



Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Signal transducing membrane complexes of photoreceptor outer segments

Theodore G. Wensel*

Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

ARTICLE INFO

Article history:

Received 20 February 2008

Received in revised form 17 March 2008

Available online xxxxx

Keywords:

Phototransduction
 Membrane complexes
 Signal transduction
 Photoreceptors

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 $\text{Na}^+/\text{Ca}^{2+}$, 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, $\text{G}_{\beta 5\text{L}}$, 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.

© 2008 Elsevier Ltd. All rights reserved.

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 struc-

ture 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

* Fax: +1 713 798 1625.

E-mail address: twensel@bcm.tmc.edu

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, $G_{\alpha\beta\gamma 1}$ (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 time-scale 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,

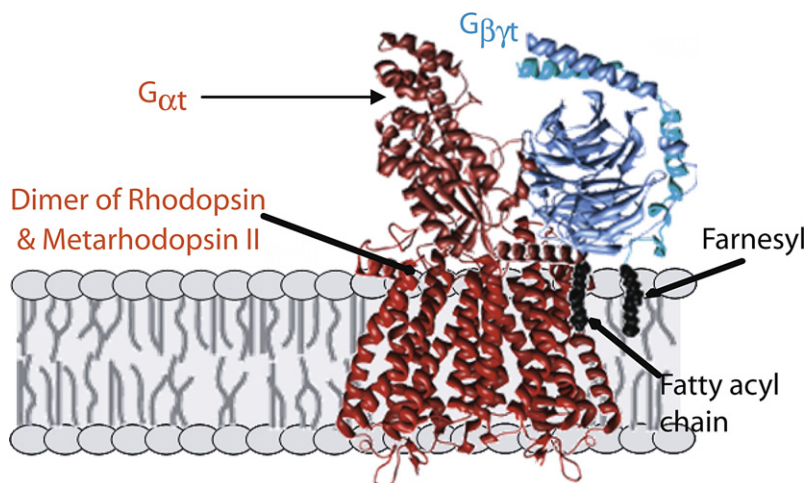


Fig. 1. Proposed model for first complex formed in photoactivation. Following absorption of light and photoisomerization by one subunit of the rhodopsin dimer, a heterodimer of rhodopsin and Metarhodopsin II (R^*) is formed, which quickly complexes with the G protein, transducin, in its heterotrimeric form. A conformational change within $G_{\alpha t}$ allows release of bound GDP, which then allows binding of GTP and dissociation of activated $G_{\alpha t}$ -GTP. The representation of the complex is purely schematic, as only indirect low-resolution information is available on the structures of the actual complexes. The major contacts with R^* are provided by $G_{\alpha t}$, with both its carboxyl and fatty acylated amino termini known to be involved. $G_{\beta\gamma t}$ are also important for R^* binding, with potential interactions with the membrane hydrocarbon phase provided by the farnesyl group on G_{γ} . For making the structure images shown, PDB files 1U19.pdb (Okada et al., 2004) and 1GOT.pdb (Lambright et al., 1996) were used with UCSF Chimera (Meng, Pettersen, Couch, Huang, & Ferrin, 2006; Pettersen et al., 2004).

Van Eps, Preininger, Hubbell, & Hamm, 2006; Ridge et al., 2006). Kinetic studies using light-scattering and other methods (Bayburt, Leitz, Xie, Oprian, & Sligar, 2007; Ernst, Gramse, Kolbe, Hofmann, & Heck, 2007; Heck & Hofmann, 2001; Herrmann et al., 2004, 2006b; Herrmann, Heck, Henklein, Hofmann, & Ernst, 2006a; Oprian, 1992) have also shed light on the mechanisms of transducin activation by R^* , but much remains to be done. There is reason for optimism that structures derived from three-dimensional or two-dimensional crystals of R^* -transducin complexes will be forthcoming in the near future.

2.3. PDE6 and complexes with $G_{\alpha t}$ -GTP

The only known physiological function of activated GTP-bound G_{α} , is activation of its downstream effector enzyme, the cGMP-specific phosphodiesterase, PDE6. PDE6 is yet another lipidated peripheral membrane protein of the phototransduction cascade. It consists of four subunits of three kinds with a stoichiometry of $PDE6_{\alpha\beta\gamma\gamma}$. $PDE6_{\alpha}$ and $PDE6_{\beta}$ are catalytic subunits with similar structures; in cones there are two identical copies of a single type of catalytic subunit, $PDE6_{\alpha}$, most closely related to $PDE6_{\beta}$. These catalytic subunits have modifications identical to those described above for $G_{\gamma t}$, except that $PDE6_{\alpha}$ is farnesylated, whereas $PDE6_{\beta}$ is geranylgeranylated in mammals (Anant et al., 1992; Catty & Deterre, 1991; Ong, Ota, Clarke, & Fung, 1989; Qin, Pittler, & Baehr, 1992; Qin, 1992), while the situation is reversed in frogs (Yamazaki et al., 2002). These modifications at the carboxyl termini are critical for positioning PDE6 on the membrane surface where it interacts with activated GTP for $G_{\alpha t}$, and proteolytic removal of the carboxyl termini, or binding of a prenyl-binding protein (Zhang et al., 2004a), also known, somewhat misleadingly, as $PDE6_s$, releases PDE6 from disk membranes (Cook, Ghomashchi, Gelb, Florio, & Beavo, 2000, 2001; Deterre, Bigay, Forquet, Robert, & Chabre, 1988; Florio, Prusti, & Beavo, 1996; Li et al., 1998; Norton et al., 2005; Wensel & Stryer, 1986). The $PDE6_{\gamma}$ subunit is a 9.7-kDa inhibitory polypeptide that keeps PDE6 catalytic activity at a very low level in the dark. Much of the action of G_{α} -GTP on the activity of PDE6 is mediated through its interactions with the $PDE6_{\gamma}$ subunit, and complexes of $PDE6_{\gamma}$ with either GDP form or GTP γ S form $PDE6_{\gamma}$ have been studied in solution (Antonny, Otto-Bruc, Chabre,

& Vuong, 1993; Artemyev et al., 1993; Skiba, Artemyev, & Hamm, 1995; Slepak et al., 1995). Indirect evidence suggests that two molecules of G_{α} -GTP bind to each PDE6 heterotetramer. In solution, the affinity of G_{α} -GTP for holo-PDE6 is relatively low, but phospholipid surfaces enhance their interactions dramatically, leading to nearly stoichiometric complex formation when both are membrane bound. This lipid-mediated interaction is enhanced by either positively charged lipids, which are not found in rods, or by phosphoinositides, especially phosphatidylinositol(4,5)bisphosphate (PIP_2) which is present in disk membranes at low levels (He et al., 2004; Melia et al., 2000; Womack et al., 2000). The physiological significance of the PIP_2 interactions remains to be determined.

Low-resolution structures of PDE6 have been determined by electron microscopy of the complex in heavy metal negative stain (Kajimura, Yamazaki, Morikawa, Yamazaki, & Mayanagi, 2002; Kameni Tcheudji et al., 2001). Electron microscope images of PDE6 in frozen solution or bound to vesicles have been recently obtained, as have images of quasi-crystalline arrays of PD6 bound to GTP γ S-form $G_{\alpha t}$. (Z. Zhang, F. He, & T. Wensel, unpublished observations), so that low- to medium-resolution structures of these membrane complexes should be forthcoming in the near future.

2.4. RGS9-1- $G_{\beta 5L}$ -R9AP and its complex with $G_{\alpha t}$ -GTP

The other important membrane-associated complex formed by $G_{\alpha t}$ in the course of phototransduction is its complex with the GTPase accelerating complex consisting of the GTPase-accelerating protein (GAP) RGS9-1, $G_{\beta 5L}$, and the membrane anchor, R9AP (Fig. 2). Indirect evidence suggests that under physiological conditions this complex may also be bound to PDE6, so that assuming a 2:1 stoichiometric ratio of G_{α} and its bound GAP complex to PDE6, one such complex could have a minimum of 12 membrane-associated polypeptides. Although high-resolution structures have been determined by X-ray crystallography for a complex of G_{α} with a C-terminal fragment of $PDE6_{\gamma}$ and the catalytic core of RGS9-1 (Slep et al., 2001; Sowa et al., 2001), as well as for a nearly full-length complex of RGS9-1 with $G_{\beta 5L}$ (Cheever et al., 2008), we are far from knowing how this large multi-subunit membrane complex is organized and how it is positioned with respect to the membrane surface. It is clear that

