytoplasm, interact with host factors, and support the early stages of viral replication. These outstanding issues ensure that capsids will remain at the forefront of retrovirology for some time to come.

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Dynamic Regulation of the INAD Signaling Scaffold Becomes Crystal Clear

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PDZ domains are common building blocks of scaffold proteins that enhance specificity and speed in signal transduction cascades. Although PDZ modules are often viewed as passive participants, Mishra et al. (2007) now show that a PDZ domain in INAD, a scaffold protein in photoreceptor cells of the fruit fly, undergoes a light-dependent conformational change, which has important consequences for signaling and animal behavior.

The organization of signaling molecules into macromolecular assemblies is essential for achieving specificity in signaling, as it is common for proteins functioning in one signal transduction cascade to be employed by others. Signaling complexes are also crucial to cascades that operate over very short time scales, such as during phototransduction in the fruit fly *Drosophila*. Without these macromolecular assemblies, the speed of signaling would be limited by stochastic collisions between activated molecules and downstream effectors. Signaling complexes are nucleated by a variety of scaffold proteins, and among the most common are those with multiple copies of ~90 amino acid PDZ domains. In many cases, interactions with PDZ domains are regulated by dynamic modifications of the target proteins. In contrast, the PDZ domains are often considered passive components during signaling. However, this concept needs to be reevaluated in light of a study reported in this issue by Mishra and colleagues. They demonstrate that one of the PDZ domains in INAD, a molecular scaffold in *Drosophila* photoreceptor cells, undergoes a light-dependent conformational change. The dynamic structural transformation disrupts the surface groove important for PDZ/target protein interactions and has important physiological effects on the light response.

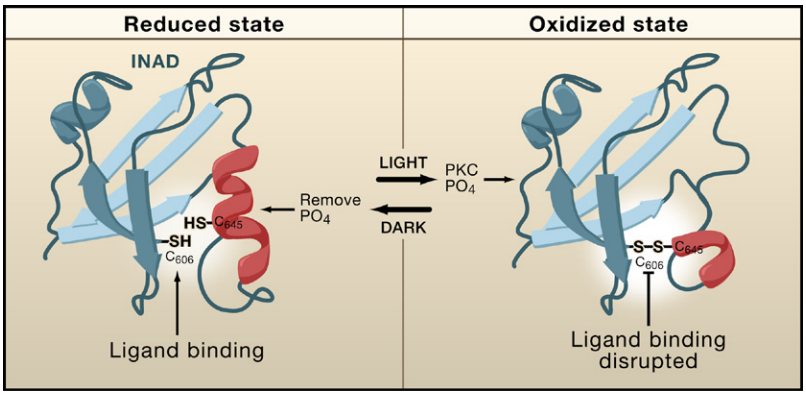

Figure 1. A Light-Dependent Conformational Switch in the PDZ5 Domain of INAD
When flies are dark-adapted, PDZ5 is in the reduced state and a surface groove is established, typical of other PDZ domain structures. In the reduced state, PDZ5 can bind to its ligand. Exposure to light triggers a PKC-induced allosteric change leading to partial unwinding of the α2 helix (red) and disulfide bond formation. The light-induced oxidized state blocks ligand binding. In the dark, the phosphate is removed and the disulfide bond is reduced.

Phototransduction in *Drosophila* is among the fastest known G protein-coupled signaling cascades, as it is maximally activated in <20 ms. The effector for the heterotrimeric G protein, G_q, is a phospholipase C (PLC; referred to as NORPA), which hydrolyzes phosphoinoside-4,5-bisphosphate (PIP_2) to generate inositol-1,4,5-trisphosphate and diacylglycerol. The cascade culminates with the opening of at least two cation channels, TRP and TRPL. Termination is also rapid and is regulated at multiple steps in the cascade.

INAD serves to link many of the proteins required in phototransduction, primarily through its five PDZ modules. INAD and three of its targets, TRP, PLC, and the protein kinase C (PKC, also referred to as INAC), comprise the core complex.

A key question is whether or not interactions with INAD are regulated in a light-dependent manner, and if so, how? The work by Mishra et al. (2007) not only answers this question in the affirmative but also reveals a mechanism that is both fascinating and unexpected. The authors used X-ray crystallography to obtain a high-resolution (~2.0 Å) structure for the fifth PDZ domain (PDZ5). Based on previous studies on other PDZ domains, the PDZ5 structure is predicted to preclude binding to target proteins. PDZ domains have a compact globular structure consisting of a β barrel comprised of five of the six antiparallel β strands and two α helices. Target proteins form hydrogen bonds with the PDZ domain in a groove situated between the α2 helix and the β2 strand. However, in PDZ5 the α2 helix is partially unwound and displaced to such an extent that association with binding partners is highly unlikely.

The conformational distortion in PDZ5 is stabilized by a disulfide bond between two cysteines situated in the β3 strand and the α2 helix (residues 606 and 645). Although disulfide bonds are relatively rare in intracellular proteins, the authors provide evidence that the bond is sufficiently strong to withstand the reducing conditions typical of most cells. However, this raises a conundrum. How can PDZ5 contribute to signaling if it is incapable of ligand binding? Mishra et al. show that PDZ5 is not locked into the oxidized state. Rather, INAD switches between two conformations in a light-dependent manner. If flies are transferred to the dark, the disulfide bond is reduced, and a standard PDZ-binding pocket is generated, thereby permitting ligand binding (Figure 1). Upon exposure to light, the cysteine bond reforms to block ligand binding.

