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Signal transducing membrane complexes of photoreceptor outer segments

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ABSTRACT

Signal transduction in outer segments of vertebrate photoreceptors is mediated by a series of reactions among multiple polypeptides that form protein–protein complexes within or on the surface of the disk and plasma membranes. The individual components in the activation reactions include the photon receptor rhodopsin and the products of its absorption of light, the three subunits of the G protein, transducin, the four subunits of the cGMP phosphodiesterase, PDE6 and the four subunits of the cGMP-gated cation channel. Recovery involves membrane complexes with additional polypeptides including the Na^+/Ca^{2+}, K^+ exchanger, NCKX2, rhodopsin kinases RK1 and RK7, arrestin, guanylate cyclases, guanylate cyclase activating proteins, GCAP1 and GCAP2, and the GTPase accelerating complex of RGS9-1, $G_{\beta 5L}$, and membrane anchor R9AP. Modes of membrane binding by these polypeptides include transmembrane helices, fatty acyl or isoprenyl modifications, polar interactions with lipid head groups, non-polar interactions of hydrophobic side chains with lipid hydrocarbon phase, and both polar and non-polar protein–protein interactions. In the course of signal transduction, complexes among these polypeptides form and dissociate, and undergo structural rearrangements that are coupled to their interactions with and catalysis of reactions by small molecules and ions, including guanine nucleotides, ATP, Ca^{2+}, Mg^{2+}, and lipids. The substantial progress that has been made in understanding the composition and function of these complexes is reviewed, along with the more preliminary state of our understanding of the structures of these complexes and the challenges and opportunities that present themselves for deepening our understanding of these complexes, and how they work together to convert a light signal into an electrical signal.

1. Introduction

The physiological function of rod and cone outer segments is the conversion of a light signal into an electrical signal. The biochemical cascade responsible for this conversion is known as the phototransduction cascade, and the major events in it are carried out by complexes of multiple polypeptides embedded in or attached to the surface of disk membranes and plasma membranes of the outer segments. Additional membrane complexes maintain the structures of these highly specialized membranes, and establish their proper spatial relationships.

Over the past two decades considerable progress has been made in identifying the major membrane proteins that make up these complexes and carry out their functions. Many of them have been purified from the retina, and all of their gene sequences are now known, allowing them to be studied in heterologous expression systems and in genetically engineered animals. Thus, despite the formidable challenges that continue to be faced in the biochemical characterization and structure determination of membrane proteins, much headway has been made in understanding the structure and function of membrane complexes important for phototransduction and outer segment structure. Understanding their structure–function relationships is important both for deepening our appreciation of the molecular mechanisms of vision, and for understanding the diseases that develop as consequences of disruption of the structures and functions of these complexes. The impact of progress in studying membrane complexes of the photoreceptors is felt well beyond the field of vision research, as these complexes have served as powerful models for understanding membrane complexes that mediate signaling pathways and membrane structures throughout the central nervous system and the rest of the body. One of the most striking examples is the enormous impact of the crystal structure of rhodopsin (Palczewski et al., 2000) on the fields of G protein-coupled receptors and membrane proteins.

An ongoing challenge and source of fascination is the role of the lipid milieu in which these complexes function. They have evolved to work optimally in an environment formed by a membrane bilayer with a highly specialized lipid composition. Most structural approaches and many biochemical studies begin by removing the complexes from the lipid bilayer, and one of the areas of research focus in the immediate future will be finding and exploiting creative approaches for determining structure

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and function in the membrane environment, and understanding the influence of the lipids on the behavior of the protein complexes.

2. The G protein, transducin, and its multiple membrane complexes

The photon receptor protein, rhodopsin, is a G protein-coupled receptor, and phototransduction is a prototypical G protein-mediated signaling cascade. At the center of this cascade lies the heterotrimeric G protein, transducin, $G_{\alpha t\beta 1\gamma 1}$ (rods) a peripheral membrane protein (the similar but distinct subunits of the rod and cone subunits will be generically referred to here as $G_{\alpha\beta\gamma}$, with distinctions between rods and cones noted as needed). As the first G protein and the first component of the phototransduction cascade to have its structure determined, its structure and function have been extensively reviewed (Arshavsky, Lamb, & Pugh, 2002; Birnbaumer, 2007; Bohm, Gaudet, & Sigler, 1997; Chen, 2005; Coleman & Sprang, 1996; Downs, Arimoto, Marshall, & Kisselev, 2006; Hargrave, Hamm, & Hofmann, 1993, 1999; Shichida & Morizumi, 2007; Sprang, 1997a, 1997b, 2000; Sprang, Chen, & Du, 2007). Much of this work has focused on soluble forms of transducin and its component subunits, whereas the focus here is on its membrane-dependent complexes.