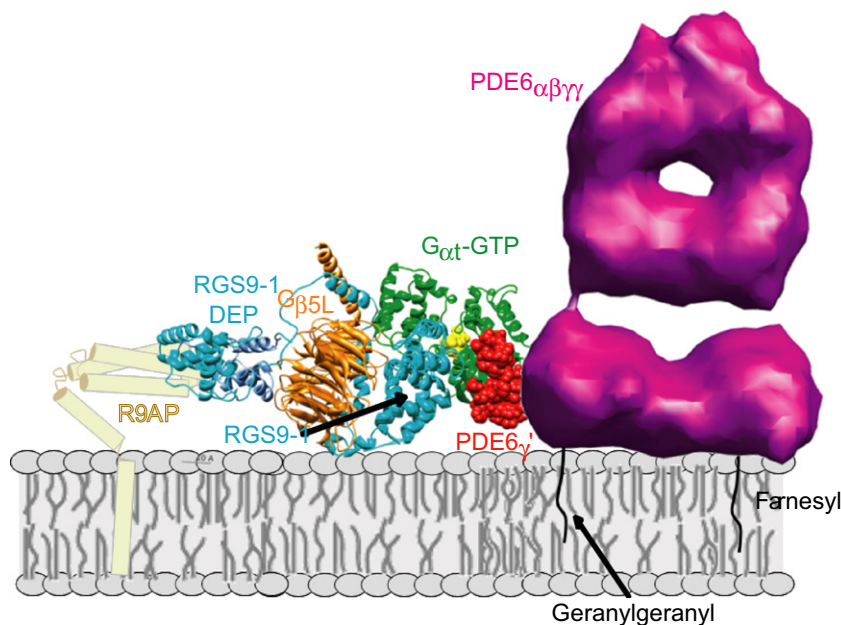


Fig. 2. A multi-subunit complex essential for normal photoresponse recovery kinetics. Sub-second GTP hydrolysis by activated $G_{\alpha t}$ -GTP is catalyzed by the GTPase accelerating complex of RGS9-1, $G_{\beta 5L}$, and single-pass trans-membrane anchor protein, R9AP. Indirect evidence suggests that formation of this complex occurs while $G_{\alpha t}$ -GTP is bound to its effector PDE6, largely through interactions with PDE6 $_{\gamma}$, whose C-terminal fragment, PDE6 $_{\gamma}'$ (Slep et al., 2001) is shown in red space-filling representation. For making the structure images shown, the RGS domains from PDB files FQJ.pdb (Slep et al., 2001) and 2PBI (Cheever et al., 2008) were aligned in UCSF Chimera (Meng, Pettersen, Couch, Huang, & Ferrin, 2006; Pettersen et al., 2004) and the resulting assembly of models positioned next to a model of holo PDE6, based on unpublished cryo-electron microscopy data kindly provided by Dr. Zhixian Zhang. The schematic representation of R9AP was loosely derived from a model presented previously (Cheever et al., 2008). The spatial relationships of PDE6 $_{\gamma}$ to holo PDE6, and of all of the polypeptides with respect to the membrane are not intended to be accurate; however, the attachment of the complex to the membrane *via* insertion of the isoprenyl tails of PDE6 and of the transmembrane segment of R9AP into the membrane is based on substantial biochemical evidence.

association of RGS9-1 with its membrane anchor, R9AP, a member of the syntaxin super-family with a single transmembrane helix (Hu & Wensel, 2002, 2004; Hu, Zhang, & Wensel, 2003), not only dramatically enhances its affinity for the membrane, but also is critical for its catalytic GAP activity and for its stability in rod cells (Baker, Martemyanov, Shavkunov, & Arshavsky, 2006; Keresztes et al., 2004; Keresztes, Mutai, Hibino, Hudspeth, & Heller, 2003; Krispel et al., 2006; Nishiguchi et al., 2004).

3. Additional complexes important for recovery

There are several membrane-associated complexes that are critical for the recovery phase of phototransduction. As their structures remain to be determined, and they have been recently reviewed, they will only be discussed briefly here.

3.1. R^* -rhodopsin kinase

An essential step for a return to the dark state of the phototransduction cascade is phosphorylation of R^* by rhodopsin kinase (GRK1; in some species a related kinase, GRK7 is found in cones), which belongs to a family of serine/threonine kinases specific for activated states of G protein-coupled receptors (Arshavsky, 2002; Hurley, Spencer, & Niemi, 1998; Maeda, Imanishi, & Palczewski, 2003; Penn, Pronin, & Benovic, 2000; Pitcher, Freedman, & Lefkowitz, 1998; Premont & Gainetdinov, 2007). The phosphorylation sites are found in the carboxyl-terminal tail of rhodopsin, which lies near the surface of the lipid membrane, and a transient complex forms between rhodopsin kinase and rhodopsin on the membrane. Both GRK1 and GRK7 are isoprenylated, and also have the other covalent modifications at their C-termini described for $G_{\alpha t}$ above, with GRK1 being predominantly farnesylated (C15) and GRK7 having a C-terminal sequence directing geranylgeranylation

(C20). An intriguing possibility is that the faster inactivation of cone pigments by phosphorylation as compared to rhodopsin (Tachibanaki, Arinobu, Shimauchi-Matsukawa, Tsushima, & Kawamura, 2005; Wada, Sugiyama, Okano, & Fukada, 2006) is related to differences in membrane binding between GRK1 and GRK7. An unresolved question in photoresponse termination is the role of the calcium-binding protein recoverin, of the neuronal calcium-binding protein family and calmodulin superfamily (Chen, 2002; Chen, Inglese, Lefkowitz, & Hurley, 1995; Kawamura & Tachibanaki, 2002; Klenchin & Bounds, 1995; Otto-Bruc, Fariss, Van Hooser, & Palczewski, 1998). It has been proposed that recoverin plays a key role in allowing lowered intracellular calcium concentration to serve as a feedback signal for activation of rhodopsin kinase in response to photoactivation. However, results of biochemical studies with permeabilized rods challenge this role (Otto-Bruc et al., 1998), and the effects of a knockout of the recoverin gene on photoresponse recovery kinetics, while in the right direction to support this hypothesis, are rather subtle (Makino et al., 2004; Sampath et al., 2005). One feature of recoverin's interactions with Ca^{2+} is clear: binding of Ca^{2+} induces a conformational change termed a "myristoyl switch" which causes extrusion of an N-terminal fatty acyl group from a pocket within the protein structure to an exposed state which would be expected to maximize interactions with the hydrocarbon phase of the phospholipids membrane. (Ames, Tanaka, Stryer, & Ikura, 1996; Zozulya & Stryer, 1992).

The ability of rhodopsin kinase to effect R^* inactivation depends to a large degree on the action of the capping protein, visual arrestin (Vishnivetskiy et al., 2007). Although there has been relatively little attention paid to interactions of arrestin with membrane lipids, an intriguing observation is that incorporation of acidic phospholipids, which are relatively abundant in disk membranes, enhances the interactions between arrestin and phosphorylated R^* in detergent micelles (Sommer, Smith, & Farrens, 2006).

3.2. Guanylate cyclase–GCAPs

Another membrane complex between transmembrane and peripheral membrane proteins is the complex between the single pass transmembrane protein, photoreceptor guanylate cyclase (GC: GC1 and GC2), and its associated guanylate cyclase-activating proteins or GCAPs (Baehr et al., 2007; Koch, 2002; Koch, Duda, & Sharma, 2002; Palczewski et al., 1994; Pugh, Duda, Sitaramayya, & Sharma, 1997; Yu et al., 1999). Unlike recoverin, deletion of the GCAP genes has a dramatic effect on photoresponse recovery kinetics, as well as on the peak amplitudes of dim flash photoresponses (Burns, Mendez, Chen, & Baylor, 2002; Howes et al., 2002; Mendez et al., 2001). GCAP complexes are necessarily formed at the membrane–cytoplasm interface with GC, which is an integral membrane protein. Like recoverin, they have an N-terminal sequence-directing fatty acylation, but do not seem to undergo a “Ca²⁺-myristoyl switch” mechanism. Structural studies by X-ray crystallography and NMR have revealed that the myristoyl group is embedded in the protein structure of GCAP1, but that the fatty acyl group of GCAP2 inserts into the membrane bilayer (Stephen, Bereta, Golczak, Palczewski, & Sousa, 2007; Vogel, Schroder, Lange, & Huster, 2007). Myristoylation influences the affinity of GCAP1 for GC and its Ca²⁺ sensitivity, but not that of GCAP2 (Hwang & Koch, 2002a, 2002b; Olshevskaya, Hughes, Hurley, & Dizhoor, 1997). In photoreceptor membranes, guanylate cyclase dimerizes with itself as well as binding GCAPs, and these interactions are critical to its activation when intracellular calcium concentrations fall in response to light (Hwang et al., 2003; Olshevskaya, Ermilov, & Dizhoor, 1999; Ramamurthy et al., 2001; Tucker et al., 1999; Yu et al., 1999).

4. Plasma membrane complexes

Multi-subunit protein complexes mediate the light regulated ionic fluxes through the plasma membrane of photoreceptor outer segments which are integral to light responses (Kaupp & Altenhofen, 1992; Molday, Warren, Loewen, & Molday, 1999). The cyclic GMP-gated cation channel is a heterotetrameric complex of three α (CNGA1) and one β (CNGB1) subunits (Trudeau & Zagotta, 2002; Weitz, Ficek, Kremmer, Bauer, & Kaupp, 2002; Zheng, Trudeau, & Zagotta, 2002; Zhong, Molday, Molday, & Yau, 2002); cones have a similar but apparently symmetric assembly of two CNGA3 and two CNGB3 subunits (Peng, Rich, & Varnum, 2004). These complexes are in turn associated with the major calcium extrusion protein of outer segments, the Na⁺/Ca²⁺,K⁺ exchanger (Bauer, 2002; Schnetkamp, 1989). Although progress has been made in establishing the stoichiometry of the participants in this complex, and in the functional roles of specific domains and residues within them (Bradley, Reisert, & Frings, 2005; Kaupp & Seifert, 2002; Matulef & Zagotta, 2003; Shibukawa et al., 2007), little is known about their structural arrangement. Electron microscopy and single-particle analysis have been used to determine a low resolution structure of the CNG channel (Higgins, Weitz, Warne, Schertler, & Kaupp, 2002), and there is a high-resolution structure of a cyclic nucleotide-binding domain similar to that of the photoreceptor CNG channel (Zagotta et al., 2003).

