Role of the intradiscal domain in rhodopsin assembly and function

(synthetic gene/site-specific mutagenesis/transmembrane receptor/signal transduction/glycosylation)

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ABSTRACT The role of the intradiscal polypeptide loops in bovine rhodopsin has been investigated by deletions in the N-terminal tail and in loops B–C, D–E, and E–F as well as by single amino acid substitutions in the D–E loop. Mutants with three types of phenotypes were observed. Type I mutants showed a rhodopsin-like chromophore and glycosylation. Type II mutants did not regenerate the chromophore and showed abnormal glycosylation. Type III mutants showed poor chromophore regeneration and abnormal glycosylation. Reduced transducin activation was shown by some type I and III mutants. Single amino acid substitutions in the D–E loop gave mostly type I mutants. Deletions in loops B–C, D–E, and F–G gave type II mutants, whereas deletions in the N-terminal tail produced type III mutants. Systematic deletions of two adjacent amino acids in loop D–E indicated that the amino acid sequences 171–182 and 189–192 were essential to rhodopsin structure. Immunofluorescence double-staining and transmission electron microscopy of one type II mutant (with residues 189 and 190 deleted) showed that it was mostly in the endoplasmic reticulum, whereas the wild-type protein was in the plasma membrane. We conclude that the first step in the assembly of the rhodopsin molecule is the formation of a three-dimensional structure in the intradiscal domain involving the bulk of the out-of-the-membrane polypeptide segments followed by the linkage of Cys-110 and Cys-187 through a disulfide bond.

Rhodopsin, the photoreceptor in the vertebrate rod cells (1), consists of a single polypeptide chain of 348 amino acids. It is believed to contain seven transmembrane helical segments (2) (Fig. 1), with roughly half of the protein embedded in the membrane and the other half distributed between the cytoplasmic and intradiscal domains, which contain peptide loops of various sizes and the N- and C-terminal tails. Light activation of rhodopsin prepares the molecule for interaction with transducin, the GTP-binding regulatory protein, and allows its phosphorylation by rhodopsin kinase and subsequent interaction with the 48-kDa protein, S antigen (3). These interactions all occur on the cytoplasmic face of rhodopsin. In contrast, the role of the intradiscal domain in rhodopsin structure and/or function has remained unclear. Recently, systematic replacements of cysteine residues in rhodopsin were carried out (4). This study showed that the intradiscal Cys-110 and Cys-187 (Fig. 1) are essential for the formation of functional rhodopsin and that these two cysteines form a disulfide bond between them. To clarify further the role of the intradiscal domain in rhodopsin function, we have now performed deletion mutagenesis in the N-terminal tail and the three intradiscal loops as well as single amino acid substitutions in loop D–E (Fig. 1). The results show that relatively short deletions in any one of the loops or in the N-terminal tail affect the assembly of functional rhodopsin.

We conclude that the role of the intradiscal domain in rhodopsin is structural. Very likely this comprises the formation of a folded structure from the bulk of the intradiscal polypeptide segments, a structure that is stabilized by the disulfide bond between Cys-110 and Cys-187. The intradiscal structure leads to the correct alignment of the membrane-embedded helices and, consequently, the correct structure at the cytoplasmic surface.

MATERIALS AND METHODS

Materials. Oligonucleotides were synthesized and characterized as described (5). Dodecyl maltoside was purchased from Sigma and was further purified (6). We are grateful to Sabine Flitsch (Oxford University) for gifts of the purified preparation. Deoxyadenosine 5'-[α -³⁵S]thio]triphosphate, [γ -³²P]GTP, and guanosine 5'-[γ -³⁵S]thio]triphosphate (GTP[γ -³⁵S]) were obtained from New England Nuclear. Goat anti-mouse IgG conjugated to alkaline phosphatase was obtained from Promega Biotec. Fluorescein-conjugated goat anti-rabbit IgG, rhodamine-conjugated goat anti-mouse IgG, endo- β -N-acetylglucosaminidase H (endo H; EC 3.2.1.96) and glycopeptidase F (EC 3.2.2.18) were from Boehringer Mannheim. rho 1D4-Sepharose 2B (7) and rod outer segments (8) were prepared as described. Transducin was isolated from rod outer segments under room light (9).

