Rhodopsin on Tracks: New Ways to Go in Signaling

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In this issue of Structure, Gunkel et al. describe cryoelectron tomography analysis of the nano-organization of the G protein-coupled receptor (GPCR) rhodopsin in the rod photoreceptor disk membranes in a near-native environment. Their data strongly suggest that rhodopsin is organized in the native rod disk as dimers arranged in parallel tracks aligned to the incisure.

The arrangement of rhodopsin in the rod disk, and in particular the question of whether rhodopsin forms dimers in a physiological context, is controversial. In order to appreciate the full significance of the paper by Gunkel et al. (2015), one has to understand the physiological context and recent findings leading to the contradicting conclusions and the controversy.

Vertebrates and humans have highly conserved visual systems. In these organisms, the neural layer in the retina is the very complex light sensor tissue consisting of multiple cell layers. The actual sensory layer for vision consists of cone- and rod-shaped cells. These elongated, specialized neural cells are either cone-shaped and mediate color vision and high-resolution detail, or they are rod-shaped and are responsible for dim-light vision letting us catch the light from the stars or find our way at night. Evolutionary pressure on the visual system to have maximum photon sensitivity is very high, and indeed, rod cells have an exceptional quantum yield.

Rod cells contain large stack of flattened sacks of membranes, the rod disk membranes, in the outer segment of the cell. These disks contain a very high concentration of rhodopsin, the actual light-sensing molecule, which is embedded in the disk membrane. Upon light stimulation, rhodopsin activates the signaling amplification cascade. Through an axonal extension, the rod cell is connected to a large synaptic region that is connected with horizontal and bipolar cells in a very special synaptic arrangement, called the ribbon synapse. At its end, the neurotransmitter (glutamate) is continuously released by synaptic vesicle fusion in the resting state. When light is absorbed in the rod outer segment, a biochemical amplification system leads to closing channels in the plasma membrane and hyper-polarization, causing a reduction in neurotransmitter release from the ribbon synapses.

The structure and function of the visual pigment rhodopsin has been an active field of research for many decades. Like all GPCRs, rhodopsin is a membrane protein with seven transmembrane helices. Upon light irradiation, the bent, banana-shaped 11-cis retinal isomerizes and straightens and triggers a series of conformational changes and distinct states. The structure of rhodopsin in the inactive and the active state (metarhodopsin II) is known to atomic detail and suggests an activation mechanism for rhodopsin (Deupi et al., 2012).

The first direct structural insights for rhodopsin were obtained by electron microscopy (EM). Corless and colleagues extracted lipids from disk membranes of frog rod cell outer segments and obtained 2D arrays that could be analyzed with negative stain EM. They observed rows of density for the protein and concluded that the rows were made of rhodopsin dimers (Corless et al., 1993). We (Schertler et al., 1992, 1993) obtained our first 2D crystals from reconstituted bovine rhodopsin and studied them with cryoelectron crystallography. In all cases an identical rhodopsin dimer was observed, and a conserved dimer was also found in several other 2D crystal forms (Schertler and Hargrave, 1995; Krebs et al., 2003). However, these results were from reconstituted or extracted membranes, and it was not clear if these dimers would also be present in the native disk membrane or if they were induced by the 2D crystallization.

In 2003, a key experiment was carried out by Fotiadis et al. (2003) with atomic force microscopy. Mica adsorbed, mouse disk membranes clearly showed rows of rhodopsin dimers. This important experiment triggered a fierce controversy in the rod disk field. So far, the free diffusion of rhodopsin in the disk membrane had been an implicit assumption in the amplification model of the light signal. What was the functional implication of dimerization? Was there a preferred dimer interface as suggested by electron crystallography? Subsequently, many experiments were carried out by many laboratories studying rhodopsin and other GPCRs. A somewhat divided picture was emerging. Whereas under a number of experimental conditions, dimerization of rhodopsin and other GPCRs could be observed and proven, comparison between monomers and dimers with respect to the activation of the G proteins showed only a minor increase in the activation for the dimer, and it was clearly shown that monomeric rhodopsin and many other GPCRs were able to fully activate the G proteins (Bayburt et al., 2007; Ernst et al., 2007).

With respect to the organization of rhodopsin in the rod disk, a number of different models evolved: an even monomer distribution throughout the disk (Chabre, 1975), a concentration of monomers at the center of the disk (Buzhynsky et al., 2011), and a random distribution of rows of dimers (Fotiadis et al., 2003) (Figures 1A–1C). However, the key question could not be answered, because it is indeed experimentally difficult to address: do dimers exist in the intact photoreceptor cell?
EM methods are developing fast as instruments have matured. Recently, new direct detectors are revolutionizing single-particle analysis, electron crystallography and electron tomography. This was nicely demonstrated by Wensel and collaborators, who described the three dimensional architecture of the rod sensory cilium by cryotomography (Gilliam et al., 2012). Additionally, advances in sample preparation, like high pressure freezing cryo sectioning, cryo Focused Ion Beam (FIB) specimen thinning, and lamella preparations enable the study of very large cells with tomography. Finally, improvements in data analysis, for example, by using sub-tomogram averaging, increase the resolution when periodic structures are present. Here, Gunkel et al. (2015) applied methods that could alter or destroy oligomers and higher order structures. Gunkel et al. are the first to address rhodopsin organization in the natural context of the intact photoreceptor cell and few, if any, artifacts would be expected during the preparation. Additionally, Gunkel et al. (2015) apply particle-based reaction-diffusion simulations to compare the activation kinetics of the G protein transducin in the presence and absence of rhodopsin tracks. Their data are intriguing and suggest that in the presence of preassembled transducin complexes, a single activated rhodopsin in a track would activate all transducins trapped in this particular track, largely independent of the rhodopsin lifetime. Because the sizes of the tracks have little variations in the disk, this could explain the experimentally observed uniform single-photon responses in rod cells. The simulation illustrates excellently how a nanostructured arrangement of visual pigments could affect activation of the transducer and supports the notion that the nanoorganization of the signaling components has a direct effect on the signaling cascade in vision.