In addition to its association with the Na/Ca exchanger, the CNG channel also binds calmodulin (Molday, 1996; Trudeau & Zagotta, 2002) at a site between the N-terminal GARP (glutamic acid-rich protein) domain and the transmembrane domain of the β subunit (CNGB1), and may be involved in interactions with an alternative protein product of the channel β subunit gene in which the glutamic acid-rich protein or GARP (Korschen et al., 1999; Molday & Molday, 1998; Pentia, Hosier, & Cote, 2006) is expressed as a solu-

ble protein without the transmembrane and cyclic nucleotide-binding domains. GARP (there are two splice variants of GARP, a minor form, GARP1, and a major form, GARP2) has been proposed to bind to multiple membrane proteins of both the plasma and disk membranes and may be involved in forming some of the connections between these non-continuous membranes (see below).

5. Membrane complexes of the disk rims

An additional membrane compartment, in addition to the planar surface of the disk membranes or the gently curved surface of the plasma membrane is the rim region of the disk and the corresponding region of the cone plasma membranes where there is an extraordinarily low radius of curvature and a unique set of membrane protein complexes. The tetraspanin proteins peripherin/rds and ROM1 are important for maintaining the unusual membrane structure in this region (Goldberg, 2006; Molday, Hicks, & Molday, 1987; Molday et al., 1999). These proteins do not act as monomers, but rather as multimers, with a non-covalent heterotetramer of peripherin/rds and ROM1 forming higher order multimers through covalent disulfide linkages. These disulfide linkages may form in the disk lumen and serve as molecular “staples” to hold together the closely apposed bilayers of either side of each disk membrane. While several studies have described the interactions among these proteins and their ability to induce sharp membrane curvature into heterologous membranes (Wrigley, Ahmed, Nevett, & Findlay, 2000) little is known about the three-dimensional structure of these important membrane protein complexes. Also confined to the disk rims is a photoreceptor-specific member of the ATP-binding cassette family known as ABCR or ABCA4 (Allikmets et al., 1997; Azarian & Travis, 1997; Illing, Molday, & Molday, 1997; Shroyer, Lewis, Yatsenko, Wensel, & Lupski, 2001; Sun & Nathans, 1997; Wiszniewski et al., 2005). Rather than playing a structural role, this transport protein has been proposed to serve as a lipid-flipase, possibly for the covalent Schiff's base adduct between all-trans-retinaldehyde and the amino group of phosphatidylethanolamine (APE). It seems likely that localization to the disk rims is mediated by complex formation between ABCR and other rim-specific proteins such as peripherin/RDS and ROM-1. Although some structural information has been obtained for other members of the ATP-binding cassette family which ABCR likely resembles in many of its structural features, its three-dimensional structure and the structure or even existence of its complexes with other rim proteins remain to be determined. This determination may be facilitated by the existence of experimental protocols for expression and purification of ABCR in functional form (Ahn & Molday, 2000; Sun, Molday, & Nathans, 1999).

6. Mystery proteins controlling membrane structure

There may be additional membrane protein complexes important for photoreceptor structure and function whose components have yet to be identified. Freeze-etch electron microscopy has revealed connections, likely formed by membrane-associated proteins, between the disks and the plasma membranes, and between the rims of adjacent disks (Roof & Heuser, 1982; Roof, Korenbrot, & Heuser, 1982). A recent study of mouse disk membranes by cryo-electron tomography revealed very large protein complexes connecting adjacent disks, randomly distributed over the disk plane (Nickell, Park, Baumeister, & Palczewski, 2007). Identifying the constituents of all these intermembrane complexes remains a fascinating challenge to be overcome in the next few years.

7. Influence of membranes on kinetics and thermodynamics of protein–protein interactions

A recurring theme that has emerged in the study of membrane protein complexes in phototransduction is that the membranes do much more than simply organize the protein components. By greatly increasing the local concentrations of protein binding partners and by reducing their conformational and orientational freedom they dramatically increase their effective affinities for one another. These effects are evident not only for the heteromeric complexes, but likely also for the rhodopsin homo-dimer, since the form it takes in disk membranes is likely incompatible with those it assumes in detergent micelles and reconstituted membranes, where it can be found either as dimers or monomers (Bayburt et al., 2007; Fotiadis et al., 2003; Jastrzebska et al., 2004; Li, Edwards, Burghammer, Villa, & Schertler, 2004; Liang et al., 2003; Schertler, Villa, & Henderson, 1993). It is worth noting that the existence and/or functional importance of rhodopsin dimers remains a subject of some controversy (Bayburt et al., 2007; Chabre, Cone, & Saibil, 2003; Chabre & le Maire, 2005; Fotiadis et al., 2003; Hanson et al., 2007).

In principle membranes can have a similar effect on kinetics both by the concentration effect and by the reduction in dimensionality from three to two dimensions. At this point the diffusion kinetics in photoreceptors have been reported only for rhodopsin (Cone, 1972; Liebman, Weiner, & Drzymala, 1982; Montal, Elliott, Poo & Cone, 1973, 1974; Wey, Cone, & Edidin, 1981), so it will be important to document the dynamic behavior of the other membrane protein components of the phototransduction cascade within intact photoreceptors. It seems likely that the varying lipid environments presented by lipid microdomains within disk membranes will exert varying effects on behavior of the protein complexes (Boesze-Battaglia, Disposito, & Kahoe, 2002; Martin, Elliott, Brush, & Anderson, 2005; Nair, Balasubramanian, & Slepak, 2002; Nickell et al., 2007; Senin et al., 2004; Seno et al., 2001). Extensive evidence exists for the modulation of rhodopsin's behavior, as well as that of the downstream phototransduction complexes by lipid composition (Alves et al., 2005; Botelho, Huber, Sakmar, & Brown, 2006; Brown, 1994; He et al., 2004; Koenig et al., 2002; Litman, Niu, Polozova, & Mitchell, 2001; Malinski & Wensel, 1992; Melia et al., 2000; Mitchell, Niu, & Litman, 2001, 2003a, 2003b; Niu, Mitchell, & Litman, 2001, 2002; Womack et al., 2000).

8. Remaining challenges and future prospects

In reviewing the current state of our understanding of membrane protein complexes of photoreceptor outer segments it is clear that the protein composition of most of the major complexes has been determined, much is known about the biochemistry of the polypeptides involved, and through spontaneous or engineered mutations in animal models or humans, much is known about their physiological functions. The weakest link in our knowledge is in the structures of these complexes, especially as they exist *in vivo*, embedded in or attached to the surface of the photoreceptor membrane. Part of the reason is clearly that multi-subunit membrane complexes remain the most challenging subjects for X-ray crystallography, which has been by far the most commonly used approach for determination of protein structure. New approaches, or new applications of existing alternative approaches, are needed if progress on this front in the next decade is to exceed the progress made over the past decade. Some of these approaches, involving cryo-electron microscopy and electron cryo-tomography are especially well suited to determining membrane structure in the environment of a membrane bilayer. Spectroscopic techniques, including solid-state NMR, electron paramagnetic spectroscopy of

spin-labeled proteins, and fluorescence energy transfer techniques are emerging as powerful methods for extracting structural information about membrane protein complexes over a range of spatial resolutions. Also promising in this regard is the development of miniature bilayer membranes stabilized by lipoproteins, known as nano-disks or bicelles (Bayburt, Grinkova, & Sligar, 2006; Boldog, Li, & Hazelbauer, 2007; De Angelis & Opella, 2007; Leitz, Bayburt, Barnakov, Springer, & Sligar, 2006; McKibbin et al., 2007; Prosser, Evanics, Kitevski, & Al-Abdul-Wahid, 2006; Struppe, Komives, Taylor, & Vold, 1998). These have begun to be exploited for the study of photoreceptor proteins (Bayburt et al., 2007; McKibbin et al., 2007) and may offer a route to application of high-resolution structural techniques to bilayer-embedded proteins. However, these alternative approaches are still in the process of development, and are being pursued by a relatively small number of laboratories. We can only hope that an appreciation of the importance of establishing and extending new approaches to membrane protein complexes will motivate sufficient support from funding agencies, scientific journals, research institutions and others to keep these efforts going through the awkward stages faced by all truly innovative scientific endeavors.

Acknowledgments

Work in the Wensel laboratory on signal transducing membrane complexes has been supported by the National Eye Institute, by NASA, and by the Welch Foundation (Q0035). Drs. Zhixian Zhang, Feng He and Qiong Wang provided access to unpublished data.

