Light-induced Reorganization of Phospholipids in Rod
Disc Membranes*

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The transbilayer redistribution of spin-labeled phospholipid analogues (SL-PL) with choline, serine, and ethanolamine head groups (PC, PS, and PE, respectively) was studied on intact disc vesicles of bovine rod outer segment membranes in the dark and after illumination. Redistribution was measured by the extraction of spin-labeled lipid analogues from the outer leaflet of membrane using the bovine serum albumin back-exchange assay. In the dark, PS was distributed symmetrically, favoring the outer leaflet, whereas PC and PE showed small if any asymmetry. Green illumination for 1 min caused lipid head group-specific reorganization of SL-PL. Extrac tion of SL-PS by bovine serum albumin showed a fast transient (<10 min) enhancement, which was further augmented by a peptide stabilizing the active metarhodopsin II conformation. The data suggest a direct release of 1 molecule of bound PS per rhodopsin into the outer leaflet and subsequent redistribution between the two leaflets. SL-PE and SL-PC showed more complex kinetics, in both cases consistent with a prolonged period of reduced extraction (2 phospholipids per rhodopsin in each case). The different phases of SL-PL reorganization after illumination may be related to the formation and decay of the active rhodopsin species and to their subsequent regeneration process.

Rhodopsin, the photoreceptor of the retinal rod, and its G-protein transducin (Gt) are archetypes of the G-protein-coupled receptor and heterotrimeric G-protein families, respectively. Rhodopsin is embedded in the membranes of the rod outer segment, which are arranged in a long, closely spaced stack of disc-like sacules. Gt is bound to the cytoplasmic surface of the disc membranes. To provide an effective target for the light, rhodopsin is very densely packed, so that the receptor accounts for half of the dry weight of the disc membranes. It is composed of the apoprotein opsin, comprising seven transmembrane helices and the chromophore 11-cis-retinal, which is covalently bound to Lys296 in the seventh helix via a protonated Schiff base and acts as a highly effective inverse agonist. Following the absorption of a photon, the retinal isomerizes to a strained all-trans conformation, which induces a series of conformational rearrangements of the opsin moiety. The final product of this reaction sequence is the active metarhodopsin II (Meta II) conformation, which contains all-trans-retinal as a covalently bound agonist, and is capable of catalyzing the activation of the retinal G-protein transducin (for reviews see Refs. 1–3). Identified physicochemical events that accompany the transition into the active state include proton transfer reactions and helix movements. Deprotonation of the Schiff base bond, protonation of its counterion Glu113, and the uptake of a proton from the aqueous solution (4) mediated by the highly conserved opsin residue Glu134 are determinants of the active state. Based on computer simulations and mutagenesis studies, similar results have been obtained for the α1b-adrenergic receptor (5, 6).

The disc membrane is distinguished by its content of large amounts of highly unsaturated (22:6n-3) acyl chains in its major phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylethanolamine (PE) (7). A lack of polyunsaturated fatty acids causes abnormalities in visual function (8, 9). The high fluidity and other properties of the membrane’s special composition may be crucial for the anchoring of G-protein and effector to the membrane surface, and thus, for proper signal transduction in the disc membrane. Understanding of the role of membrane properties in signal transduction is, at the physicochemical level, still incomplete. However, with regard to photoreceptor function, there are two identified determinants, namely, the formation of the active intermediate and the transport of the hydrophobic retinal ligand.

Rhodopsin’s active Meta II conformation depends on the composition (10) and the physical properties of the lipid bilayer (11, 12). Its formation goes along with a number of physicochemical alterations, including a considerable reaction volume, and changes of the interfacial potential, positive relative to the aqueous exterior of the disc, within a boundary region of the native isolated disc membrane near the cytoplasmic surface. Electrical effects can be measured in situ, namely, as a component of the “early receptor potential.” The most recent development is the “early receptor current” technique, realized in an unicellular expression system (13). Fourier transform infrared spectroscopy showed changes in the molecular environment of lipid after transition of rhodopsin to Meta II (14). After the decay of the active photoprotein into the apoprotein opsin and
the photolyzed chromophore, all-trans-retinal, the phospholipid PE forms the condensation product N-retinylidene-PE (15), suggested to be the putative substrate for the ABC transporter. A concurrent process is the formation of an active complex between the retinal and the apoprotein, which depends on the presence of the palmitates at C-terminal cysteines, thus suggesting a role of lipid host in its formation (16).