Construction of Rhodopsin Gene Mutants. Deletions and amino acid substitutions were introduced into the synthetic rhodopsin gene (7) in a pSP65-derived cloning vector (10) or directly in the expression vector pMT4 (11) by restriction fragment replacement. The mutant genes constructed in the cloning vector were transferred to the expression vector by means of *Eco*RI and *Not* I cloning sites (11). Mutants Δ 4 and Δ 10 (Fig. 1) have one additional amino acid, histidine and serine, respectively, in addition to the deletions as a result of blunt-end ligation of *Fsp* I and *Sca* I sites or ligation of *Xba* I and *Sph* I sites with one oligonucleotide. The DNA sequences around the replaced oligonucleotides were confirmed by the chain-termination method (12).

Expression and Purification of Rhodopsin Mutants. The wild-type synthetic rhodopsin gene and mutant genes were expressed in COS-1 cells as described (7), and the cells were harvested 3 days after transfection. For mutants that showed normal glycosylation, the transfected cells were incubated with 11-*cis*-retinal (2–10 μ M) and solubilized in 1% (wt/vol) dodecyl maltoside, and the proteins were purified by immunoaffinity chromatography in 0.1% or 0.2% dodecyl mal-

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate; endo H, endo- β -N-acetylglucosaminidase H; GTP[γ -³⁵S], guanosine 5'-[γ -³⁵S]thio]triphosphate. Rhodopsin mutants with amino acid substitutions are designated by the one-letter abbreviations for amino acids. The amino acid to the left of the residue number is the original amino acid, while the one to the right is the substituted amino acid.

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FIG. 1. Secondary structure model for the polypeptide chain of bovine rhodopsin (2). The putative transmembrane helices are in vertical boxes. Deletions involving two or more amino acids are shown by lines close to the amino acids deleted and are numbered after the symbol Δ . Substitutions of single amino acids are shown by arrows. All the deletion and substitution mutations are on the intradiscal side.

side as described (4). The regenerated pigments showed A_{280}/A_{495} ratios in the range of 1.7–3.0. For mutants that showed abnormal glycosylation, incubation with 11-*cis*-retinal was performed after homogenization of transfected cells or by using the postnuclear supernatants of disrupted cells to ensure the accessibility of 11-*cis*-retinal to all the membranes. Disruption of cells was followed by phase-contrast microscopy. For mutants $\Delta 14$, $\Delta 1$ – $\Delta 4$, and Asp-190 $\rightarrow$ Ala (D190A), after absorption of the protein to immunoaffinity matrix, washes and elution were carried out in the presence of 0.075% (wt/vol) egg phosphatidylcholine (Avanti Polar Lipids), 0.05% (wt/vol) 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS), and 0.1% (wt/vol) dodecyl maltoside to increase the stability of the solubilized proteins. The purified proteins were characterized by UV/visible spectroscopy and by NaDodSO₄/polyacrylamide gel electrophoresis followed by immunoblotting with rho 1D4, a mouse monoclonal antibody specific for the C terminus of rhodopsin. An extinction coefficient of $42,700 \text{ M}^{-1}\text{cm}^{-1}$ at 495 nm (13) was used for rhodopsin. Photobleaching of the chromophore was performed with a Fiber-Lite A-200 (Dolan-Jenner Industries) light source equipped with a cut-off glass filter of 495 nm.

Transducin Activation Assay. Purified rhodopsin mutants were assayed for their ability to activate transducin by (i) the GTPase activity and (ii) the exchange of the bound GDP for the nonhydrolyzable GTP analogue GTP[γ -³⁵S]. The rhodopsin mutants were diluted to 5–100 nM in a solution containing 50 mM Tris-HCl, pH 6.8/0.1 M NaCl/1 mM CaCl₂/0.07% egg phosphatidylcholine/0.05% CHAPS/0.1% dodecyl maltoside at room temperature. Inclusion of phosphatidylcholine enhanced the activation of transducin by 2- to 3-fold, but preincubation with rhodopsin for about 30 min was required for optimal results. The final concentrations of phosphatidylcholine, CHAPS, and dodecyl maltoside in the reaction

mixture of both assays were 0.0075%, 0.005%, and 0.01%, respectively (10).

The concentration of rhodopsin mutants used in the GTPase assay (4) was 2.5 nM, which is within the linear range of the assay. The concentrations of transducin and [γ -³²P]GTP were 3.6 μ M and 10–11 μ M, respectively.