References

- Abdulaev, N. G., Ngo, T., Ramon, E., Brabazon, D. M., Marino, J. P., & Ridge, K. D. (2006). The receptor-bound "empty pocket" state of the heterotrimeric G-protein alpha-subunit is conformationally dynamic. *Biochemistry*, 45(43), 12986–12997.
- Abdulaev, N. G., Ngo, T., Zhang, C., Dinh, A., Brabazon, D. M., Ridge, K. D., et al. (2005). Heterotrimeric G-protein alpha-subunit adopts a "preactivated" conformation when associated with betagamma-subunits. *Journal of Biological Chemistry*, 280(45), 38071–38080.
- Ahn, J., & Molday, R. S. (2000). Purification and characterization of ABCR from bovine rod outer segments. *Methods Enzymology*, 315, 864–879.
- Allikmets, R., Singh, N., Sun, H., Shroyer, N. F., Hutchinson, A., Chidambaram, A., et al. (1997). A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nature Genetics*, 15(3), 236–246.
- Alves, I. D., Salgado, G. F., Salamon, Z., Brown, M. F., Tollin, G., & Hruby, V. J. (2005). Phosphatidylethanolamine enhances rhodopsin photoactivation and transducin binding in a solid supported lipid bilayer as determined using plasmon-waveguide resonance spectroscopy. *Biophysics Journal*, 88(1), 198–210.
- Ames, J. B., Tanaka, T., Stryer, L., & Ikura, M. (1996). Portrait of a myristoyl switch protein. *Current Opinion in Structural Biology*, 6(4), 432–438.
- Anant, J. S., Ong, O. C., Xie, H. Y., Clarke, S., O'Brien, P. J., & Fung, B. K. (1992). *In vivo* differential prenylation of retinal cyclic GMP phosphodiesterase catalytic subunits. *Journal of Biological Chemistry*, 267(2), 687–690.
- Antony, B., Otto-Bruc, A., Chabre, M., & Vuong, T. M. (1993). GTP hydrolysis by purified alpha-subunit of transducin and its complex with the cyclic GMP phosphodiesterase inhibitor. *Biochemistry*, 32, 8646–8653.
- Arshavsky, V. Y. (2002). Rhodopsin phosphorylation: From terminating single photon responses to photoreceptor dark adaptation. *Trends in Neurosciences*, 25(3), 124–126.
- Arshavsky, V. Y., Lamb, T. D., & Pugh, E. N. Jr. (2002). G proteins and phototransduction. *Annual Reviews of Physiology*, 64, 153–187.
- Artemyev, N. O., Mills, J. S., Thornburg, K. R., Knapp, D. R., Schey, K. L., & Hamm, H. E. (1993). A site on transducin alpha-subunit of interaction with the polycationic region of cGMP phosphodiesterase inhibitory subunit. *Journal of Biological Chemistry*, 268(31), 23611–23615.
- Azarian, S. M., & Travis, G. H. (1997). The photoreceptor rim protein is an ABC transporter encoded by the gene for recessive Stargardt's disease (ABCR). *FEBS Letters*, 409(2), 247–252.
- Baehr, W., Karan, S., Maeda, T., Luo, D. G., Li, S., Bronson, J. D., et al. (2007). The function of guanylate cyclase 1 and guanylate cyclase 2 in rod and cone photoreceptors. *Journal of Biological Chemistry*, 282(12), 8837–8847.
- Baker, S. A., Martemyanov, K. A., Shavkunov, A. S., & Arshavsky, V. Y. (2006). Kinetic mechanism of RGS9-1 potentiation by R9AP. *Biochemistry*, 45(35), 10690–10697.