Clearly, understanding the role of phospholipids in these functions will require information about the lateral and transbilayer distribution and dynamics of lipids in the disc membrane. The transbilayer distribution of the major phospholipids in disc membranes in the dark is nearly symmetric for PC and PE but asymmetric for PS, favoring the outer (cytoplasmic) leaflet of the membrane (17, 18). The electrical asymmetry of the rhodopsin molecule, normal to the membrane plane, has been made responsible for the asymmetry of the lipid distribution strongly supported by studies on disc membranes in the dark (19, 20). Thus, rhodopsin plays an essential role in organization of the disc membrane lipid phase. Accordingly, it is reasonable to anticipate that activation of rhodopsin affects its interaction with and the organization of the lipid phase as well. However, despite the quite detailed literature about the resting dark state, the problem of measuring possible effects of the light stimulus on the distribution of the different lipids has never been addressed.

In this study, we therefore explored the possibility to assay the distribution of phospholipids in disc membranes after illumination, using spin-labeled lipid analogues (SL-PC, SL-PS, and SL-PE) and the BSA back-exchange assay (21–23). Analyzing the ESR spectra of both the supernatants and the membranes, we found characteristic time-dependent changes in the amount of SL-PL extractable from the outer membrane leaflet after illumination with activating wavelengths. Peptides from the G_{12} sequence, which are known to stabilize active Meta II, enhance the extraction of PS from the outer leaflet, supporting a direct role of the active photoprotein. The effects observed with SL-PC and SL-PE are complex and may reflect an influence of these lipids in retinal binding and transport.

**EXPERIMENTAL PROCEDURES**

**Materials**—Spin-labeled phospholipid analogues were synthesized as described previously (21–23). Synthesis and purification of the peptide G_{12}-(340–350)HAA were as described before (24). The amino acid sequence of the high-affinity analogue of G_{12}-(340–350) was G_{12}-(340–350)HAA; VLDLKSCEGLF. All chemicals were purchased from Merck (Darmstadt, Germany), Fluka (Neu-Ulm, Germany), Roche Molecular Biochemicals (Mannheim, Germany), and Sigma Chemical Co. (St. Louis, MO).

**Preparation of Disc Vesicles**—All procedures were carried out in dim red light. The bovine ROS membranes were isolated from frozen bovine retina (50 per preparation) and prepared according to standard procedure (25). Disc vesicles were prepared following a previous procedure (26) with modifications. Briefly, fresh prepared ROS were shocked hypotonically in aqueous and centrifuged, and the resulting pellet was resuspended in 3.5% (w/v) Ficoll 400. The sample was left for 12 h at 4 °C to allow complete osmotic bursting of the ROS. The intact discs were harvested from the surface of the Ficoll 400 solution after centrifugation at 132,000  ×  g for 2 h at 4 °C. Subsequently, the discs were washed in 5% (w/v) sucrose and resuspended in sucrose/Hepes buffer (5% (w/v) sucrose, 100 mM Hepes, pH 7.0). To prepare isolated discs the suspension was pressed through a filter with 2-μm pores (Nucleopore filter) and were used immediately. All measurements were done in the vesicle suspension medium (5% (w/v) sucrose solution, 100 mM Hepes buffer, 1 mM Mg-ATP, pH 7.0 ± 0.1, osmotic pressure of 380 ± 20 mosmol) in the dark at 20 °C.

**Rhodopsin Determination**—The rhodopsin concentration was determined spectrophotometrically (extinction coefficient of 40,000 cm m⁻¹ in the presence of 5% (w/v) lauryldimethylamine oxide. The disc suspensions were diluted in their respective medium to a final concentration of 120 μM rhodopsin (stock suspension).

**ATP Assay**—The ATP content of preparations was measured using the luminescent assay kit HS II containing the luciferin-luciferase assay. The emitted light was detected using a luminometer (LB 9500, Berthold Co., Germany).

**Incubation with Peptide**—The interaction of the synthetic peptide G_{12}-(340–350)HAA with rhodopsin was used to study the influence on the phospholipid distribution in disc membranes (pH 7.0) after illumination at 20 °C. The vesicle suspension containing 20 μM rhodopsin was preincubated with 200 μM G_{12}-(340–350)HAA in the dark. The kinetics of the spin-labeled phospholipid distribution was followed as described.

**Spin Labeling of Disc Membranes with SL-PL**—Spin-labeled phospholipid analogues carrying a long-chain fatty acid at the sn-1 position and a short spin-labeled fatty acid at the sn-2 position with a paramagnetic nitroxide group at the fourth carbon position were synthesized as described above.