The GTP[γ -³⁵S] exchange assay by filter binding was carried out basically as described (14). The initial velocities of the exchange reaction were measured for various transducin concentrations (0.5, 0.9, 1.45, and 2.5 μ M) at 5–7 μ M GTP[γ -³⁵S]. The recovery of transducin bound to the nitrocellulose filters was not included in calculations. The apparent K_m and v_{max} were obtained by plotting (initial velocities)⁻¹ as a function of [transducin]⁻²; a linear relation was obtained (14).

Immunofluorescent Double-Staining of COS-1 Cells. COS-1 cells were grown on glass slips in plates, transfected as described, and further cultured for 67 hr. Cells were fixed and permeabilized in methanol for 5 min at -20°C and in acetone for 5 min at -20°C , then dried and rehydrated in phosphate-buffered saline (10 mM sodium phosphate buffer, pH 7/150 mM NaCl/0.02% NaN₃). The fixed cells were incubated with mouse monoclonal antibody rho 1D4 (10 μ g/ml) followed by staining with rhodamine-conjugated goat anti-mouse IgG (10 μ g/ml). Cells were further incubated with rabbit polyclonal antibody for endoplasmic reticulum resident proteins (15) and then washed and stained with fluorescein-conjugated goat anti-rabbit IgG. Finally, the cells were washed with phosphate-buffered saline and mounted on glass microscope slides.

Immunogold-Dextran Labeling of COS-1 Cells. Transfected COS-1 cells were fixed in 1.25% glutaraldehyde/phosphate-buffered saline, indirectly labeled with the N-terminal-specific rhodopsin monoclonal antibody rho 4D2 and protein A-gold-dextran conjugates, and processed for transmission electron microscopy as described (7). Hybridoma cell culture fluid containing the rho 4D2 antibody was diluted 1:10 in phosphate-buffered saline for the first labeling step. Stock protein A-gold-dextran conjugate having an approximate diameter of 10 nm and an A_{520} of 10 was diluted 1:20 for the second labeling step.

RESULTS

The total deletions and single amino acid substitutions made are shown in Fig. 1. The expression levels of all the mutants were comparable to that of the wild-type proteins. The mutants could be divided into three types (Table 1) as described below.

Type I: Mutants Whose Phenotype Resembles That of the Wild-Type Opsin. These included the two-amino-acid-deletion mutants $\Delta 18$, $\Delta 19$, $\Delta 21$, $\Delta 22$, and $\Delta 23$, one four-amino-acid-deletion mutant, $\Delta 20$, and the substitution mutants Y191L, Y192L, T193V, P194A, and P194G. These mutations, which are all located in loop D–E near helix E, generated the typical rhodopsin chromophore ($\lambda_{max} = 495$ nm) (Fig. 2A). However, while the wild-type and most of the type I mutant chromophores shifted quantitatively to 380 nm (Fig. 2A) on irradiation for 10 + 10 sec, the mutants $\Delta 18$, Y191L, and Y192L showed distinctly different photobleached spectra (Fig. 2B).

The glycosylation pattern of type I mutants in COS-1 cells is exemplified by the immunoblotting of the cell lysate containing mutant $\Delta 18$ proteins (Fig. 3A). The protein pattern showed an unglycosylated band (31 kDa), the major glycosylated band (41 kDa), and a series of protein bands with successively decreasing mobility from 41 kDa to 68 kDa. This is very similar to the wild-type glycosylation pattern (Fig. 3A).

The above type I mutants, which regenerated the chromophore, were studied for their ability to activate transducin. Kinetics of typical GTPase activity are shown in Fig. 4.

Table 1. Mutants with amino acid deletions and substitutions in the intradiscal domain

Mutant designation	Amino acid(s) substituted or deleted	Mutant designation	Amino acid(s) substituted or deleted
Phenotype I		$\Delta 10$	177–182
$\Delta 18$	193–194	$\Delta 11$	179–180
$\Delta 19$	195–196	$\Delta 12$	181–182
$\Delta 20$	195–198	$\Delta 13$	181–197
$\Delta 21$	197–198	$\Delta 15$	189–190
$\Delta 22$	199–200	$\Delta 16$	189–198
$\Delta 23$	201–202	$\Delta 17$	191–192
Y191L	191	$\Delta 24$	203–204
Y192L	192	$\Delta 25$	205–206
T193V	193	$\Delta 26$	207–208
P194A	194	$\Delta 27$	290–293
P194G	194	Phenotype III	
Phenotype II		$\Delta 1$	7–10
$\Delta 5$	101–108	$\Delta 2$	11–14
$\Delta 6$	171–172	$\Delta 3$	18–20
$\Delta 7$	173–174	$\Delta 4$	21–29
$\Delta 8$	175–176	$\Delta 14$	183–184
$\Delta 9$	177–178	D190A	190

