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Assignment of groups responsible for the "opsin shift" and light absorptions of rhodopsin and red, green, and blue iodopsins (cone pigments)

[opsin structural model/rhodopsin catalysis ("photoswitch")]

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ABSTRACT **A modified structural model of rhodopsin is presented. Seven (α-helical) segments of 24 largely hydrophobic amino acid residues are assembled with exobilayer connecting strands into an aligned set, using the sequences of human red, green, and blue iodopsins (cone pigments) and human and bovine rod rhodopsins. (Aligned set numbering is used in this article.) The inner region of the heptahelical hydrophobic domain includes one His-Glu (Asp) ion pair (red, green rod) near the retinylidene moiety in addition to an iminium ion Asp-99 pair. The negative charges posited in the "point-charge model" to cause the shift of the retinylidene iminium ion light absorption to longer wavelengths in the protein ("opsin shift") are Asp-99 (red, green rod), Glu-102 (red, green), and Glu-138 (rod). Blue iodopsin lacks both an ion pair and a counter charge to the iminium ion in the inner region, a fact that explains its absorption relative to rod rhodopsin. The spectroscopic difference between rod rhodopsin and the red/green iodopsins is due to the influence of Glu-102 in the latter. The red–green difference is due to the net effect of seven OH groups around the chromophore, all such groups being found within one helix turn of the retinylidene location. The tryptophan, which rotates as the retinylidene group isomerizes, may be Trp-142 or Trp-177. The geometric change (the rhodopsin "photoswitch") resulting from cis–trans isomerization in the first excited electronic state (S_1), ultimately leads to RX (photoactivated rhodopsin, metarhodopsin II) and changes the activity of exobilayer groups, possibly causing dissociation of Lys-83 and Arg-85 from the carboxylate groups at positions 263 and 265.**

Visible light (400–700 nm) is converted into a neural signal in animals via light absorption by rhodopsin (R) in retinal rod cells (iodopsin in cone cells) (1, 2). The 11-*cis*-retinylidene chromophore is converted to the 11-trans structure in the first excited electronic state (S_1), which then decays to photorhodopsin (3). Photorhodopsin changes within 40 ps (3) to bathorhodopsin, which is transformed via lumirhodopsin and metarhodopsin I to metarhodopsin II. Metarhodopsin II [the phototransformed protein, RX (4)] initiates processes that temporarily decrease cyclic guanosine 3′,5′-monophosphate (cGMP) within the cell (5, 6). The initial step is the phototransformed rhodopsin (iodopsin)-promoted displacement of guanosine diphosphate (GDP) by guanosine triphosphate (GTP) from the transducin [T; G-protein (5–7)] complex with GDP. The flow of information to the nervous system about a photon absorbed by a rod or cone may be expressed in the steps, photon + R → RX → T–GTP → (−cGMP) → (−signal to nervous system). A "point-charge" model (a negative charge stabilizing the retinylidene excited state more than the ground state) has been proposed to explain the shift of the retinylidene iminium ion light absorption to longer wavelengths in the protein ("opsin shift") (8–11).

On the basis of the alignment of the amino acid sequences of human rod rhodopsin and red, green, and blue iodopsins (12), we are able to develop a modified structural model by specifying charge–charge interactions (cf. refs. 13–16). With this model, we can identify (*i*) the groups responsible for spectroscopic differences between the rhodopsins and iodopsins (consistent with the general idea of the point-charge model), (*ii*) the nature of the change in rhodopsin produced by the S_1 cis–trans isomerization, and (*iii*) part of the conformational change that produces RX, the catalyst for the formation of T–GTP.

RESULTS AND DISCUSSION

The aligned sequences [the bovine rod rhodopsin sequence (14, 15, 17) has been added] are examined for hydrophobic character. The bilayer thickness is taken as 36 Å (24 amino acids in an α-helix). Such segments can be selected without difficulty by inspection of the aligned set; more formal methods lead to essentially the same choice (14). The N-terminal sequence carrying a known carbohydrate sequence (18, 19) is inside the disk (20, 21) (outside the cone); an odd number of transmembrane segments puts the C-terminal end in the cell cytoplasm, as found (22). The cytoplasmic links will be examined below. A schematic structure for the rhodopsins is presented in Fig. 1. Other ungulate rhodopsins have amino acid sequences very similar to that of bovine rhodopsin; in particular, ovine rhodopsin has Asp-99, Glu-138, His-227, and Lys-312 (23). Even the more distantly related *Drosophila* rhodopsins have the equivalent of Asp-99 and Lys-312 [rho_{1-6}: Asp-96, Lys-319 (24); rho_8: Asp-103, Lys-326 (25, 26)].

