Scaffold proteins as dynamic switches

Mingjie Zhang

Multimodular scaffold proteins are ideally suited for assembling the various proteins in signaling pathways into supramolecular complexes. A recent study demonstrates that, in addition to a passive scaffolding role, a PDZ domain in a photoreceptor scaffold protein actively regulates fruit fly visual signaling via light-dependent conformational cycling.

Multiple proteins in signaling pathways are often assembled into their supramolecular complexes and targeted to specific regions of cells by multimodular scaffold proteins. The success of this organization and localization is critical for signaling efficiency and specificity. The catalytically inactive scaffold proteins that perform these tasks are traditionally viewed as passive molecules that "glue" signaling proteins together. However, research in a recent article by Mishra *et al.*[1] reveals that one of the PDZ domains in the fruit fly photoreceptor scaffold protein INAD can cycle between two functionally distinct conformations in a light-dependent manner and actively participates in the G protein–coupled visual signaling process.

PDZ domain scaffold proteins are highly abundant in eukaryotic genomes. Each PDZ domain contains ~90 residues and folds into a globular module capable of binding to short peptide fragments situated at the extreme carboxyl tail of target proteins. INAD uses its five PDZ domains to assemble the core proteins required for fruit fly visual signal transduction[2,3]. The scaffolding of the Ca^{2+}-permeable transient receptor potential (TRP) channel, phospholipase C and eye-specific protein kinase C (PKC) to INAD at the inner membrane surface of *Drosophila melanogaster* photoreceptor cells efficiently links the G protein–coupled receptor rhodopsin (the photon detector) with the Ca^{2+}-mediated second messenger signaling processes. PKC in the INAD-organized complex has a key role in the termination of the light stimulation signal by negative feedback inhibition of the TRP channel.

To learn more about the structure and function of INAD, Mishra *et al.*[1] solved the crystal structure of the fifth PDZ domain in INAD in the absence of reducing reagent; surprisingly, they discovered a stable disulfide bond formed between two cysteines in the αB-helix and the βB-strand near the bottom of the canonical target-binding groove (**Fig. 1a**). In this oxidized PDZ5, the target-binding groove is severely deformed, apparently because of the melting of the C-terminal half of the αB-helix normally seen in canonical PDZ domains. The authors also crystallized PDZ5 in the presence of 10 mM DTT and unexpectedly found that reduced and oxidized states coexist within each asymmetric unit. The binding groove of the reduced form of PDZ5 adopts the canonical conformation of PDZ domains, and thus is predicted to be able to bind to target proteins (**Fig. 1a**). Together with additional biochemical data, the authors concluded that PDZ5 exists in equilibrium between the two structural states, likely with distinct target binding activities in each conformation.

Looking further into the biological system, the authors used a cysteine-reactive fluorescent probe and two-dimensional gel electrophoresis to demonstrate that PDZ5 can indeed exist in the reduced and oxidized forms in fly photoreceptor cells and that the two structural states interconvert in a light-dependent manner. If flies are kept in the dark, PDZ5 is in the reduced form. Upon exposure to light, at least some portion of PDZ5 is converted to the oxidized conformation. The authors further showed that PKC is required for this light-dependent conformational interconversion. Introduction of an *inaD* mutant with its PDZ5 trapped in a permanently reduced form by substituting one of the cysteine residues with serine resulted in animals that were defective in terminating the stimulation signal produced by bright (but not dim) light. This correlated with fly behavior more globally: the same mutant flies reacted sluggishly in an escape jump assay that monitored jumping upon the sudden switch off of light (**Fig. 1b**). Therefore, the rapid disulfide bond conversion of INAD PDZ5 is critical for the adaptation of flies to broad dynamic ranges of light intensities.