- Bauer, P. J. (2002). The complex of cGMP-gated channel and $\text{Na}^+/\text{Ca}^{2+}$, K^+ exchanger in rod photoreceptors. *Advances in Experimental Medicine and Biology*, 514, 253–274.
- Bayburt, T. H., Grinkova, Y. V., & Sligar, S. G. (2006). Assembly of single bacteriorhodopsin trimers in bilayer nanodiscs. *Archives of Biochemistry and Biophysics*, 450(2), 215–222.
- Bayburt, T. H., Leitz, A. J., Xie, G., Oprian, D. D., & Sligar, S. G. (2007). Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins. *Journal of Biological Chemistry*, 282(20), 14875–14881.
- Bigay, J., Faurobert, E., Franco, M., & Chabre, M. (1994). Roles of lipid modifications of transducin subunits in their GDP-dependent association and membrane binding. *Biochemistry*, 33(47), 14081–14090.
- Birnbaumer, L. (2007). Expansion of signal transduction by G proteins. The second 15 years or so: From 3 to 16 alpha subunits plus betagamma dimers. *Biochimica et Biophysica Acta*, 1768(4), 772–793.
- Boesze-Battaglia, K., Dispoto, J., & Kahoe, M. A. (2002). Association of a photoreceptor-specific tetraspanin protein, ROM-1, with triton X-100-resistant membrane rafts from rod outer segment disk membranes. *Journal of Biological Chemistry*, 277(44), 41843–41849.
- Bohm, A., Gaudet, R., & Sigler, P. B. (1997). Structural aspects of heterotrimeric G-protein signaling. *Current Opinion in Biotechnology*, 8(4), 480–487.
- Boldog, T., Li, M., & Hazelbauer, G. L. (2007). Using nanodiscs to create water-soluble transmembrane chemoreceptors inserted in lipid bilayers. *Methods Enzymology*, 423, 317–335.
- Botelho, A. V., Huber, T., Sakmar, T. P., & Brown, M. F. (2006). Curvature and hydrophobic forces drive oligomerization and modulate activity of rhodopsin in membranes. *Biophysical Journal*, 91(12), 4464–4477.
- Brabazon, D. M., Abdulaev, N. G., Marino, J. P., & Ridge, K. D. (2003). Evidence for structural changes in carboxyl-terminal peptides of transducin alpha-subunit upon binding a soluble mimic of light-activated rhodopsin. *Biochemistry*, 42(2), 302–311.
- Bradley, J., Reisert, J., & Frings, S. (2005). Regulation of cyclic nucleotide-gated channels. *Current Opinion in Neurobiology*, 15(3), 343–349.
- Brown, M. F. (1994). Modulation of rhodopsin function by properties of the membrane bilayer. *Chemistry and Physics of Lipids*, 73(1–2), 159–180.
- Burns, M. E., Mendez, A., Chen, J., & Baylor, D. A. (2002). Dynamics of cyclic GMP synthesis in retinal rods. *Neuron*, 36(1), 81–91.
- Catty, P., & Deterre, P. (1991). Activation and solubilization of the retinal cGMP-specific phosphodiesterase by limited proteolysis. Role of the C-terminal domain of the beta-subunit. *European Journal of Biochemistry*, 199(2), 263–269.
- Chabre, M., Cone, R., & Saibil, H. (2003). Biophysics: Is rhodopsin dimeric in native retinal rods? *Nature*, 426(6962), 30–31. discussion 31.
- Chabre, M., & le Maire, M. (2005). Monomeric G-protein-coupled receptor as a functional unit. *Biochemistry*, 44(27), 9395–9403.
- Cheever, M. L., Snyder, J. T., Gershburg, S., Siderovski, D. P., Harden, T. K., & Sondek, J. (2008). Crystal structure of the multifunctional Gbeta5-RGS9 complex. *Nature Structural and Molecular Biology*, 15(2), 155–162.
- Chen, C. K. (2002). Recoverin and rhodopsin kinase. *Advances in Experimental and Medical Biology*, 514, 101–107.
- Chen, C. K. (2005). The vertebrate phototransduction cascade: Amplification and termination mechanisms. *Reviews of Physiology Biochemistry and Pharmacology*, 154, 101–121.
- Chen, C. K., Inglese, J., Lefkowitz, R. J., & Hurley, J. B. (1995). Ca^{2+} -dependent interaction of recoverin with rhodopsin kinase. *Journal of Biological Chemistry*, 270(30), 18060–18066.
- Chen, J., Wu, M., Sezate, S. A., & McGinnis, J. F. (2007). Light threshold-controlled cone alpha-transducin translocation. *Investigative Ophthalmology & Visual Science*, 48(7), 3350–3355.
- Coleman, D. E., & Sprang, S. R. (1996). How G proteins work: A continuing story. *Trends in Biochemical Sciences*, 21(2), 41–44.
- Coleman, J. E., & Sempole-Rowland, S. L. (2005). GC1 deletion prevents light-dependent arrestin translocation in mouse cone photoreceptor cells. *Investigative Ophthalmology & Visual Science*, 46(1), 12–16.
- Cone, R. A. (1972). Rotational diffusion of rhodopsin in the visual receptor membrane. *Nature New Biology*, 236(63), 39–43.
- Cook, T. A., Ghomashchi, F., Gelb, M. H., Florio, S. K., & Beavo, J. A. (2000). Binding of the delta subunit to rod phosphodiesterase catalytic subunits requires methylated, prenylated C-termini of the catalytic subunits. *Biochemistry*, 39(44), 13516–13523.
- Cook, T. A., Ghomashchi, F., Gelb, M. H., Florio, S. K., & Beavo, J. A. (2001). The delta subunit of type 6 phosphodiesterase reduces light-induced cGMP hydrolysis in rod outer segments. *Journal of Biological Chemistry*, 276(7), 5248–5255.
- De Angelis, A. A., & Opella, S. J. (2007). Bicelle samples for solid-state NMR of membrane proteins. *Nature Protocol*, 2(10), 2332–2338.
- DeMar, J. C., Jr., Rundle, D. R., Wensel, T. G., & Anderson, R. E. (1999). Heterogeneous N-terminal acylation of retinal proteins. *Progress in Lipid Research*, 38(1), 49–90.
- DeMar, J. C., Jr., Wensel, T. G., & Anderson, R. E. (1996). Biosynthesis of the unsaturated 14-carbon fatty acids found on the N termini of photoreceptor-specific proteins. *Journal of Biological Chemistry*, 271(9), 5007–5016.
- Deterre, P., Bigay, J., Forquet, F., Robert, M., & Chabre, M. (1988). cGMP phosphodiesterase of retinal rods is regulated by two inhibitory subunits. *Proceedings of the National Academy of Sciences of the United States of America*, 85(8), 2424–2428.
- Downs, M. A., Arimoto, R., Marshall, G. R., & Kisselev, O. G. (2006). G-protein alpha and beta-gamma subunits interact with conformationally distinct signaling states of rhodopsin. *Vision Research*, 46(27), 4442–4448.
- Ernst, O. P., Gramse, V., Kolbe, M., Hofmann, K. P., & Heck, M. (2007). Monomeric G protein-coupled receptor rhodopsin in solution activates its G protein transducin at the diffusion limit. *Proceedings of the National Academy of Sciences of the United States of America*, 104(26), 10859–10864.
- Florio, S. K., Prusti, R. K., & Beavo, J. A. (1996). Solubilization of membrane-bound rod phosphodiesterase by the rod phosphodiesterase recombinant delta subunit. *Journal of Biological Chemistry*, 271(39), 24036–24047.
- Fotiadis, D., Liang, Y., Filipek, S., Saperstein, D. A., Engel, A., & Palczewski, K. (2003). Atomic-force microscopy: Rhodopsin dimers in native disc membranes. *Nature*, 421(6919), 127–128.
- Fukada, Y. (1995). Prenylation and carboxymethylation of G-protein gamma subunit. *Methods Enzymology*, 250, 91–105.
- Fukada, Y., Takao, T., Ohguro, H., Yoshizawa, T., Akino, T., & Shimonishi, Y. (1990). Farnesylated gamma-subunit of photoreceptor G protein indispensable for GTP-binding. *Nature*, 346(6285), 658–660.
- Goldberg, A. F. (2006). Role of peripherin/rds in vertebrate photoreceptor architecture and inherited retinal degenerations. *International Review of Cytology*, 253, 131–175.
- Hanson, S. M., Gurevich, E. V., Vishnivitskiy, S. A., Ahmed, M. R., Song, X., & Gurevich, V. V. (2007). Each rhodopsin molecule binds its own arrestin. *Proceedings of the National Academy of Sciences of the United States of America*, 104(9), 3125–3128.
- Hargrave, P. A., Hamm, H. E., & Hofmann, K. P. (1993). Interaction of rhodopsin with the G-protein, transducin. *Bioessays*, 15(1), 43–50.
- He, F., Mao, M., & Wensel, T. G. (2004). Enhancement of phototransduction G protein-effector interactions by phosphoinositides. *Journal of Biological Chemistry*, 279(10), 8986–8990.
- Heck, M., & Hofmann, K. P. (2001). Maximal rate and nucleotide dependence of rhodopsin-catalyzed transducin activation: Initial rate analysis based on a double displacement mechanism. *Journal of Biological Chemistry*, 276(13), 10000–10009.
- Herrmann, R., Heck, M., Henklein, P., Henklein, P., Kleuss, C., Hofmann, K. P., et al. (2004). Sequence of interactions in receptor-G protein coupling. *Journal of Biological Chemistry*, 279(23), 24283–24290.
- Herrmann, R., Heck, M., Henklein, P., Hofmann, K. P., & Ernst, O. P. (2006a). Signal transfer from GPCRs to G proteins: Role of the G alpha N-terminal region in rhodopsin-transducin coupling. *Journal of Biological Chemistry*, 281(40), 30234–30241.
- Herrmann, R., Heck, M., Henklein, P., Kleuss, C., Wray, V., Hofmann, K. P., et al. (2006b). Rhodopsin-transducin coupling: Role of the G alpha C-terminus in nucleotide exchange catalysis. *Vision Research*, 46(27), 4582–4593.
- Hessel, E., Heck, M., Muller, P., Herrmann, A., & Hofmann, K. P. (2003). Signal transduction in the visual cascade involves specific lipid-protein interactions. *Journal of Biological Chemistry*, 278(25), 22853–22860.
- Higgins, M. K., Weitz, D., Warne, T., Schertler, G. F., & Kaupp, U. B. (2002). Molecular architecture of a retinal cGMP-gated channel: The arrangement of the cytoplasmic domains. *EMBO Journal*, 21(9), 2087–2094.
- Hofmann, K. P. (1999). Signalling states of photoactivated rhodopsin. *Novartis Foundation Symposium*, 224, 158–175. discussion pp. 175–180.
- Howes, K. A., Pennesi, M. E., Sokal, I., Church-Kopish, J., Schmidt, B., Margolis, D., et al. (2002). GCAP1 rescues rod photoreceptor response in GCAP1/GCAP2 knockout mice. *EMBO Journal*, 21(7), 1545–1554.
- Hu, G., & Wensel, T. G. (2002). R9AP, a membrane anchor for the photoreceptor GTPase accelerating protein, RGS9-1. *Proceedings of the National Academy of Sciences of the United States of America*, 99(15), 9755–9760.
- Hu, G., & Wensel, T. G. (2004). Characterization of R9AP, a membrane anchor for the photoreceptor GTPase-accelerating protein, RGS9-1. *Methods Enzymology*, 390, 178–196.
- Hu, G., Zhang, Z., & Wensel, T. G. (2003). Activation of RGS9-1GTPase acceleration by its membrane anchor, R9AP. *Journal of Biological Chemistry*, 278(16), 14550–14554.
- Hurley, J. B., Spencer, M., & Niemi, G. A. (1998). Rhodopsin phosphorylation and its role in photoreceptor function. *Vision Research*, 38(10), 1341–1352.
- Hwang, J. Y., & Koch, K. W. (2002a). Calcium- and myristoyl-dependent properties of guanylate cyclase-activating protein-1 and protein-2. *Biochemistry*, 41(43), 13021–13028.
- Hwang, J. Y., & Koch, K. W. (2002b). The myristoylation of the neuronal Ca^{2+} -sensors guanylate cyclase-activating protein 1 and 2. *Biochimica et Biophysica Acta*, 1600(1–2), 111–117.
- Hwang, J. Y., Lange, C., Helten, A., Hoppner-Heitmann, D., Duda, T., Sharma, R. K., et al. (2003). Regulatory modes of rod outer segment membrane guanylate cyclase differ in catalytic efficiency and Ca^{2+} -sensitivity. *European Journal of Biochemistry*, 270(18), 3814–3821.
- Illing, M., Molday, L. L., & Molday, R. S. (1997). The 220-kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. *Journal of Biological Chemistry*, 272(15), 10303–10310.
- Jastrzebska, B., Maeda, T., Zhu, L., Fotiadis, D., Filipek, S., Engel, A., et al. (2004). Functional characterization of rhodopsin monomers and dimers in detergents. *Journal of Biological Chemistry*, 279(52), 54663–54675.
- Johnson, R. S., Ohguro, H., Palczewski, K., Hurley, J. B., Walsh, K. A., & Neubert, T. A. (1994). Heterogeneous N-acylation is a tissue- and species-specific posttranslational modification. *Journal of Biological Chemistry*, 269(33), 21067–21071.
- Kajimura, N., Yamazaki, M., Morikawa, K., Yamazaki, A., & Mayanagi, K. (2002). Three-dimensional structure of non-activated cGMP phosphodiesterase 6 and