Several features are immediately apparent in the model. The retinylidene chain bound to Lys-312 (27, 28) is close to the middle of the bilayer. A small number of charged groups is found well within the bilayer. It is proposed that these groups are functionally involved with the retinylidene group located in the inner region of the helices. Ion pairing will occur when possible for oppositely charged groups in a moderately nonpolar environment. Four of five opsins have a negatively charged group (Asp) at position 99, which is therefore the counterion to the 312 iminium ion. (Both *Drosophila* rhodopsins have homologous ion pairs, as noted above.) The same four opsins have another ion pair, in addition to $99^- - 312^+$, in the center of the bilayer. The additional ion pair in rod rhodopsins is composed of Glu-138 and His-227; in red–green iodopsins, the pair consists of

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Abbreviations: RX, photoactivated rhodopsin; T, transducin.

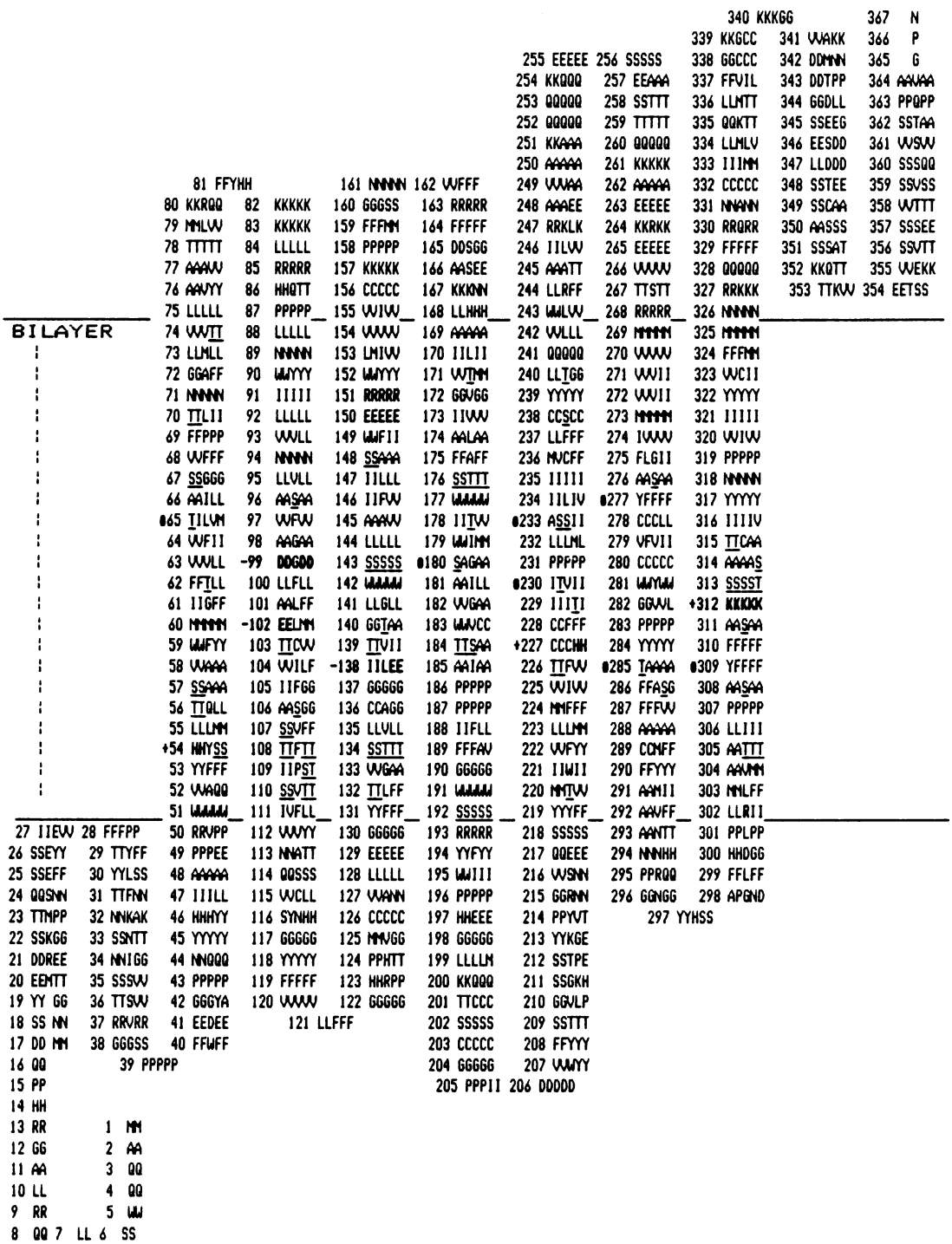

FIG. 1. A linearized structure of human red, green, and blue iodopsins (cone pigments) and human and bovine rhodopsins (rod pigments) showing the distribution of the protein between the membrane bilayer and the aqueous compartment of the rod disk (cone invagination). The interior of the disk (corresponds to the cell exterior in a cone) is at the bottom of the figure, together with the N-terminal residue of the opsin. The single-letter code for amino acids is used for clarity. Aligned sequences are shown using the homology numbering based on red–green iodopsins. To obtain the position in the rhodopsin sequence, subtract 16 (19 for the blue iodopsin). No skips are required for the alignment. Membrane bilayer segments of 24 amino acids (36 Å) are chosen on the basis of regions of hydrophobicity in the aligned sequences. Seven segments are found, a result that agrees with more formal methods of choosing hydrophobic segments. It is assumed that these segments occur as α-helices (numbered 1–7). The Lys-312, known to carry the retinylidene moiety as an iminium ion, is located in the middle of helix 7 and is common to all known opsins. The second charged group common to 4 of the 5 opsins is Asp-99, which must be