The implications of this work are multifaceted. First, it clearly demonstrates that, in

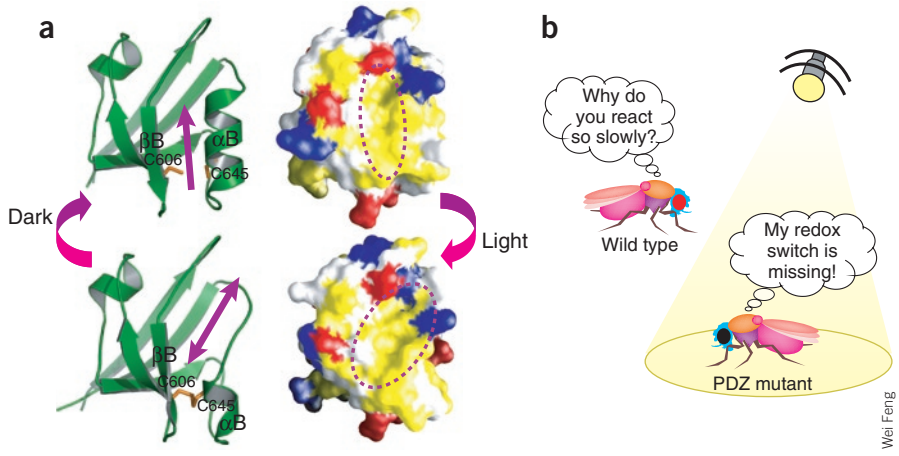

Figure 1 Conformational cycling of INAD PDZ5 via disulfide bond formation. (**a**) The light-induced conformational interconversion of INAD PDZ5. When flies are kept in the dark, two cysteine residues (Cys606 and Cys645) located in the αB/βB groove of PDZ5 are in the reduced form, and the domain contains a canonical PDZ domain binding pocket. Under bright light, the cysteine residues are oxidized and form a stable disulfide bond, which leads to a distortion of the target-binding groove. This groove is highlighted by an arrow in the ribbon diagram and an oval in the surface representation. (**b**) A cartoon presentation showing the loss of jump escape behavior in *inaD*^{C645S} flies.

Mingjie Zhang is in the Department of Biochemistry, Hong Kong University of Science and Technology, Clearwater Bay, Kowloon, Hong Kong, China.
e-mail: mzhang@ust.hk

addition to well-accepted assembling capacities, scaffold proteins can actively participate in the regulation of signaling events. Amino acid sequence analysis of the complete set of PDZ domains in the human genome reveals that several PDZ domains (for example, the PDZ domain of regulator of G protein signaling 12 and the fourth PDZ domain of ubiquitin ligase LNX1) contain a pair of cysteine residues at positions similar to those found in INAD PDZ5. It is possible that this disulfide bond–mediated conformational switching may also exist in these proteins, although the triggering factors are likely to be different. Second, the study also suggests that various biochemical mechanisms can be built into scaffold proteins to time signaling events appropriately. In the case of INAD PDZ5, interconversion of a disulfide bond in response to light stimulation operates at the millisecond timescale. It remains to be seen whether other conformational fluctuation mechanisms (for example, phosphorylation, lipidation, prolyl *cis-trans* isomerization or protein degradation) have been used to regulate the functional status of scaffold proteins either on faster or slower timescales. Third, the concept that scaffold proteins can actively regulate signaling events via different conformational states offers new opportunities to chemically manipulate signaling activities by directly targeting scaffold proteins. For example, one might be able to selectively stabilize one of the two conformations using small molecules. Given that numerous scaffold protein–organized signaling pathways are intimately linked to human diseases[4], modifications to the regulatory properties of scaffold proteins may be a new direction for future development of therapeutic compounds.

Like other groundbreaking discoveries, the work also raises many new questions. For example, although a PKC-INAD interaction and PKC's kinase activity are required for the conformational switch of PDZ5, the molecular basis of PKC's action is not known. PDZ5 does not contain any obvious PKC phosphorylation sites, which immediately suggests that PKC indirectly regulates the conformational cycling of PDZ5. One possible scenario is that the conformation of PDZ5 is allosterically regulated by its neighboring PDZ domains. It has been shown that tandem PDZ domains often display distinct structural and functional properties when compared with isolated PDZ domains[5,6]. Sequence analysis of INAD reveals that PDZ4 and PDZ5 are linked by a very short and rigid peptide sequence; consideration of both domains as a supramodule may be important in further elucidating INAD function. Considering again the intact biological system, it is not clear how PDZ5 maintains the reduced state in the dark or under dim light, as the disulfide bond of PDZ5 is expected to be stable at the cellular redox potential. Similarly, the study raises the question of how the level of PDZ5 oxidation and the intensity of light stimulation are correlated; perhaps PDZ5 functions as a "capacitor" for the fly photoreceptors to adapt to different intensities of lights. Further research will undoubtedly shed light on this interesting conformational trigger.

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