Ten minutes before labeling, the disc membranes were supplemented with 5 μM DFP at 20 °C to minimize hydrolysis of spin-labeled phospholipids. For labeling in the dark, disc membrane suspensions (20 μM rhodopsin concentration) were mixed with the label suspension representing time zero for all kinetic measurements. The final label concentration corresponded to 2 mol% of total phospholipids.

The rapid incorporation of analogues into the disc membrane (data not shown) was in agreement with our recent study found in various disc membrane preparations (pH 7.4) at 25 °C (18). After insertion of spin-labeled phospholipids into the outer membrane leaflet of disc membranes in the dark state, the problem of measuring possible effects of the light stimulus on the distribution of the different lipids has never been addressed.

In this study, we therefore explored the possibility to assay the distribution of phospholipids in disc membranes after illumination, using spin-labeled lipid analogues (SL-PC, SL-PS, and SL-PE) and the BSA back-exchange assay (21–23). Analyzing the ESR spectra of both the supernatants and the membranes, we found characteristic time-dependent changes in the amount of SL-PL extractable from the outer membrane leaflet after illumination with activating wavelengths. Peptides from the G_{12} sequence, which are known to stabilize active Meta II, enhance the extraction of PS from the outer leaflet, supporting a direct role of the active photoprotein. The effects observed with SL-PC and SL-PE are complex and may reflect an influence of these lipids in retinal binding and transport.
of the extractable lipid and a final maximum level with a value of 4% at \( t = 70 \text{ min} \) that decayed slowly to the dark level.

As a control, the vesicle suspension was illuminated with 670-nm light, which does not activate rhodopsin. Under this condition, no significant light-induced effect was found with any of the SL-PLs.

To elucidate whether the effect of illumination with 520 nm on extraction of SL-PS and SL-PC depends on ATP, the experiments were performed in the absence of Mg-ATP. No influence was found for SL-PS and SL-PC as presented in Fig. 4, A and B. To study the effect of rapid Meta II decay (29), some suspensions were incubated with hydroxylamine (0.2 and 1 mM for 10 min at 20 °C) before addition of the SL-PL. However, these samples gave unstable data, presumably because of a direct effect of hydroxylamine on the spin-label compounds (data not shown).

**Influence of the Peptide \( G_\alpha-(340-350)\text{HAA} \) on the Phospholipid Distribution after Illumination**—The synthetic peptide \( G_\alpha-(340-350)\text{HAA} \) interacts specifically with Meta II and can therefore be used to specify the measured effects of illumination. The vesicle suspension (20 \( \mu \text{M} \) rhodopsin) was preincubated with 200 \( \mu \text{M} \) \( G_\alpha-(340-350)\text{HAA} \). In the dark, SL-PL redistribution occurred with the same kinetics in the presence or absence of the peptide (data not shown). Fig. 5 shows measurements for SL-PS in the membrane pellet that followed the same protocol as was applied in Figs. 2 and 3. \( G_\alpha-(340-350)\text{HAA} \) seems to enhance the effect of illumination on extractable SL-PS, although no significant effect was found for SL-PC (Fig. 6).

**DISCUSSION**

In this study we examined the effect of a functional associated conformational change of a receptor membrane protein, the rhodopsin molecule on the organization of the phospholipid bilayer. Rhodopsin belongs to that class of membrane-spanning receptors, which transmit their signal into the cell interior by coupling to heterotrimeric guanine nucleotide-binding proteins or G-proteins (G-protein-coupled receptors). The G-protein het-

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**Fig. 1.** Distribution of phospholipids PC, PS, and PE between the two leaflets of bovine disc membranes in the dark. Values are expressed in mol% of total phospholipid (see text for details).

**Fig. 2.** Spin-labeled phosphatidylserine extracted by BSA from disc vesicles after illumination with 520-nm light. SL-PS (2 mol% of total phospholipid) was incorporated into the outer leaflet of disc membranes (20 \( \mu \text{M} \) rhodopsin, disc medium, pH 7.0, with 1 mM Mg-ATP) in the dark at 20 °C (\( t = 0 \)). At the times indicated, aliquots of the disc suspensions were collected and the fraction of analogues present in the membrane was assayed by back-exchange on BSA. After 60 min, the suspensions were illuminated (520 nm, 1 min). The further redistribution of SL-PS was immediately determined in 1-min intervals in the dark at 20 °C. The percentage of SL-PS that remained nonextractable is plotted versus time, for the sample in the dark (means and standard deviations, filled symbols, \( n = 11 \)) compared with two illuminated samples (open symbols for the largest and gray symbols for the smallest effect after illumination, respectively). The illuminated samples showed an increased extraction of SL-PS by BSA.
tetrailer, composed of the α-subunit (Gα) and the undisso-
ciable βγ-subunit complex (Gβγ) reaches the receptor by dif-
fusion on the cytoplasmic surface of the membrane and couples