the counterion to the iminium ion. The next most salient feature is the Glu-138 found in the rhodopsins. At the same level in both rhodopsins is His-227, which thus is a good choice as the counterion (in protonated form) to Glu-138. In place of this ion pair, we find Glu-102 at a location appropriate for pairing (weakly) with His-54. No counterion is found for the iminium ion in blue iodopsin. Amino acids with OH groups with an influence on the spectroscopic properties of the rhodopsins are marked with a solid rectangle.

Glu-102 and His-54. The Glu-102–His-54 separation is between 7 and 9.5 Å, depending on conformation. Although the charge–charge separation is a bit large for an ion pair, the choice of His-54 is validated by the occurrence of Lys at the homologous positions in both *Drosophila* rhodopsins, even though there is no identifiable intrabilayer counterion in the latter. The partners in the ion pairs may fluctuate; certain charged groups may not have partners. Given the angle of

18° between the chromophore transition moment and the bilayer plane (29), the retinylidene moiety should be located not far from the amino acid side chains at positions 102, 138, and 227.

The bilayer helices are numbered 1 to 7 from the N-terminal end of the protein. By assuming that ion pairing occurs in a hydrogen-bonded arrangement within the cavity containing the retinylidene chain, we are able to orient bilayer helices 3 and 5 and 1 and 2 with respect to one another. Asp-99 must be directed in the general direction of the chromophore since it is one helix turn above Glu-102. By packing the helices around the 11-*cis*-retinylidene imine, we arrive at the central bilayer region structure depicted in Fig. 2. The orientations of helices 1, 3, and 5 are different from those chosen on the basis of hydrophobicity (16). The arrangement in Fig. 2 is only approximate, arbitrarily counterclockwise in helix order and schematic, since the overall structure of rhodopsin is not known. Bacteriorhodopsin, for which both fine and overall structural details are established (30–33), and rhodopsin may be similar, but the similarity hypothesis cannot be applied without further information in view of the rather different functions of the two proteins.