2.1. Lipid modifications and structure of membrane-bound heterotrimer in GDP state

The α subunit of transducin, $G_{\alpha t}$, is the more dynamic half of the heterotrimer, moving rapidly among at least three conformational states, and shuttling back and forth between binding partners on the membrane surface. It has one of four different 12- or 14-carbon fatty acids (DeMar, Rundle, Wensel, & Anderson, 1999; DeMar, Wensel, & Anderson, 1996; Kokame, Fukada, Yoshizawa, Takao, & Shimonishi, 1992; Neubert, Johnson, Hurley, & Walsh, 1992; Yang & Wensel, 1992) attached in an amide linkage to its N-terminal glycine residue, and these provide for modest membrane-binding affinity, which varies somewhat depending on the hydrophobicity of the fatty acid (Johnson et al., 1994; Lobanova et al., 2007; Neubert & Hurley, 1998; Neubert et al., 1992). However, it is its interactions with other membrane proteins that keep it tethered to the disk membrane in rods under dim light conditions (where it functions in signaling) and likely membrane-bound in cones over most illumination conditions (Coleman & Semple-Rowland, 2005; Kennedy, Dunn, & Hurley, 2004); however, see (Chen, Wu, Sezate, & McGinnis, 2007). In its inactive GDP-bound form, which predominates in the dark, the arrangement of its "switch" domains favors binding to its partner subunits, $G_{\beta\gamma}$. G_{β} and G_{γ} bind to one another very tightly and have a mutual dependence for proper folding and stability. The intrinsic affinity of $G_{\beta\gamma}$ for the disk membrane is higher than that of $G_{\alpha t}$ and is partly mediated by the presence of two hydrophobic modifications on G_{γ}: The cysteine residue which is the fourth residue from the carboxyl terminus in the initial translation product is methyl esterified after the last three residues are proteolytically cleaved, and a farnesyl group is attached in a thioether linkage to this same residue (Bigay, Faurobert, Franco, & Chabre, 1994; Fukada, 1995; Fukada et al., 1990; Lai, Perez-Sala, Canada, & Rando, 1990; Ohguro et al., 1991). Both G_{α} and $G_{\beta\gamma}$ bind membranes, with a higher affinity displayed by $G_{\beta\gamma}$ than G_{α}-GDP (Bigay et al., 1994), which binds more tightly than G_{α}-GTP. However, for the heterotrimer, it is G_{α}-GDP that provides most of the membrane binding interactions (Seitz et al., 1999; Zhang et al., 2004b).

Studies with reconstituted vesicles or with spin-labeled lipids in disk membranes have revealed specificity in the interaction of transducin complexes with phospholipids (He, Mao, & Wensel, 2004; Hessel, Heck, Muller, Herrmann, & Hofmann, 2003; Malinski & Wensel, 1992; Melia, Malinski, He, & Wensel, 2000; Melia, Sowa, Schutze, & Wensel, 1999; Murray, McLaughlin, & Honig, 2001).

A structure of the $G_{\alpha\beta\gamma}$ complex bound to GDP and a membrane bilayer was determined by cryo-electron microscopy of two-dimensional (helical) crystals of the complex bound to tubules of lipid bilayers (Melia et al., 1999; Zhang et al., 2004b). The structure reveals lipid interactions of both the amino-terminal and carboxyl terminal regions of G_{α}, and of the carboxyl terminus of G_{γ}, with no apparent lipid contact with G_{β}. Two caveats of this structure are 1) that it reveals a static picture, whereas in reality, in the absence of crystal contacts there is likely considerable dynamic motion of the hydrophilic surface of the heterotrimer with respect to the membrane surface; and 2) that the contacts shown may be biased toward those favored by electrostatic attraction to positively charged lipids used for crystallization. Studies of transducin complexes in micelles and vesicles suggest that a lipid-like milieu enhances the effective affinity of $G_{\beta\gamma}$ and G_{α} for one another, likely as a result of both having lipid moieties. The combination of interactions of the two lipid tails with the membranes and of the G_{α} and $G_{\beta\gamma}$ polypeptides with one another, produces a cooperativity of membrane binding of the two subunits (Bigay et al., 1994).

2.2. Complex with rhodopsin and photoexcited rhodopsin (R^*)-progress and challenges

The affinity of transducin for disk membranes is not likely to be due entirely to its interactions with lipids. Although it can diffuse freely between photoreceptor outer and inner segments on a timescale of minutes, in the dark all three subunits of rod transducin are found almost exclusively in the outer segment. When a substantial portion of rhodopsin is bleached (i.e., to a level well beyond the point of saturation for rod vision), all three subunits translocate passively to the inner segment, most likely as separate G_{α}-GTP and $G_{\beta\gamma}$ units (Lobanova et al., 2007; Rosenzweig et al., 2007). One possible explanation for these results is a higher affinity of G_{α} for dark disk membranes than for partially bleached membranes, which would suggest that the relatively low affinity of the heterotrimeric form of transducin for the dark state of rhodopsin (Alves et al., 2005) is sufficient to allow sequestration of the G protein to the outer segments in the dark. This rhodopsin–transducin–GDP complex has received relatively little attention, and is deserving of more thorough characterization. Alternatively, the lower membrane affinities of G_{α} and $G_{\beta\gamma}$ separately for membranes, as compared to the higher membrane affinity of the heterotrimer, may be important in the net translocation.

The most attention has been focused on the complex between photoexcited rhodopsin, metarhodopsin II or R^*, and the transducin heterotrimer (Fig. 1). Likely there are multiple states of this complex, and currently there are high-resolution structures for none of them. The highest affinity form seems to involve the nucleotide-free form of $G_{\alpha\beta\gamma}$, which is a key intermediate in the nucleotide exchange reaction (release of GDP and binding of GTP) catalyzed by R^* as its central role in phototransduction. However, even this complex may exist in multiple conformations, and the complexes involving bound GDP or GTP must be considered as well. The structural and kinetic characterization of all of these complexes will be an important area of research in the coming years. Significant insights into their properties have been obtained by electron paramagnetic resonance (EPR) studies of the related protein G_i bound to rhodopsin in detergent, and by NMR and fluorescence studies of complex formation (Abdulaev et al., 2005, 2006; Brabazon, Abdulaev, Marino, & Ridge, 2003; Knierim, Hofmann, Ernst, & Hubbell, 2007; Medkova, Preininger, Yu, Hubbell, & Hamm, 2002; Oldham,