FIG. 3. Spin-labeled phospholipids (SL-PL) extracted by BSA
from disc vesicles after illumination with 520-nm light. Protocol
was analogous to Fig. 2. The percentage of SL-PL that remained
non-extractable is plotted versus time, for the sample in the dark and the
illuminated sample (filled and open symbols, respectively) in A–C (A:
SL-PS, n = 11; B: SL-PC, n = 9; C: SL-PE, n = 7; means and standard
deviations, n represents number of preparations). Insets show the ki-
etics for a time interval of 50–75 min for the SL-PL distribution with
and without illumination. (*Significant at p > 0.05 compared with the
dark level.)

FIG. 4. Influence of ATP on nonextractable SL-PL after illumi-
nation with 520-nm light. Protocol was analogous to Figs. 2 and 3,
but with (filled symbols) and without (open symbols) Mg-ATP in the disc
medium (final concentration: 1 mM Mg-ATP). Data are for SL-PS (A)
and SL-PC (B) (data points are means and standard deviations, from
independent measurements of at least three different preparations).

FIG. 5. Influence of Gα(340–350)HAA-peptide on nonextract-
able SL-PS. Spin-labeled lipids (2 mol% of total phospholipids; calcu-
lated from the ESR spectra of the membrane pellet) were added in the
dark with (black symbols) and without (white symbols) preincubation
with 200 μM Gα(340–350)HAA-peptide, presented for four inde-
pendent measurements. Illumination and BSA extraction were as in Figs. 2
and 3. The change of SL-PS extraction after illumination corresponded
to an increased extraction by BSA.

FIG. 6. Influence of Gα(340–350)HAA-peptide on SL-PC ex-
traction. Protocol was analogous to Fig. 5, but data are for SL-PC
presented for two independent measurements. Illumination and BSA
extraction were as in Figs. 2 and 3. The change of SL-PC extraction was
equivalent to a decreased extraction by BSA.

its seven-helix transmembrane structure at the cytoplasmic
face. Stimulus receptors induce exchange of GTP for GDP on
Gα and dissociation of Gα from Gβγ. Both GαGTP and Gβγ
can interact with various membrane-bound effector molecules.

We have studied the effect of the light signal on the organi-
zation of spin-labeled phospholipid analogues in the rod disc
membrane, in particular, the kinetics of phospholipid trans-
membrane movement and their distribution of those analogues
in the dark and after illumination. The transmembrane (re)distrib-
ution was assessed by back-exchange assay of analogues to
BSA. We find a substantial rearrangement of phospholipid
organization as a consequence of the reception of the light signal and formation of the active intermediate in rhodopsin. Although we did not attempt to provide mechanistic conclusions in this initial work, our results provide strong evidence for the dynamic interaction of rhodopsin with the lipid phase of the disc membranes.

The Transbilayer Distribution of SL-PL in the Dark—To obtain a basis for the study of the light-induced effect, we first tested the SL-PL distribution of discs in the dark. It was found that, with the experimental conditions, optimal for studying the light effect (pH 7.0, 20 °C), the SL-PL transbilayer movement and distribution did not significantly differ from those reported previously in media of pH 7.4 of different disc preparations at 25 °C, including those appearing as a preserved native membrane stack (18). After addition of the spin-labeled analogue lipid (2 mol% of the total phospholipid) to the outer leaflet, the transmembrane redistribution of the label between the two leaflets of the disc membrane occurred with a half time of less than 4 min at 20 °C. In the dark, PC and PE are almost symmetrically distributed, but PS shows a pronounced asymmetry with a preference for the outer leaflet (76%). Neither the kinetics nor the final equilibrium level of lipid redistribution depended on ATP, and it did not appear to be affected by cytoskeletal components, asymmetric salt solutions, or other transport systems (18). This establishes a difference between the disc organelle and plasma membrane systems (21, 30–32). The disc membrane is distinguished by its high contents of both the transmembrane protein rhodopsin and of long-chain, polyunsaturated fatty acids. With regard to the fatty acid content, docosahexaenoic acid is with a total fraction of 43 mol% unusually enriched (33). In bovine disc membranes, PS, PE, and PC contain a fraction of polyunsaturated fatty acid of 70.5, 49, and 34.7 mol%, respectively (7). Likely, this composition facilitates transmembrane motion of lipids, which generally appears to depend on lipid chain length and saturation. Bilayers made up from lipids with at least one unsaturated chain have a more rapid flip-flop, as compared with those with saturated lipids (see Refs. 34 and 35 and references therein).