Spectroscopic Parameters

Among the questions that must be addressed in terms of the model are (*i*) the origin of the opsin shift and (*ii*) the origin of the differences among iodopsins.

The model places groups and charges in specific locations on the basis of which various types of interactions may be estimated. Two serious difficulties stand in the way of an exact calculation of electrostatic and other effects. First, the amino acid side chains can exist in a number of different local conformations that may interconvert by "single group rotations" (34). The distances between charged groups then cannot be exactly specified. Second, the dielectric constant of the regions between the charges may vary in a complex way from 2 to 78 (35–39). The electrostatic interaction difference between the ground state and the excited state can thus be estimated only roughly. The parameters used are distances measured between charged atoms (NH^+) and the centers of complex groups (His/COO^-), 0.5 as the excited state charge, either 2 or 4 for the dielectric constant (depending on proximity), and 10 in the case of His-54, for which Ser-57 should influence the interaction. Charge–charge interactions were calculated by Coulomb's law for each pair of charges and the net stabilization (or destabilization) was obtained for the ground and excited states. Estimates for the model suggest that the stabilization of the excited state (positive charge appearing near the Asp-99 and Glu-138 in the rod) should be greater than the stabilization of the ground state, 11–12 kcal/mol versus the 7.1 kcal/mol found experimentally (1 cal = 4.18 J). These results are consistent with the basic premises of the point-charge model, albeit with a charge arrangement somewhat different from that used in the model (8, 10).

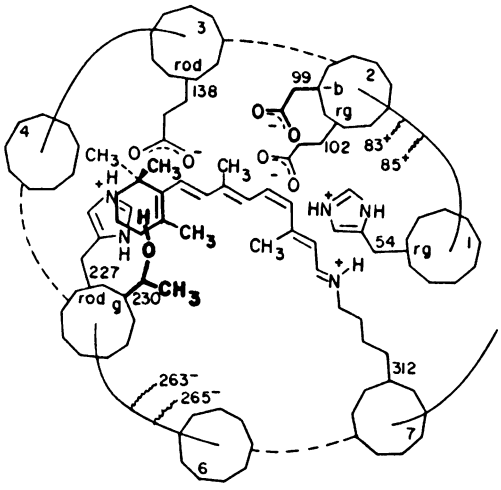

FIG. 2. An approximate and schematic arrangement of the critical groups in the central bilayer region of all of the iodopsins and rhodopsins. Ion pairs in red–green iodopsins (His-54, Glu-102) and rod rhodopsins (His-227, Glu-138) are used to orient the helices, the net result being that the Glu and Asp carboxylate groups must be located very close to the retinylidene moiety. The OH group of Thr-230 is close to the cyclohexene double bond. Other OH groups that differ in the red and green sequences are cited in the text and are marked on the sequences in Fig. 1. The positions of the 83^+, 85^+, 263^-, and 265^- groups are shown schematically.

The sensitivity of the rhodopsin (iodopsin) maximum to microenvironment is borne out by a striking property of retinylidene iminium systems: the shift of the absorption maximum to longer wavelengths in the presence of excess acid in solution. It is likely that the excess acid hydrogen bonds to the anion associated with the positive ions and diminishes ion pairing. Weaker ion pairing is equivalent to deshielding the positive charges (40). The exact magnitude for the "opsin shift" may be smaller than that just cited if the 6,7-bond proves to be *s-trans* as in the case of bacteriorhodopsin (refs. 10 and 41; cf. also refs. 42–44) but the 6-*s-cis* form is currently favored for rhodopsin (45).