- comparison of its image with those of activated forms. *Journal of Structural Biology*, 139(1), 27–38.
- Kameni Tcheudji, J. F., Lebeaux, L., Virmaux, N., Maftei, C. G., Cote, R. H., Lugnier, C., et al. (2001). Molecular organization of bovine rod cGMP-phosphodiesterase 6. *Journal of Molecular Biology*, 310(4), 781–791.
- Kaupp, U. B., & Altenhofen, W. (1992). Cyclic nucleotide-gated channels of vertebrate photoreceptor cells and olfactory epithelium. *Society of General Physiology Series*, 47, 133–150.
- Kaupp, U. B., & Seifert, R. (2002). Cyclic nucleotide-gated ion channels. *Physiological Reviews*, 82(3), 769–824.
- Kawamura, S., & Tachibanaki, S. (2002). S-modulin. *Advances in Experimental and Medical Biology*, 514, 61–68.
- Kennedy, M. J., Dunn, F. A., & Hurley, J. B. (2004). Visual pigment phosphorylation but not transducin translocation can contribute to light adaptation in zebrafish cones. *Neuron*, 41(6), 915–928.
- Keresztes, G., Martemyanov, K. A., Krispel, C. M., Mutai, H., Yoo, P. J., Maison, S. F., et al. (2004). Absence of the RGS9.Gbeta5 GTPase-activating complex in photoreceptors of the R9AP knockout mouse. *Journal of Biological Chemistry*, 279(3), 1581–1584.
- Keresztes, G., Mutai, H., Hibino, H., Hudspeth, A. J., & Heller, S. (2003). Expression patterns of the RGS9-1 anchoring protein R9AP in the chicken and mouse suggest multiple roles in the nervous system. *Molecular and Cellular Neuroscience*, 24(3), 687–695.
- Klenchin, V. A. C. P. D., & Bounds, M. D. (1995). Inhibition of rhodopsin kinase by recoverin. *Journal of Biological Chemistry*, 270, 16147–16152.
- Knierim, B., Hofmann, K. P., Ernst, O. P., & Hubbell, W. L. (2007). Sequence of late molecular events in the activation of rhodopsin. *Proceedings of the National Academy of Sciences of the United States of America*, 104(51), 20290–20295.
- Koch, K. W. (2002). Target recognition of guanylate cyclase by guanylate cyclase-activating proteins. *Advances in Experimental and Medical Biology*, 514, 349–360.
- Koch, K. W., Duda, T., & Sharma, R. K. (2002). Photoreceptor specific guanylate cyclases in vertebrate phototransduction. *Molecular and Cellular Biochemistry*, 230(1–2), 97–106.
- Koenig, B. W., Kontaxis, G., Mitchell, D. C., Louis, J. M., Litman, B. J., & Bax, A. (2002). Structure and orientation of a G protein fragment in the receptor bound state from residual dipolar couplings. *Journal of Molecular Biology*, 322(2), 441–461.
- Kokame, K., Fukada, Y., Yoshizawa, T., Takao, T., & Shimonishi, Y. (1992). Lipid modification at the N terminus of photoreceptor G-protein alpha-subunit. *Nature*, 359(6397), 749–752.
- Korschen, H. G., Beyermann, M., Muller, F., Heck, M., Vantler, M., Koch, K. W., et al. (1999). Interaction of glutamic-acid-rich proteins with the cGMP signalling pathway in rod photoreceptors. *Nature*, 400(6746), 761–766.
- Krispel, C. M., Chen, D., Melling, N., Chen, Y. J., Martemyanov, K. A., Quillinan, N., et al. (2006). RGS expression rate-limits recovery of rod photoresponses. *Neuron*, 51(4), 409–416.
- Lai, R. K., Perez-Sala, D., Canada, F. J., & Rando, R. R. (1990). The gamma subunit of transducin is farnesylated. *Proceedings of the National Academy of Sciences of the United States of America*, 87(19), 7673–7677.
- Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., & Sigler, P. B. (1996). The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature*, 379(6563), 311–319.
- Leitz, A. J., Bayburt, T. H., Barnakov, A. N., Springer, B. A., & Sligar, S. G. (2006). Functional reconstitution of Beta2-adrenergic receptors utilizing self-assembling Nanodisc technology. *Biotechniques*, 40(5), 601–602. 604, 606, passim.
- Li, J., Edwards, P. C., Burghammer, M., Villa, C., & Schertler, G. F. (2004). Structure of bovine rhodopsin in a trigonal crystal form. *Journal of Molecular Biology*, 343(5), 1409–1438.
- Li, N., Florio, S. K., Pettenati, M. J., Rao, P. N., Beavo, J. A., & Baehr, W. (1998). Characterization of human and mouse rod cGMP phosphodiesterase delta subunit (PDE6D) and chromosomal localization of the human gene. *Genomics*, 49(1), 76–82.
- Liang, Y., Fotiadis, D., Filipek, S., Saperstein, D. A., Palczewski, K., & Engel, A. (2003). Organization of the G Protein-coupled receptors rhodopsin and opsin in native membranes. *Journal of Biological Chemistry*, 278(24), 21655–21662.
- Liebman, P. A., Weiner, H. L., & Drzymala, R. E. (1982). Lateral diffusion of visual pigment in rod disk membranes. *Methods Enzymology*, 81, 660–668.
- Litman, B. J., Niu, S. L., Polozova, A., & Mitchell, D. C. (2001). The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: Visual transduction. *Journal of Molecular Neuroscience*, 16(2–3), 237–242. discussion 279–284.
- Lobanova, E. S., Finkelstein, S., Song, H., Tsang, S. H., Chen, C. K., Sokolov, M., et al. (2007). Transducin translocation in rods is triggered by saturation of the GTPase-activating complex. *Journal of Neuroscience*, 27(5), 1151–1160.
- Maeda, T., Imanishi, Y., & Palczewski, K. (2003). Rhodopsin phosphorylation: 30 years later. *Progress in Retinal and Eye Research*, 22(4), 417–434.
- Makino, C. L., Dodd, R. L., Chen, J., Burns, M. E., Roca, A., Simon, M. I., et al. (2004). Recoverin regulates light-dependent phosphodiesterase activity in retinal rods. *Journal of General Physiology*, 123(6), 729–741.
- Malinski, J. A., & Wensel, T. G. (1992). Membrane stimulation of cGMP phosphodiesterase activation by transducin: Comparison of phospholipid bilayers to rod outer segment membranes. *Biochemistry*, 31(39), 9502–9512.
- Martin, R. E., Elliott, M. H., Brush, R. S., & Anderson, R. E. (2005). Detailed characterization of the lipid composition of detergent-resistant membranes from photoreceptor rod outer segment membranes. *Investigative Ophthalmology & Visual Science*, 46(4), 1147–1154.
- Matulef, K., & Zagotta, W. N. (2003). Cyclic nucleotide-gated ion channels. *Annual Review of Cell and Developmental Biology*, 19, 23–44.
- McKibbin, C., Farmer, N. A., Jeans, C., Reeves, P. J., Khorana, H. G., Wallace, B. A., et al. (2007). Opsin stability and folding: Modulation by phospholipid bicelles. *Journal of Molecular Biology*, 374(5), 1319–1332.
- Medkova, M., Preininger, A. M., Yu, N. J., Hubbell, W. L., & Hamm, H. E. (2002). Conformational changes in the amino-terminal helix of the G protein alpha(11) following dissociation from Gbetagamma subunit and activation. *Biochemistry*, 41(31), 9962–9972.
- Melia, T. J., Malinski, J. A., He, F., & Wensel, T. G. (2000). Enhancement of phototransduction protein interactions by lipid surfaces. *Journal of Biological Chemistry*, 275(05), 3535–3542.
- Melia, T. J., Sowa, M. E., Sokal, I., Dizhoor, A. M., Baehr, W., Palczewski, K., et al. (1999). Formation of helical protein assemblies of IgG and transducin on varied lipid tubules. *Journal of Structural Biology*, 128(1), 119–130.
- Mendez, A., Burns, M. E., Sokal, I., Dizhoor, A. M., Baehr, W., Palczewski, K., et al. (2001). Role of guanylate cyclase-activating proteins (GCAPs) in setting the flash sensitivity of rod photoreceptors. *Proceedings of the National Academy of Sciences of the United States of America*, 98(17), 9948–9953.
- Meng, E. C., Pettersen, E. F., Couch, G. S., Huang, C. C., & Ferrin, T. E. (2006). Tools for integrated sequence-structure analysis with UCSF Chimera. *BMC Bioinformatics*, 7(1), 339.
- Mitchell, D. C., Niu, S. L., & Litman, B. J. (2001). Optimization of receptor-G protein coupling by bilayer lipid composition. I: Kinetics of rhodopsin-transducin binding. *Journal of Biological Chemistry*, 276(46), 42801–42806.
- Mitchell, D. C., Niu, S. L., & Litman, B. J. (2003a). DHA-Rich phospholipids optimize G-Protein-coupled signaling. *Journal of Pediatrics*, 143(4 Suppl.), S80–S86.
- Mitchell, D. C., Niu, S. L., & Litman, B. J. (2003b). Enhancement of G protein-coupled signaling by DHA phospholipids. *Lipids*, 38(4), 437–443.
- Molday, R. S. (1996). Calmodulin regulation of cyclic-nucleotide-gated channels. *Current Opinion in Neurobiology*, 6(4), 445–452.
- Molday, R. S., Hicks, D., & Molday, L. (1987). Peripherin. A rim-specific membrane protein of rod outer segment discs. *Investigative Ophthalmology & Visual Science*, 28(1), 50–61.
- Molday, R. S., & Molday, L. L. (1998). Molecular properties of the cGMP-gated channel of rod photoreceptors. *Vision Research*, 38(10), 1315–1323.
- Molday, R. S., Warren, R., Loewen, C., & Molday, L. (1999). Cyclic GMP-gated channel and peripherin/rds-rom-1 complex of rod cells. *Novartis Foundation Symposium*, 224, 249–261. discussion 261–244.
- Montal, M. (1976). Rhodopsin in bilayer membranes. *Biochemical Society Transactions*, 4(4), 560–561.
- Murray, D., McLaughlin, S., & Honig, B. (2001). The role of electrostatic interactions in the regulation of the membrane association of G protein beta gamma heterodimers. *Journal of Biological Chemistry*, 276(48), 45153–45159.
- Nair, K. S., Balasubramanian, N., & Slepak, V. Z. (2002). Signal-dependent translocation of transducin, RGS9-1-Gbeta5L complex, and arrestin to detergent-resistant membrane rafts in photoreceptors. *Current Biology*, 12(5), 421–425.
- Neubert, T. A., & Hurley, J. B. (1998). Functional heterogeneity of transducin alpha subunits. *FEBS Letters*, 422(3), 343–345.
- Neubert, T. A., Johnson, R. S., Hurley, J. B., & Walsh, K. A. (1992). The rod transducin alpha subunit amino terminus is heterogeneously fatty acylated. *Journal of Biological Chemistry*, 267(26), 18274–18277.
- Nickell, S., Park, P. S., Baumeister, W., & Palczewski, K. (2007). Three-dimensional architecture of murine rod outer segments determined by cryoelectron tomography. *Journal of Cell Biology*, 177(5), 917–925.
- Nishiguchi, K. M., Sandberg, M. A., Kooijman, A. C., Martemyanov, K. A., Pott, J. W., Hagstrom, S. A., et al. (2004). Defects in RGS9 and its anchor protein R9AP in patients with slow photoreceptor deactivation. *Nature*, 427(6969), 75–78.
- Niu, S. L., Mitchell, D. C., & Litman, B. J. (2001). Optimization of receptor-G protein coupling by bilayer lipid composition. II: Formation of metarhodopsin II-transducin complex. *Journal of Biological Chemistry*, 276(46), 42807–42811.
- Niu, S. L., Mitchell, D. C., & Litman, B. J. (2002). Manipulation of cholesterol levels in rod disk membranes by methyl-beta-cyclodextrin: Effects on receptor activation. *Journal of Biological Chemistry*, 277(23), 20139–20145.
- Norton, A. W., Hosier, S., Terew, J. M., Li, N., Dhingra, A., Vardi, N., et al. (2005). Evaluation of the 17-kDa prenyl-binding protein as a regulatory protein for phototransduction in retinal photoreceptors. *Journal of Biological Chemistry*, 280(2), 1248–1256.
- Ohguro, H., Fukada, Y., Takao, T., Shimonishi, Y., Yoshizawa, T., & Akino, T. (1991). Carboxyl methylation and farnesylation of transducin gamma-subunit synergistically enhance its coupling with metarhodopsin II. *EMBO Journal*, 10(12), 3669–3674.
- Okada, T., Sugihara, M., Bondar, A. N., Elstner, M., Entel, P., & Buss, V. (2004). The retinal conformation and its environment in rhodopsin in light of a new 2.2 Å crystal structure. *Journal of Molecular Biology*, 342(2), 571–583.
- Oldham, W. M., Van Eps, N., Preininger, A. M., Hubbell, W. L., & Hamm, H. E. (2006). Mechanism of the receptor-catalyzed activation of heterotrimeric G proteins. *Nature Structural and Molecular Biology*, 13(9), 772–777.
- Olshetskaya, E. V., Ermilov, A. N., & Dizhoor, A. M. (1999). Dimerization of guanylyl cyclase-activating protein and a mechanism of photoreceptor guanylyl cyclase activation. *Journal of Biological Chemistry*, 274(36), 25583–25587.
- Olshetskaya, E. V., Hughes, R. E., Hurley, J. B., & Dizhoor, A. M. (1997). Calcium binding, but not a calcium-myristoyl switch, controls the ability of guanylyl cyclase-activating protein GCAP-2 to regulate photoreceptor guanylyl cyclase. *Journal of Biological Chemistry*, 272(22), 14327–14333.