Taken together, the findings by Gunkel et al. (2015) have far-reaching consequences. First, they consolidate the many controversial findings in the rhodopsin field regarding dimer formation in a native environment. Interestingly, the concept of rhodopsin dimer formation and higher order structures of rhodopsin is also supported by two independent studies on arrestin, a regulatory rhodopsin-binding protein. Both studies suggest two distinct rhodopsin-binding sites, indicating the binding of phosphorylated rhodopsin dimers to terminate the signal (Ostermaier et al., 2014), (Sommer et al., 2012). This strengthens the suggestion that dimer and track formation in other GPCRs may be a phenomenon linked to the nanostructuring of signaling components (Terrillon and Bouvier, 2004).

Second, they convincingly suggest a highly organized structural assembly of rhodopsin throughout the whole rod disk. This could be the beginning of a consensus that nano-organization of signaling components is important and necessary to build a biologically functioning system. Whether a “track” organization of rows of dimers is a unique feature of the vertebrate visual system is an interesting question for further study.

Lastly, the simulation part of the paper strongly suggests that rhodopsin tracks provide a platform that coordinates the spatiotemporal interaction of signaling molecules. Tracks can act as traps for G proteins, giving rise to biphasic kinetics and creating a robust G protein response that is largely independent of the rhodopsin lifetime. They may also play a role in detecting polarized light, of which many species are capable of.

Given the controversies in the field, we can expect that a number of labs will have another look at the nanoorganization of rhodopsin and other GPCRs. The development of cryotomography is rapid; therefore, stay tuned and look forward to more interesting publications improving our understanding of this fascinating class of integrated receptor systems.

Figure 1. Suggested Rhodopsin Organization in the Rod Disk Membrane
(A) Random monomer distribution.
(B) Concentration of randomly distributed monomers at the center of the disk.
(C) Random distribution of oligomeric dimer rows.
(D) Parallel arrangement of tracks consisting of 2 rows of rhodopsin dimers as suggested by Gunkel et al. Tracks are roughly parallel to the central incisure.
Figure used with the kind permission of Benjamin Kaupp and Ashraf Al-Amoudi.
The Molecular Basis for the Substrate Specificity of Protein Tyrosine Phosphatase PTPN3

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In this issue of Structure, Chen et al. present structures of the FERM-containing protein tyrosine phosphatase PTPN3 in complex with a phosphopeptide fragment of substrate epidermal growth factor receptor pathway substrate, providing detailed information on substrate specificity.

Protein tyrosine phosphatases (PTPs) play critical roles in cell signaling pathways. Together with the kinases, they control the balance of phosphorylated species, enabling specific and varied signaling responses. A large number of enzymes and substrates are involved in these pathways, and knowledge of the specific cellular substrates of the specific PTPs is important for a more complete understanding of the complex interactions that provide the signaling cascades. Dysfunction of PTPs has been associated with a number of human diseases including cancers, autoimmune disorders, diabetes, and neurological diseases.

The work presented by Chen et al. (2015) describes studies that help provide a more coherent understanding of the molecular basis of substrate specificity in the FERM-containing subfamily of nonreceptor PTPs (Chen et al., 2015). This subfamily contains PTPs N3, N4, N13, N14, and N21. These enzymes are characterized by the presence of an N-terminal FERM (4.1 protein [F], ezrin, radixin, and moesin) plasma membrane-localization domain and a C-terminal catalytic domain. Specifically, the work here examines the molecular interactions that govern the interaction of the PTP N3 (PTPN3) with substrate epidermal growth factor receptor (EGFR) pathway substrate (Eps15). Eps15 is a scaffolding adaptor that regulates endocytosis and trafficking of the EGFR that has recently been identified as a substrate for PTPN3 (Li et al., 2014).

By taking a small phosphopeptide fragment of the substrate Eps15 and examining its interaction with a series of variants of the catalytic domain of PTPN3, the authors have revealed key interactions that likely determine both substrate recognition and the catalytic activity of the complex. Several structures are presented, which, combined with the kinetic assessment of the dephosphorylation reaction of the phosphopeptide substrate, provide interesting new insight into the molecular recognition processes involved.

The structure of a catalytically inactive variant of the PTPN3 in combination with the phosphopeptide fragment of Eps15 (Figure 1) reveals an important interaction between H812 of the enzyme and a proline residue adjacent to the phospho-tyrosine of the peptide substrate, which results in an atypical conformation of the C-terminal part of the peptide substrate. The importance of proline in delivering this conformation was evaluated by assessment of a variant synthetic phosphopeptide fragment in which the