The spectroscopic shift between rod rhodopsins and red–green iodopsins is here accounted for by the same mechanism, but with a negative charge (Glu-102) closer to the chromophore and a counterion that is farther away. A shift of 16 kcal/mol is estimated (5–6 kcal/mol more than that for the rod). The average of the measured red and green shifts is 13 kcal/mol. We ascribe the difference between red (565 nm) and green (535 nm) iodopsins to hydroxyl groups in the immediate vicinity (± 1 helix turn) of the retinylidene group. Hydroxyl groups at the cyclohexene end of the chromophoric moiety raise the transition energy (positive hydrogen closer to the region in which positive charge appears in the excited state), while those at the iminium should lower the transition energy (net stabilization of the excited state in α,β-conjugated systems). Thr-230, Ser-233 (green), and Ser-180 (red) destabilize the excited state (positive end of OH dipole hydrogen-bonded to cyclohexene double bond; cf. ref. 46). Thr-65, Tyr-277, Thr-285, and Tyr-309 (red) can stabilize the excited state more than the ground state by providing a polar environment for the iminium end of the chromophore. It is striking that essentially all of the differences between red and green iodopsins with respect to hydroxyl-bearing amino acids occur within the local interaction region of the retinylidene group. (See the dot-marked amino acids in Fig. 1.) Serine, threonine, and cysteine may also stabilize hydrophobic α-helices (47); there are 26 such groups in the iodopsin bilayer helices and 15 in the rhodopsin bilayer helices. The sequence of the second red iodopsin implied by the genetics of color matching (48, 49) would be of great interest in connection with the present analysis.

Surprisingly, there is no counterion (no negative charge) for the blue iodopsin iminium ion within the bilayer. The absorption maximum for blue iodopsin (440 nm) corresponds to that of a retinylidene iminium ion without charge stabilization (no opsin shift). The 6,7-conformation is not known. In the *Drosophila* rhodopsins, we estimate that the charges [Asp-99, Lys-54, and Lys-172 (alignment number)] will cause an opsin shift to 480 nm, not far from the value of 470 nm reported for rho_{1-6} (50).

Linear dichroism measurements have suggested that an indole moiety of a tryptophan residue rotates as the retinylidene group reorients (51). Our model suggests that either

Trp-142 (helix 3) or Trp-177 (helix 4) should be near the cyclohexene ring of the retinylidene group.

Consequences of Rhodopsin/Iodopsin Photoisomerization

The cis–trans isomerization in the rhodopsin S_1 state occurs within a restricted volume to yield bathorhodopsin, the close contact between the chiral protein and the retinylidene group being shown by circular dichroism (52). Taking into account the direction of the seventh α-helix, an extended conformation for the Lys-312, the transition dipoles for light absorption of rhodopsin (≈18° from the bilayer plane) and bathorhodopsin (0° from the plane), and the normal photoisomerization exhibited by a C_{9-11} restricted rhodopsin (53), we arrive at the formulation shown in Fig. 3. The initial photoisomerization product is tentatively labeled photorhodopsin, already noted as an intermediate that decays rapidly to bathorhodopsin. The increase in the length of the conjugated system is compensated by the decrease in lysine side-chain extension. The motion (Fig. 3) close to the bicycle pedal scheme (54, 55) is less related to a concerted twist (56, 57). In our model, the iminium nitrogen would be located at a position between helices 1 and 7 and at a level one helix turn below the position at which Lys-312 joins helix 7. The iminium nitrogen would move ≈4.5 Å toward the cytoplasmic surface of the bilayer to a position closer to helix 1 and at the level at which Lys-312 joins helix 7. The occurrence of normal photoisomerization in the same C_{9-11} restricted rhodopsin alluded to above has been interpreted as suggesting a pathway different from that of natural rhodopsin (58).

We now take note of the genetically homologous positive charges (Arg-85 and Lys-83) in the connecting link between helices 1 and 2 and the negative charges (Glu-263 and Glu-265) in the link between helices 5 and 6. Other G-protein receptors, human and hamster β_2-adrenergic receptors (Lys-60, Arg-63) (59, 60) and muscarinic acetylcholine receptor (Lys-51, Lys-57) (61) have considerable homology with bovine opsin (62) and have charged groups in the helix 1–helix 2 cytoplasmic loop. Functional homology seems to be more important than sequence homology in the case of the nicotinic acetylcholine receptor (63) so we may infer that such charge centers may be involved in the biochemical function of rhodopsin—i.e., in the interaction of RX with the G-protein α-transducin. We believe that catalytically inactive rhodopsin and iodopsin have a conformation in which the 83,85-(+)-pair interacts with the 263,265-(−)-pair. The