In principle, the conformation of PDZ5 could be regulated directly by changes in the redox potential in the photoreceptor cell as light can cause an increase in reactive oxygen species (ROS) (Kagan et al., 1973). Furthermore, ROS have also been shown to promote the formation of lipid rafts in some cell types (Lu et al., 2007), and a recent study indicates that the INAD signaling complex translocates to lipid rafts in a light-dependent manner (Sanxaridis et al., 2007). It is unclear whether movements into lipid rafts would promote a conformational change in PDZ5, but this is a possibility given that many PDZ domains bind PIP_2 (reviewed in Zimmermann, 2006), and membrane lipids can affect protein structure (reviewed in Tillman and Cascio, 2003). Although not yet established, if PDZ5 binds PIP_2, one possibility is that hydrolysis of PIP_2 and release of PDZ5 from the plasma membrane following light stimulation might contribute to an allosteric change in PDZ5. This latter mechanism could occur independently of changes in redox potential.

Interestingly, Mishra et al. propose that the dynamic switch in PDZ5 is independent of the redox state and provide evidence for a mechanism involving protein phosphorylation. PKC (INAC), which is tethered to another PDZ domain in INAD, is required for the light-dependent formation of the disulfide bond in PDZ5. Thus, the authors suggest that the light-dependent phosphorylation of INAD by INAC (Matsumoto et al., 1999) results in an allosteric change that promotes formation of the disulfide bond in PDZ5. However, the phosphorylation sites have not been mapped. Although there are multiple consensus PKC sites in INAD, and two flanking either end of PDZ5 (residues 553 and 666), there are no consensus PKC sites in the domain itself. It also cannot be excluded that the requirement for INAC is a consequence of phosphorylation of another protein, which in turn modulates the conformational switch. An intriguing candidate to consider might be a disulfide isomerase.

What is the physiological significance of the light- and PKC-dependent distortion of the binding pocket in PDZ5? Because this structural alteration appears incompatible with target protein interactions, it might cause negative feedback on signaling. To test this hypothesis, Mishra et al. introduced an INAD derivative into *inaD*^{1} null flies in which cysteine 645 was changed to a serine. The INAD^{C645S} substitution prevents disulfide formation, thereby locking PDZ5 in the conformation permissive for target binding. Upon exposure to single photons or to dim light, the photoresponse in *inaD*^{C645S} photoreceptor cells is indistinguishable from wild-type. However, in response to bright light the mutant flies exhibit a pronounced delay in signal termination.

The delayed termination in *inaD*^{C645S}-expressing flies appears to be a manifestation of a fundamental, but poorly understood phenomenon. In wild-type flies, a single photon activates many TRP channels in one of the ~50,000 microvilli in a photoreceptor cell. There appears to be a refractory period during which exposure of the same microvillus to a subsequent photon does not elicit a response. The refractory period is diminished or absent in *inaD*^{C645S}, and as a result of excessive signaling, the mutant flies exhibit hyperadaptation to modest light, and loss of the photoresponse to bright light, possibly as a result of exhaustion of a critical signaling molecule.

A failure to properly turn off signaling through the PDZ5 conformational switch has behavioral consequences. Detection of motion in wild-type flies is crucial for a variety of behaviors, including an acute escape response. Normally, flies jump in response to an encroaching shadow, or when lights are turned off. However, this escape behavior is dramatically reduced in *inaD*^{C645S} flies. As pointed out by Mishra et al., the cysteines in PDZ5 are conserved only in fast and not slow flying flies, suggesting that the structural dynamics in PDZ5 are of particular importance to insects that must escape during rapid flight.

The work on PDZ5 raises many new questions. In addition to addressing whether PKC regulates the dynamic state of PDZ5 directly or indirectly, the relevant targets that bind PDZ5 are not known with certainty. According to one report, the NORPA PLC binds to both PDZ1 and PDZ5 (van Huizen et al., 1998); however, other groups have not found interactions with PDZ5 (Xu et al., 1998; Mishra et al., 2007). Therefore, the question arises as to the bona fide targets for PDZ5 and whether they interact exclusively with the reduced state of PDZ5. Although the data support a model in which the PDZ5 conformational switch depends on INAC, it remains possible that a changing redox state makes an additional contribution. Thus, it would be of interest to test whether there is a light-dependent alteration in redox potential using dynamic redox sensors (Dooley et al., 2004) and to determine whether the PDZ5 conformational switch is affected by altering the endogenous expression of proteins that affect the redox status. The identity of the relevant protein phosphatase that removes the phosphate from PDZ5 also remains to be identified. Finally, although two or more cysteines are not common among PDZ domains, the work by Mishra et al. raises the intriguing possibility that phosphorylation of other PDZ domains may induce allosteric changes that regulate the binding of target proteins and signaling in other cell types and organisms.

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