The Role of Rhodopsin in Rapid Phospholipid Reorganization—A high content of intrinsic membrane proteins introduced into pure lipid bilayers generally enhances permeability as well as transversal reorientation of lipids (36, 37). The effect has been interpreted as a mismatch between lipid and protein, decreasing the hydrophobic barrier for the polar head group of lipids. In disc membranes, rhodopsin represents the major protein, accounting for more than 70% of the total ROS protein (38).

The asymmetric charge distribution in rhodopsin may play an important role in the PS distribution at equilibrium. At neutral pH, rhodopsin is expected to be strongly bipolar, with the cytoplasmic surface positively charged and the intradiscal surface negatively charged (19), resulting in a transbilayer potential equal in magnitude to the difference of the surface potentials (20). In the presence of a charged lipid, a new equilibrium state results, with altered charge densities and surface potentials and a reduced transbilayer potential with an asymmetric distribution of the charged lipid. Hubbell and coworkers (19) have specifically suggested the orientation of PS in disc membrane to be driven and stabilized by the electrostatic potential gradient. They predicted an asymmetry for PS with a preference of 75% in the outer leaflet, in agreement with the experiment (17, 18) and with results obtained with other cell membranes (39–41). Hubbell (20) also discussed a coupled movement of other lipids, especially affecting the distribution of PE.

Illumination Specifically Affects the Distribution of Different SL-PL—Illumination of disc vesicles with light of 520 nm, bleaching more than 85% of the rhodopsin, leads to a high concentration of active Meta II in the membranes, in equilibrium with Meta I. Under the conditions (pH 7.0, 20 °C) Meta II is the predominant species. For all lipid analogues investigated, a rapid change in the amount of extractable labeled lipid was seen after illumination, not observed with 670 nm of control illumination. These results yield strong evidence that the origin of the effects is associated with the formation of the active Meta II species in its lipid environment.

Specific properties of the light effect on analogue extraction depend on the head group of the spin-labeled phospholipids. SL-PS was up to 6% more extractable, as compared with dark conditions. The whole phenomenon lasted less than 10 min. The change in extraction was fully reversed within this time. Incubation of the disc vesicles with the G_{α}(340–350) C-terminal peptide resulted in a slightly enhanced SL-PS effect. These observations are consistent with the known peptide stabilization of the Meta II intermediate (24, 42). We thus interpret the results with PS as a direct influence of the conformation adopted in Meta II on the distribution of this lipid between an extractable phase and a BSA-inaccessible phase, presumably the inner and outer leaflet of the membrane (see below).

In contrast to SL-PS, the amount of SL-PC extracted from the membrane’s outer leaflet decreased after illumination. The light induced reorganization decays less rapidly than with PS. The half mean time is ca. 15–20 min under the conditions, consistent with the decay of the active Meta II. With SL-PE, a complicated time course was seen in some samples, which eventually also led to a higher extraction. In terms of phospholipid extraction, 4% of SL-PC and of SL-PE were less extractable. When related to the total amount of phospholipid present in the membranes (28) (rhodopsin is surrounded by about 65 lipids (43)), this would correspond to a reversible outward release of 1 PS and a reduced extraction of 2 PC and 2 PE per activated rhodopsin.

Role of the Active Conformation, Meta II—Several mechanisms may be considered for explaining the altered extraction of analogues upon illumination at 520 nm. In particular, two explanations arise for the observations with the negatively charged PS. It is known that, with the formation of Meta II, an electric transmembrane potential is generated, the so-called R2 wave of the “early receptor potential” (13). This potential change is superimposed to the transmembrane potential that exists in the dark and is due to the asymmetry of the rhodopsin molecule (see above). To explain our observations, we could assume that PS follows the potential change generated by Meta II formation, thus producing a new distribution of PS between outer and inner leaflet, and an altered asymmetry. However, neither the extent (15%) nor the polarity of the light effect fits to the redistribution of 1 PS molecule per Meta II formed.