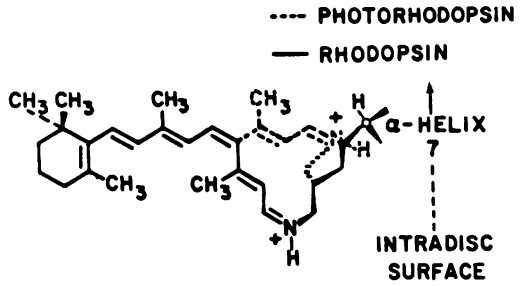

FIG. 3. A representation of the geometric change that occurs on the photoisomerization of rhodopsin to photorhodopsin. Photorhodopsin forms bathorhodopsin within 40 ps. Given the stereochemistry of the α-helix and an extended lysine side chain, the iminium ion will be found ≈1 helix turn below the point at which the lysine side chain is attached to the helix. A bicycle pedal (see refs. 54 and 55) isomerization between C_{12} of the retinylidene chain and the penultimate carbon of the lysine in the S_1 state converts the cis-C_{11-12} double bond to a trans double bond in the ground state photorhodopsin, which forms by radiationless internal conversion. The iminium ion is moved up ≈4.5 Å to a position on the same level as the Lys-312. The lower part of the figure is on the side of the intradiscal surface (exterior of a cone cell).

fact that charge-preserving lysine modifications and 88% lysine acetylation do not interfere with G-protein activation (64) implies that (*i*) arginine is the key charged group and/or (*ii*) an acetamido group can interact strongly enough to maintain the pathway. The amino acid side chains found in the 20 Å separating the iminium ion from the cytoplasmic surface (the upper side in Fig. 1) are highly nonpolar (dielectric constant, ≈2). A change of almost 4 kcal/mol in the interaction energy for the iminium ion with the 85–263 ion pair at the bilayer "surface" could be expected, corresponding to a possible change of a factor of 1000 in stability of the initial conformation of rhodopsin. The photoisomerization can then be regarded as the operational mechanism for a "photoswitch."

Since the iminium ion is the only charge that moves within the bilayer in the blue iodopsin, its role in initiating the conformational changes that eventually produce catalytically active rhodopsin would seem to be essential. Although direct electrostatic interaction is an obvious consequence of the isomerization, other changes mediated through the helix may also contribute to the operation of the opsin photoswitch. The fact of photoregeneration of rhodopsin from intermediates such as metarhodopsin (1) suggests that the conformational changes are limited in extent and closely linked to the geometric arrangement of the chromophore.

With a specific structure for rhodopsins (and iodopsins) in hand and a physical picture for the photoswitch, we suggest that metarhodopsin II might associate with transducin to catalyze the removal of GDP. The assumption of a common mechanism in all rhodopsins and iodopsins has been confirmed (65, 66), although cone transducin is different from that in the rod (67). Positive pairs can be found on the helix 1–helix 2 link on the cytoplasmic side in all opsins and other G-protein receptors. A pair of negatively charged groups (Glu-263 and Glu-265) within the connecting link between helices 5 and 6 could serve as counterions to positions 83 and 85. Their interaction would draw the connecting links together. A close association of T_α unit of T with RX must exist on the basis of the inhibition of activity on blocking one of the T_α-thiol groups (68).

Absorption of a photon and operation of the photoswitch would lead to a change in the stability of the 85,265 pair, effectively freeing the 83,85-positive pair for interaction with the negative charges of the GDP with the T–GDP complex. Direct contact between the effector metarhodopsin II and the GDP binding site may not be necessary (7). The activity of acetylated rhodopsin in G-protein activation (64) might depend on the partial positive charge on the nitrogen of the $NHCOCH_3$ group. A detailed scheme will be given elsewhere.

Conclusions

Genetic homology, detailed knowledge about the photophysics and chemistry of rhodopsin, and chemical logic have been combined to yield a fairly detailed picture of certain aspects of rhodopsin action. The occurrence of genes homologous to the opsin gene in a wide variety of species (Archeobacteria, algae, invertebrates, vertebrates) (69) and the importance of G-protein receptors [ref. 62; muscarinic acetylcholine receptor (61), β_2-adrenergic receptor (59, 60), olfactory receptor (70)] emphasizes the significance of the structure and function of these molecular systems to molecular neurobiology.

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