- Ong, O. C., Ota, I. M., Clarke, S., & Fung, B. K. (1989). The membrane binding domain of rod cGMP phosphodiesterase is posttranslationally modified by methyl esterification at a C-terminal cysteine. *Proceedings of the National Academy of Sciences of the United States of America*, 86(23), 9238–9242.
- Oprian, D. D. (1992). Molecular determinants of spectral properties and signal transduction in the visual pigments. *Current Opinion in Neurobiology*, 2(4), 428–432.
- Otto-Bruc, A. E., Fariss, R. N., Van Hooser, J. P., & Palczewski, K. (1998). Phosphorylation of photolyzed rhodopsin is calcium-insensitive in retina permeabilized by alpha-toxin. *Proceedings of the National Academy of Sciences of the United States of America*, 95(25), 15014–15019.
- Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., et al. (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*, 289(5480), 739–745.
- Palczewski, K., Subbaraya, I., Gorczyca, W. A., Helekar, B. S., Ruiz, C. C., Ohguro, H., et al. (1994). Molecular cloning and characterization of retinal photoreceptor guanylyl cyclase-activating protein. *Neuron*, 13(2), 395–404.
- Peng, C., Rich, E. D., & Varnum, M. D. (2004). Subunit configuration of heteromeric cone cyclic nucleotide-gated channels. *Neuron*, 42(3), 401–410.
- Penn, R. B., Pronin, A. N., & Benovic, J. L. (2000). Regulation of G protein-coupled receptor kinases. *Trends in Cardiovascular Medicine*, 10(2), 81–89.
- Pentia, D. C., Hosier, S., & Cote, R. H. (2006). The glutamic acid-rich protein-2 (GAR2) is a high affinity rod photoreceptor phosphodiesterase (PDE6)-binding protein that modulates its catalytic properties. *Journal of Biological Chemistry*, 281(9), 5500–5505.
- Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., et al. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, 25(13), 1605–1612.
- Pitcher, J. A., Freedman, N. J., & Lefkowitz, R. J. (1998). G protein-coupled receptor kinases. *Annual Review of Biochemistry*, 67, 653–692.
- Poo, M., & Cone, R. A. (1973). Lateral diffusion of rhodopsin in the visual receptor membrane. *Journal of Supramolecular Structure*, 1(4), 354.
- Poo, M., & Cone, R. A. (1974). Lateral diffusion of rhodopsin in the photoreceptor membrane. *Nature*, 247(441), 438–441.
- Premont, R. T., & Gainetdinov, R. R. (2007). Physiological roles of G protein-coupled receptor kinases and arrestins. *Annual Review of Physiology*, 69, 511–534.
- Prosser, R. S., Evanics, F., Kitevski, J. L., & Al-Abdul-Wahid, M. S. (2006). Current applications of bicelles in NMR studies of membrane-associated amphiphiles and proteins. *Biochemistry*, 45(28), 8453–8465.
- Pugh, E. N., Jr., Duda, T., Sitarayayya, A., & Sharma, R. K. (1997). Photoreceptor guanylate cyclases: A review. *Bioscience Report*, 17(5), 429–473.
- Qin, N., Pittler, S. J., & Baehr, W. (1992). In vitro isoprenylation and membrane association of mouse rod photoreceptor cGMP phosphodiesterase alpha and beta subunits expressed in bacteria. *Journal of Biological Chemistry*, 267, 8458–8463.
- Qin, N. P. S. J. B. W. (1992). In vitro isoprenylation and membrane association of mouse rod photoreceptor cGMP phosphodiesterase alpha and beta subunits expressed in bacteria. *Journal of Biological Chemistry*, 267, 8458–8463.
- Ramamurthy, V., Tucker, C., Wilkie, S. E., Daggett, V., Hunt, D. M., & Hurley, J. B. (2001). Interactions within the coiled-coil domain of RetGC-1 guanylyl cyclase are optimized for regulation rather than for high affinity. *Journal of Biological Chemistry*, 276(28), 26218–26229.
- Ridge, K. D., Marino, J. P., Ngo, T., Ramon, E., Brabazon, D. M., & Abdulaev, N. G. (2006). NMR analysis of rhodopsin–transducin interactions. *Vision Research*, 46(27), 4482–4492.
- Roof, D. J., & Heuser, J. E. (1982). Surfaces of rod photoreceptor disk membranes: Integral membrane components. *Journal of Cell Biology*, 95(2 Pt 1), 487–500.
- Roof, D. J., Korenbrot, J. I., & Heuser, J. E. (1982). Surfaces of rod photoreceptor disk membranes: Light-activated enzymes. *Journal of Cell Biology*, 95(2 Pt 1), 501–509.
- Rosenzweig, D. H., Nair, K. S., Wei, J., Wang, Q., Garwin, G., Saari, J. C., et al. (2007). Subunit dissociation and diffusion determine the subcellular localization of rod and cone transducins. *Journal of Neuroscience*, 27(20), 5484–5494.
- Sampath, A. P., Strissel, K. J., Elias, R., Arshavsky, V. Y., McGinnis, J. F., Chen, J., et al. (2005). Recoverin improves rod-mediated vision by enhancing signal transmission in the mouse retina. *Neuron*, 46(3), 413–420.
- Schertler, G. F., Villa, C., & Henderson, R. (1993). Projection structure of rhodopsin. *Nature*, 362(6422), 770–772.
- Schnetkamp, P. P. (1989). Na–Ca or Na–Ca–K exchange in rod photoreceptors. *Progress in Biophysics and Molecular Biology*, 54(1), 1–29.
- Seitz, H. R., Heck, M., Hofmann, K. P., Alt, T., Pellaud, J., & Seelig, A. (1999). Molecular determinants of the reversible membrane anchorage of the G-protein transducin. *Biochemistry*, 38(25), 7950–7960.
- Senin, I. I., Hoppner-Heitmann, D., Polkovnikova, O. O., Churumova, V. A., Tikhomirova, N. K., Philippov, P. P., et al. (2004). Recoverin and rhodopsin kinase activity in detergent-resistant membrane rafts from rod outer segments. *Journal of Biological Chemistry*, 279(47), 48647–48653.
- Seno, K., Kishimoto, M., Abe, M., Higuchi, Y., Mieda, M., Owada, Y., et al. (2001). Light- and guanosine 5'-3-O-(thio)triphosphate-sensitive localization of a G protein and its effector on detergent-resistant membrane rafts in rod photoreceptor outer segments. *Journal of Biological Chemistry*, 276(24), 20813–20816.
- Shibukawa, Y., Kang, K. J., Kinjo, T. G., Szerencsei, R. T., Altimimi, H. F., Pratikhya, P., et al. (2007). Structure–function relationships of the NCKX2 Na⁺/Ca²⁺–K⁺ exchanger. *Annals of the New York Academy of Sciences*, 1099, 16–28.
- Shichida, Y., & Morizumi, T. (2007). Mechanism of G-protein activation by rhodopsin. *Photochemistry and Photobiology*, 83(1), 70–75.
- Shroyer, N. F., Lewis, R. A., Yatsenko, A. N., Wensel, T. G., & Lupski, J. R. (2001). Coregulation and functional analysis of mutant ABCR (ABCA4) alleles in families that manifest both Stargardt disease and age-related macular degeneration. *Human and Molecular Genetics*, 10(23), 2671–2678.
- Skiba, N. P., Artemyev, N. O., & Hamm, H. E. (1995). The carboxyl terminus of the gamma-subunit of rod cGMP phosphodiesterase contains distinct sites of interaction with the enzyme catalytic subunits and the alpha-subunit of transducin. *Journal of Biological Chemistry*, 270(22), 13210–13215.
- Slep, K. C., Kercher, M. A., He, W., Cowan, C. W., Wensel, T. G., & Sigler, P. B. (2001). Structural determinants for regulation of phosphodiesterase by a G protein at 2.0 Å. *Nature*, 409(6823), 1071–1077.
- Slepak, V. Z., Artemyev, N. O., Zhu, Y., Dumke, C. L., Sabacan, L., Sondek, J., et al. (1995). An effector site that stimulates G-protein GTPase in photoreceptors. *Journal of Biological Chemistry*, 270(24), 14319–14324.
- Sommer, M. E., Smith, W. C., & Farrens, D. L. (2006). Dynamics of arrestin–rhodopsin interactions: Acidic phospholipids enable binding of arrestin to purified rhodopsin in detergent. *Journal of Biological Chemistry*, 281(14), 9407–9417.
- Sowa, M. E., He, W., Slep, K. C., Kercher, M. A., Lichtarge, O., & Wensel, T. G. (2001). Prediction and confirmation of a site critical for effector regulation of RGS domain activity. *Nature Structural Biology*, 8(3), 234–237.
- Sprang, S. R. (1997a). G protein mechanisms: Insights from structural analysis. *Annual Review of Biochemistry*, 66, 639–678.
- Sprang, S. R. (1997b). G proteins, effectors and GAPS: Structure and mechanism. *Current Opinion in Structural Biology*, 7(6), 849–856.
- Sprang, S. R. (2000). Conformational display: A role for switch polymorphism in the superfamily of regulatory GTPases. *Science's STKE*, 2000(50), PE1.
- Sprang, S. R., Chen, Z., & Du, X. (2007). Structural basis of effector regulation and signal termination in heterotrimeric Gα proteins. *Advances in Protein Chemistry*, 74, 1–65.
- Stephen, R., Bereta, G., Golczak, M., Palczewski, K., & Sousa, M. C. (2007). Stabilizing function for myristoyl group revealed by the crystal structure of a neuronal calcium sensor, guanylate cyclase-activating protein 1. *Structure*, 15(11), 1392–1402.
- Struppe, J., Komives, E. A., Taylor, S. S., & Vold, R. R. (1998). 2H NMR studies of a myristoylated peptide in neutral and acidic phospholipid bicelles. *Biochemistry*, 37(44), 15523–15527.
- Sun, H., Molday, R. S., & Nathans, J. (1999). Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. *Journal of Biological Chemistry*, 274(12), 8269–8281.
- Sun, H., & Nathans, J. (1997). Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. *Nature Genetics*, 17(1), 15–16.
- Tachibanaki, S., Arinobu, D., Shimauchi-Matsukawa, Y., Tsushima, S., & Kawamura, S. (2005). Highly effective phosphorylation by G protein-coupled receptor kinase 7 of light-activated visual pigment in cones. *Proceedings of the National Academy of Sciences of the United States of America*, 102(26), 9329–9334.
- Trudeau, M. C., & Zagotta, W. N. (2002). Mechanism of calcium/calmodulin inhibition of rod cyclic nucleotide-gated channels. *Proceedings of the National Academy of Sciences of the United States of America*, 99(12), 8424–8429.
- Tucker, C. L., Woodcock, S. C., Kellsell, R. E., Ramamurthy, V., Hunt, D. M., & Hurley, J. B. (1999). Biochemical analysis of a dimerization domain mutation in RetGC-1 associated with dominant cone-rod dystrophy. *Proceedings of the National Academy of Sciences of the United States of America*, 96(16), 9039–9044.
- Vishnivetskiy, S. A., Raman, D., Wei, J., Kennedy, M. J., Hurley, J. B., & Gurevich, V. V. (2007). Regulation of arrestin binding by rhodopsin phosphorylation level. *Journal of Biological Chemistry*, 282(44), 32075–32083.
- Vogel, A., Schroder, T., Lange, C., & Huster, D. (2007). Characterization of the myristoyl lipid modification of membrane-bound GCAP-2 by 2H solid-state NMR spectroscopy. *Biochimica et Biophysica Acta*, 1768(12), 3171–3181.
- Wada, Y., Sugiyama, J., Okano, T., & Fukada, Y. (2006). GRK1 and GRK7: Unique cellular distribution and widely different activities of opsin phosphorylation in the zebrafish rods and cones. *Journal of Neurochemistry*, 98(3), 824–837.
- Weitz, D., Ficek, N., Kremmer, E., Bauer, P. J., & Kaupp, U. B. (2002). Subunit stoichiometry of the CNG channel of rod photoreceptors. *Neuron*, 36(5), 881–889.
- Wensel, T. G., & Stryer, L. (1986). Reciprocal control of retinal rod cyclic GMP phosphodiesterase by its gamma subunit and transducin. *Proteins*, 1(1), 90–99.
- Wey, C. L., Cone, R. A., & Edidin, M. A. (1981). Lateral diffusion of rhodopsin in photoreceptor cells measured by fluorescence photobleaching and recovery. *Biophysical Journal*, 33(2), 225–232.
- Wiszniewski, W., Zaremba, C. M., Yatsenko, A. N., Jamrich, M., Wensel, T. G., Lewis, R. A., et al. (2005). ABCA4 mutations causing mislocalization are found frequently in patients with severe retinal dystrophies. *Human and Molecular Genetics*, 14(19), 2769–2778.
- Womack, K. B., Gordon, S. E., He, F., Wensel, T. G., Lu, C. C., & Hilgemann, D. W. (2000). Do phosphatidylinositides modulate vertebrate phototransduction? *Journal of Neuroscience*, 20(8), 2792–2799.
- Wrigley, J. D., Ahmed, T., Nevett, C. L., & Findlay, J. B. (2000). Peripherin/rds influences membrane vesicle morphology. Implications for retinopathies. *Journal of Biological Chemistry*, 275(18), 13191–13194.
- Yamazaki, M., Li, N., Bondarenko, V. A., Yamazaki, R. K., Baehr, W., & Yamazaki, A. (2002). Binding of cGMP to GAF domains in amphibian rod photoreceptor cGMP phosphodiesterase (PDE). Identification of GAF domains in PDE alpha subunits and distinct domains in the PDE gamma subunit involved in

