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The INAD Scaffold Is a Dynamic, Redox-Regulated Modulator of Signaling in the *Drosophila* Eye

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SUMMARY

INAD is a scaffolding protein that regulates signaling in *Drosophila* photoreceptors. One of its PDZ domains, PDZ5, cycles between reduced and oxidized forms in response to light, but it is unclear how light affects its redox potential. Through biochemical and structural studies, we show that the redox potential of PDZ5 is allosterically regulated by its interaction with another INAD domain, PDZ4. Whereas isolated PDZ5 is stable in the oxidized state, formation of a PDZ45 "supramodule" locks PDZ5 in the reduced state by raising the redox potential of its Cys606/Cys645 disulfide bond by ~330 mV. Acidification, potentially mediated via light and PLCβ-mediated hydrolysis of PIP_2, disrupts the interaction between PDZ4 and PDZ5, leading to PDZ5 oxidation and dissociation from the TRP Ca^{2+} channel, a key component of fly visual signaling. These results show that scaffolding proteins can actively modulate the intrinsic redox potentials of their disulfide bonds to exert regulatory roles in signaling.

INTRODUCTION

Scaffold proteins serve as platforms in signaling pathways by nucleating multiple proteins into macromolecular complexes and targeting them to specific regions within cells, and are critical for both efficiency and specificity of signaling events (Bhattacharyya et al., 2006; Pawson and Nash, 2003; Zhang and Wang, 2003). The *Drosophila* visual PDZ domain protein Inactivation, no after-potential D (INAD) is one of the best understood model systems for the roles of scaffolding in signal transduction (Huber, 2001; Montell, 1999; Tsunoda and Zuker, 1999). Within the microvilli of fly photoreceptor cells, INAD organizes the core components of the phototransduction pathway into a supramolecular complex, involving the Ca^{2+}-permeable transient receptor potential (TRP) channel, phospholipase Cβ (PLCβ/NORPA), and eye-specific protein kinase C (ePKC) via distinct PDZ domains, thus efficiently linking the G protein-coupled receptor rhodopsin to the Ca^{2+}-mediated signaling cascades (Adamski et al., 1998; Chevesich et al., 1997; Huber et al., 1996a; Kimple et al., 2001; Montell, 2005; Peng et al., 2008; Shieh and Zhu, 1996; Tsunoda et al., 1997; van Huizen et al., 1998; Wang and Montell, 2007). In this cascade, light photoisomerises rhodopsin, which activates PLCβ via the heterotrimeric Gq protein. PLCβ hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2), releasing inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG) and a proton, which leads to opening of TRP channels, and depolarization of photoreceptor cells (Huang et al., 2010; for reviews, see Hardie and Raghu 2001; Wang and Montell 2007; Hardie and Postma 2008). DAG is also a potent activator of eye-PKC which phosphorylates both TRP and INAD and plays a key role in the termination of the light response (Popescu et al., 2006; Smith et al., 1991; Hardie et al., 1993; Gu et al., 2005; Huber et al., 1996b; Peng et al., 2008). Via the INAD-mediated assembly of the signaling complex, fly photoreceptors can respond to light with extremely fast kinetics (for reviews, see Tsunoda and Zuker 1999; Huber 2001; Hardie and Raghu 2001; Wang and Montell 2007; Hardie and Postma 2008) and adapt to a huge dynamic range of light intensities (Juusola and Hardie, 2001).

A recent study indicated that in addition to acting as a master scaffolding protein, INAD plays a critical dynamic role in regulating phototransduction on a millisecond timescale (Mishra et al., 2007). Namely, a pair of Cys residues in INAD PDZ5 (Cys606 and Cys645) undergo reversible, disulfide-mediated oxidation in response to light. When flies are kept in the dark, the two Cys residues in PDZ5 are in the reduced form. Upon exposure to bright light, PDZ5 is converted to the oxidized state by formation of the intramolecular Cys606-Cys645 disulfide