Transducin activation was measured both by the GTPase activity assay and, in some cases, by GDP–GTP exchange. In the GTPase assay, type I mutants comprising two-amino-acid deletions, $\Delta 19$, $\Delta 21$, $\Delta 22$, and $\Delta 23$, as well as $\Delta 20$, with a deletion of four amino acids, showed essentially wild-type activity (Fig. 4), whereas $\Delta 18$ with a two-amino-acid deletion showed about 27% of wild-type initial velocity. Substitution mutants Y191L and Y192L showed initial rates somewhat less (55–75%) than that of the wild type but also showed faster decay of activity. The substitution mutants T193V, P194A, and P194G, in contrast, showed completely wild-type activity. The mutants $\Delta 18$ and Y191L were also studied for their ability to stimulate GDP exchange for GTP[γ - 35 S] by transducin. The apparent K_m for the wild-type protein, $\Delta 18$, and Y191L were similar, but the apparent v_{max} values showed distinct differences (data not shown). These and the above results suggest that the active species formed on photolysis of the mutants $\Delta 18$ and Y191L are less stable than the wild type.

Type II: Mutants That Fail to Generate Rhodopsin Chromophore and Cause Abnormal Glycosylation. These mutations comprised relatively large deletions ($\Delta 5$ in loop B–C; $\Delta 10$, $\Delta 13$, and $\Delta 16$ in loop D–E; and $\Delta 27$ in loop F–G as well as two-amino-acid deletions, $\Delta 6$ – $\Delta 9$, $\Delta 11$ – $\Delta 12$, $\Delta 15$, $\Delta 17$, and $\Delta 24$ – $\Delta 26$ in loop D–E) (Table 1). None of these mutants regenerated rhodopsin-like chromophore when, after expression in COS-1 cells, the membranes were incubated with 11-*cis*-retinal, as shown, for example, for mutant $\Delta 15$ (Fig. 2C).

The mutants showed a markedly different glycosylation pattern [e.g., mutant $\Delta 15$ (Fig. 3A)]. Thus, three major protein bands of 31, 36, and 41 kDa were observed, and the minor bands (smear) between 41 and 68 kDa characteristic of the wild-type proteins were absent. The difference in the glycosylation pattern between the wild-type and type II mutants is further shown in Fig. 3B by the sensitivity of the glycosylated proteins to the enzyme endo H. The wild-type protein was only partly sensitive to the enzyme, whereas the 36- and 41-kDa proteins from mutant $\Delta 16$ disappeared and were replaced by the 31-kDa band, corresponding to the deglycosylated opsin. The sensitivity of the type II mutants to endo H indicates that the carbohydrate is high-mannose type and that it has not been converted to the complex carbohydrate.

Type III: Mutants That Regenerate the Chromophore Poorly and Are Abnormally Glycosylated. Type III mutants com-

Fig. 2. UV/visible absorption spectra of rhodopsin (wild-type) and mutants expressed in COS-1 cells. (A) Wild-type rhodopsin. (B) The $\Delta 18$ mutant, which has a deletion of amino acids 193 and 194. (C) The $\Delta 15$ mutant, which has a deletion of amino acids 189 and 190; this mutant generated no chromophore. (D) The $\Delta 3$ mutant, which has a deletion of amino acids 18–20. This mutant generated essentially the wild-type chromophore but in 10–20% yield. Bleaching of the chromophores in the mutants was carried out for the times indicated (10 sec, 10 + 10 sec, or 10 + 20 sec). A light source equipped with a 495-nm cut-off filter was used.

prised deletions in the N-terminal tail ($\Delta 1$ – $\Delta 4$), $\Delta 14$ with a two-amino-acid deletion in loop D–E, and a single amino acid substitution (D190A) mutant (Fig. 1). After purification, the above mutants generated the rhodopsin chromophore only to the extent of 10–20% of the wild type, as shown for $\Delta 3$ in Fig. 2D. Presence of phospholipids during purification made no difference.