Another explanation seems more plausible, namely, PS that is bound to rhodopsin in the dark is released into the bulk lipid phase when Meta II is formed. The time course of the reorganization measured with this lipid (Figs. 2 and 3) would then suggest a mechanism as follows: 1) during redistribution in the dark, labeled PS is incorporated into a lipid-binding site of rhodopsin and becomes nonextractable to rapid BSA extraction; 2) when Meta II is formed, bound PS is released into the bulk phase of the outer leaflet, thus explaining its immediate availability for BSA extraction; and 3) PS redistributes between both leaflets, reflected in a decrease of the amount of extractable PS.
Consistently, the return to the dark level occurs with a rate similar to the initial redistribution after addition of the analogue (t = 0; Fig. 3) (18). PS remains in equilibrium with its binding site during redistribution and early decay of Meta II. The effect seen with PC also implies a reorganization of the analogue upon illumination. Its monophasic transition into a more extractable form is followed by a slow decay, with a time course similar to the decay of Meta II (42). The complicated time course seen with PE cannot yet be interpreted. In most samples, a biphasic time course was seen, which was similar to the one seen with PS, but went over into a steady-phase similar to PC. This may reflect different roles of this lipid during and after the lifetime of the active Meta II (see below). Clearly, in the frame of our experimental set up, the time resolution of the back-exchange sets limitations to a detailed quantitative kinetic scheme.

A general feature of the model, namely, the release of lipid from a binding site at rhodopsin on formation of the active intermediate, was also seen in a recent investigation by Fourier transform infrared difference spectroscopy (14). Specific changes in the C=O stretching vibration of an ester group were interpreted as a change in the environment of one single phospholipid molecule from a binding site occupied in the ground state. Because the observations were not significantly different with rhodopsin in its native disc membrane environment or reconstituted into egg PC, a direct comparison with our results is not yet possible. However, the fact that the PS is immediately available for BSA extraction argues for a binding site of SL-PE found in the present study.

Residual Lipid Redistribution after Meta II Decay—Part of the asymmetry arising from the light signal persists over a time longer than the decay of Meta II. With the decay of Meta II and release of the photolyzed chromophore, all-trans-retinal, a noncovalent complex with the apoprotein opsin is formed. It involves a site that is different from the original binding site and depends on rhodopsin palmitoylation (16). Moreover, the PE of the disc membrane can form the Schiff base condensation product N-retinylidene-PE when all-trans-retinal is released (15, 46–48). Even higher complexity is introduced by the rim protein (49, 50), a retina-specific ABC transporter (51), which may function as an outwardly directed flipase for the N-retinylidene-PE, whereas in the absence of this active transport mechanism the Schiff base adduct is thought to accumulate on the luminal face of the disc membrane (15, 48). It remains to be studied how this relates to the redistribution of SL-PE found in the present study.

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REFERENCES

1. Hofmann, K. P. (2000) in Handbook of Biological Physics (Hoff, A. J., Stavenga, D. G., De Grip, W. J., and Pugh, E. N., Jr., eds) pp. 91–142, Elsevier, Amsterdam
2. Stryer, L. (1986) Annu. Rev. Neurosci. 9, 87–119
3. Sakmar, T. P. (1998) Prog. Nucleic Acid Res. Mol. Biol. 59, 1–34
4. Arns, S., and Hofmann, K. P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7549–7553
5. Scheer, A., Fanelli, F., Costa, I., De-Benedetti, P. G., and Cotechi, S. (1997) Proc. Natl. Acad. U. S. A. 94, 808–813
6. Scheer, A., Fanelli, F., Diviani, D., De-Benedetti, P. G., and Cotechi, S. (1999) Eur. J. Biochem. 266, 13–18
7. Fliesler, S. J., and Anderson, R. E. (1983) Prog. Lipid Res. 22, 79–131
8. Bush, R. A., Malnoe, A., Rene, C. E., and Williams, T. P. (1994) Invest. Ophthalmol. Vis. Sci. 35, 51–96, 4898–4903
9. Anderson, R. E., Maude, M. B., Närstrom, K., and Nilsson, S. E. (1997) Exp. Eye Res. 64, 181–187
10. Geisler, N. J., and Brown, M. F. (1993) Biochemistry 32, 2438–2454
11. Mitchell, D. C., Kibbeck, J., and Litman, B. J. (1992) Biochemistry 31, 8107–8111
12. Mitchell, D. C., Lawrence, J. T. R., and Litman, B. J. (1996) J. Biol. Chem. 271, 20907–20916