- stimulation of cGMP binding to GAF domains. *Journal of Biological Chemistry*, 277(43), 40675–40686.
- Yang, Z., & Wensel, T. G. (1992). N-myristoylation of the rod outer segment G protein, transducin, in cultured retinas. *Journal of Biological Chemistry*, 267(32), 23197–23201.
- Yu, H., Olshevskaya, E., Duda, T., Seno, K., Hayashi, F., Sharma, R. K., et al. (1999). Activation of retinal guanylyl cyclase-1 by Ca^{2+} binding proteins involves its dimerization. *Journal of Biological Chemistry*, 274(22), 15547–15555.
- Zagotta, W. N., Olivier, N. B., Black, K. D., Young, E. C., Olson, R., & Gouaux, E. (2003). Structural basis for modulation and agonist specificity of HCN pacemaker channels. *Nature*, 425(6954), 200–205.
- Zhang, H., Liu, X. H., Zhang, K., Chen, C. K., Frederick, J. M., Prestwich, G. D., et al. (2004a). Photoreceptor cGMP phosphodiesterase delta subunit (PDEdelta) functions as a prenyl-binding protein. *Journal of Biological Chemistry*, 279(1), 407–413.
- Zhang, Z., Melia, T. J., He, F., Yuan, C., McGough, A., Schmid, M. F., et al. (2004b). How a G protein binds a membrane. *Journal of Biological Chemistry*, 279(32), 33937–33945.
- Zheng, J., Trudeau, M. C., & Zagotta, W. N. (2002). Rod cyclic nucleotide-gated channels have a stoichiometry of three CNGA1 subunits and one CNGB1 subunit. *Neuron*, 36(5), 891–896.
- Zhong, H., Molday, L. L., Molday, R. S., & Yau, K. W. (2002). The heteromeric cyclic nucleotide-gated channel adopts a 3A:1B stoichiometry. *Nature*, 420(6912), 193–198.
- Zozulya, S., & Stryer, L. (1992). Calcium-myristoyl protein switch. *Proceedings of the National Academy of Sciences of the United States of America*, 89(23), 11569–11573.