Fig. 3. Expression and NaDodSO₄/polyacrylamide gel electrophoresis of selected rhodopsin mutants. (A) After centrifugation the supernatants of 1% dodecyl maltoside-solubilized cells were subjected to 10% (wt/vol) NaDodSO₄/polyacrylamide gel electrophoresis and visualized by immunoblotting with monoclonal antibody rho 1D4. The mutants $\Delta 15$ and $\Delta 3$ are strikingly free from the smear immediately above the 41-kDa bands. The wild type (W.T.) and $\Delta 18$ mutant have the smear typical of the wild-type rhodopsin glycosylation pattern in COS-1 cells. (B) Purified wild-type and $\Delta 15$ proteins and their endo H-treated materials were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis and subsequent immunoblotting. – or +, the absence or presence of endo H. The greater sensitivity of $\Delta 15$ glycoproteins relative to the wild-type protein is evident.

FIG. 4. Kinetics of activation of transducin as assayed by GTPase activity catalyzed by the wild-type rhodopsin and the different mutants. All the N-terminal deletions were severely defective as shown for $\Delta 3$. The mutants $\Delta 18$, Y191L, and Y192L showed activity that underwent fast decay. Other mutants showed activity similar to that of the wild type.

The glycosylation pattern exemplified in Fig. 3A for mutant $\Delta 3$ was typical for these mutants: it was different from those of type I and type II mutants. Thus, while the major bands, 41 and 31 kDa, were present, the bands between 41 and 68 kDa, characteristic of normal opsin glycosylation in COS-1 cells, and the 36-kDa band were all minor or absent. Also, type III mutants, like type II mutants, tended to form dimers and trimers.

In GTPase assay, deletion mutants of the N-terminal tail and $\Delta 14$ showed 10–20% of the wild-type initial rate, and the activity decayed quickly. However, the mutant D190A showed wild-type kinetics. In the GDP–GTP[γ -S] exchange assay, mutant $\Delta 3$ (Fig. 1) did not show any detectable activity (data not shown).

Localization of the Type II Mutant, $\Delta 15$, in COS-1 Cells. The sensitivity of the carbohydrates of type II mutant proteins to endo H indicated that these proteins were not transported from the endoplasmic reticulum to the Golgi for processing of the high-mannose carbohydrate. Therefore, localization of the type II mutant $\Delta 15$ in COS-1 cells was studied, as an example, by indirect immunofluorescence double-staining (Fig. 5) and transmission electron microscopy (Fig. 6).

For immunofluorescence labeling, permeabilized COS-1 cells were stained with rhodamine for opsin proteins and fluorescein for endoplasmic reticulum proteins. The cells expressing the wild-type proteins showed different distributions of the two types of fluorescence. Thus, rhodamine stained the whole region and the edge of the cells (Fig. 5B), while fluorescein stained the region around the nuclei (Fig. 5C). In contrast, in cells expressing the mutant $\Delta 15$, rhodamine and fluorescein stained similar regions of the cells around the nuclei (Fig. 5D–F), indicating the location of $\Delta 15$ protein around the endoplasmic reticulum.

COS-1 cells transfected with the wild-type, $\Delta 15$ (type II), or $\Delta 18$ (type I) plasmids were also labeled with the N-terminal-specific rho 4D2 rhodopsin monoclonal antibody and protein A–gold conjugates. As visualized by transmission electron microscopy, COS-1 cells expressing the wild-type protein (Fig. 6 Top) and $\Delta 18$ mutant (Fig. 6 Bottom) showed dense gold labeling of the plasma membrane, whereas most of the cells transfected with the $\Delta 15$ plasmid showed sparse labeling at the cell surface (Fig. 6 Middle). Since the efficiency of transfection and expression of the mutant $\Delta 15$ were

FIG. 5. Immunofluorescence double-staining of COS-1 cells expressing wild-type rhodopsin (A–C) and of COS-1 cells expressing mutant $\Delta 15$ (D–F). Observed were fluorescein fluorescence (C and F) and rhodamine fluorescence (B and E) using appropriate optics as well as phase-contrast microscopy (A and D). Numbers indicate identical cells. Fluorescein conjugated to goat anti-rabbit antibody was probed for polyclonal anti-endoplasmic reticulum protein antibody, and rhodamine conjugated to goat anti-mouse antibody followed the labeling by monoclonal mouse antibody 1D4 for rhodopsin.

similar to those of the wild type as shown by immunofluorescence labeling and immunoblotting, the above results

FIG. 6. Transmission electron micrographs of COS-1 cells transfected with the plasmid containing the wild-type rhodopsin gene (Top), mutant $\Delta 15$ gene (Middle), and mutant $\Delta 18$ gene (Bottom). Immunogold–dextran particles conjugated to goat anti-mouse antibody followed the labeling by monoclonal mouse antibody 4D2 for rhodopsin.

indicate that while the wild-type and $\Delta 18$ rhodopsin mutant were transported to the plasma membrane of COS-1 cells, the $\Delta 15$ mutant protein was not.

DISCUSSION

To investigate the role of the intradiscal domain in rhodopsin structure/function, we have carried out deletions in every one of the intradiscal loops and in the N-terminal tail. Of all of the mutants characterized, only a small number (type I, Table 1) showed essentially wild-type phenotype, and these comprised single amino acid replacements (amino acids 191–194) and a set of two-amino-acid deletions between amino acids 193 and 202. This portion of the D–E loop obviously is not critical to rhodopsin function and it is not conserved (16, 17) in the opsin family. Most of the deletion mutations (Fig. 1 and Table 1) produced defective proteins. Deletions with phenotype II (Table 1) failed to generate rhodopsin-like chromophore and showed mannose-rich oligosaccharides characteristic of glycosylation in endoplasmic reticulum (Fig. 3). This class of mutations comprised relatively large deletions ($\Delta 5$, $\Delta 10$, and $\Delta 27$) and two-amino-acid deletions between positions 171 and 192 (Fig. 1) and between positions 203 and 208 (Table 1 and Fig. 1). Type III mutations were also defective in that they regenerated the chromophore very poorly and their glycosylation pattern was different from those of type I and type II mutants. Type III mutants represented deletions in the N-terminal tail (Table 1), the two-amino-acid deletion $\Delta 14$, and the amino acid substitution D190A.

One type II mutant, $\Delta 15$, was examined for its cellular location in COS-1 cells, the system used for expression of all of the mutants. The mutant protein was located mostly in the endoplasmic reticulum and not in the plasma membrane where the wild-type rhodopsin was present (Fig. 6). We believe that all the defective proteins, like $\Delta 15$, remain in the endoplasmic reticulum, as is the case for such proteins in general (18–21).

The fact that defective rhodopsin structures of type II and type III can arise by single relatively short deletions in any one of the loops, B–C, D–E, or F–G, or the N-terminal tail (Table 1) suggests that these segments are all cooperatively involved in forming a specific folded structure on the intradiscal face. We believe that this structure is stabilized by the disulfide bond (S. Karnik and H.G.K., unpublished work) between Cys-110 and Cys-187. The deletions now described produce defective opsins because they do not allow the formation of the specific tertiary structure. Recently, rhodopsin in one form of retinitis pigmentosa has been shown to carry the point mutation P23H (22). We hypothesize that this mutation also does not allow the formation of the above intradiscal structure.

We have also carried out a large number of deletion and point mutations in loops C–D and E–F on the cytoplasmic face (R. Franke, T. Sakmar, and H.G.K., unpublished work). In contrast with the deletion mutations in the intradiscal face, mutations in the cytoplasmic loops do not affect regeneration of the rhodopsin chromophore. We propose that the mode of assembly and functions of the cytoplasmic and intradiscal domains are very different. The function of the intradiscal domain is structural, while the cytoplasmic face carries out the biochemical and regulatory functions. In summary, we postulate the following main steps in the assembly and function of rhodopsin: (i) separate and uncoordinated insertion of the seven helical segments into the membrane in endoplasmic reticulum and glycosylation to form mannose-rich oligosaccharides, (ii) formation of the intradiscal tertiary structure including the Cys-110 and Cys-187 disulfide bond

followed by alignment and formation of the seven-helix bundle in the membrane, (iii) transport to the Golgi and processing of glycosylation and palmitoylation, and (iv) migration to the plasma membrane and addition of the 11-*cis*-retinal.

Light transduction initiated by *cis* $\rightarrow$ *trans*-retinal isomerization will cause perturbation of the helices, the effect will be transmitted specifically to the cytoplasmic face, and the new “open” structures formed will allow the interaction of transducin and other proteins with the “activated” rhodopsin.

A large number of transmembrane receptors that couple to GTP-binding regulatory proteins contain disulfide bonds equivalent to the intradiscal disulfide bond in rhodopsin (23). Deletions in the loops involving the disulfide bond result in decreased affinity for the ligand binding and in the abnormal glycosylation of the receptors (24). The structural evidence on this superfamily of receptors suggests a common principle for their architecture and function as we have now proposed for rhodopsin.

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