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Rhodopsin: Structure, Function, and Genetics†

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Rhodopsin is the light-absorbing protein that mediates dim light vision. It has been a source of fascination for biochemists ever since the incisive experiments of Wilhelm Kuhne demonstrated its photolability and regeneration (Kuhne, 1878). In 1894 Konig reported the coincidence between the absorption spectrum of human rhodopsin and the action spectrum of night vision (Konig, 1894), the first experiment to explain visual performance in terms of protein chemistry. In the 1930s Wald discovered that rhodopsin consists of two parts, an apoprotein, opsin, joined to a chromophore, retinal (Wald, 1935a,b; Figure 1). In 1958 the photoisomerization of retinal from 11-cis to all-trans was identified as the initiating event in visual excitation (Hubbard & Kropf, 1958); indeed, it is the only light-sensitive event in all of vision.

How does photoisomerization of retinal lead to the production of a neural signal? Thirty years ago Wald proposed "if rhodopsin were...a proenzyme, activated by the action of light, then it might catalyze the formation of many molecules of product in return for the absorption of a single photon. This would consitute one stage of amplification. If the prouct of this catalysis were a second enzyme, prepared to catalyze in turn a further reaction, that would constitute a second stage of amplification" (Wald, 1961). This remarkable insight was realized 20 years later with the discovery that photoexcited rhodopsin catalyzes the activation of a G-protein (transducin) which in turn activates a cyclic GMP phosphodiesterase (Wheeler & Bitensky, 1977; Leibman & Pugh, 1979; Fung & Stryer, 1980; Fung et al., 1981). The resulting decline in free intracellular cyclic GMP closes cyclic GMP-activated channels in the outer segment plasma membrane, leading to a membrane hyperpolarization (Fesenko et al., 1985; Haynes & Yau, 1985).

Intense selective pressure has optimized many of the functional characteristics of photoreceptor cells. These include low thermal noise, efficient photon capture, rapid activation and inactivation, and variable gain control. Many of these attributes are embodied within the rhodopsin molecule and will be emphasized here. Later steps in phototransduction are reviewed in Stryer (1986), Pugh and Lamb (1990), McNaughton (1990), and Yau and Baylor (1989).

A dark-adapted human can detect a flash of light that produces only 5–7 photoisomerizations, corresponding to an energy of 6×10^{-22} kcal (Hecht et al., 1942). By requiring an ensemble of approximately 500 rods to register 5–7 isomerizations within a several hundred millisecond integration time, the retina works as a coincidence detector. This strategy is necessary because the threshold for signal detection is limited by noise derived from thermal activation of rhodopsin, most likely as a result of thermal isomerization of retinal [Ashmore & Falk, 1977; Baylor et al., 1979, 1980; Aho et al., 1989; see Birge (1990) for a discussion of the energetics of isomerization]. Not surprisingly, the rate of thermal activation of rhodopsin is very low, approximately 1 per 400 years per rhodopsin at 37 °C (Baylor et al., 1980). This corresponds to an energy barrier of 22 kcal/mol. Photons of 500 nm (the wavelength of maximal absorption) carry 57 kcal/mol, resulting in a 40% efficient utilization of available energy in setting the signal-to-noise ratio. The low thermal noise allows for a large number of rhodopsins per cell. A human rod has approximately 4×10^7 rhodopsins, and the rods of some amphibia have 10^9 rhodopsins.

Despite the large energy barrier to thermal activation, photoactivation is extremely efficient: the quantum efficiency is 0.67 for isomerization from 11-cis to all-trans when retinal is part of rhodopsin (Kropf, 1967; Dartnall, 1968). Approximately 20% of photons at a wavelength of 500 nm that strike the human retina lead to a transduction event (Alpern & Pugh, 1974; Zwas & Alpern, 1976), an efficiency comparable to that of the best photomultiplier tubes.

Everyday experience tells us that visual transduction occurs on a time scale of tens to hundreds of milliseconds. Photoisomerization occurs within picoseconds of photon capture

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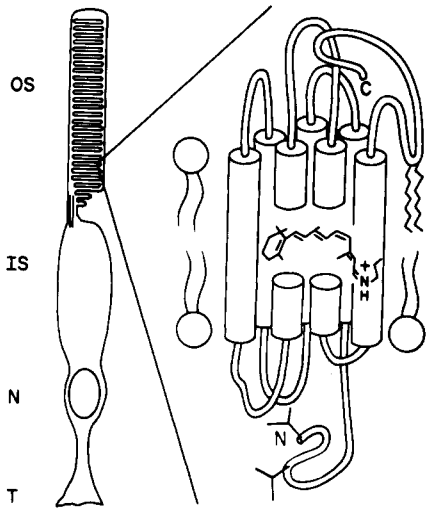

FIGURE 1: Structure of rhodopsin in the photoreceptor [modified from Hargrave et al. (1984)]. The photoreceptor cell (left) is approximately 100 μm in length; the rhodopsin molecule (enlarged, right) resides within the outer segment disk and plasma membranes. In this illustration, the two helices in front have been partly cut away to reveal the 11-*cis*-retinal chromophore. Abbreviations: OS, outer segment; IS, inner segment; N, nucleus; T, synaptic terminal.

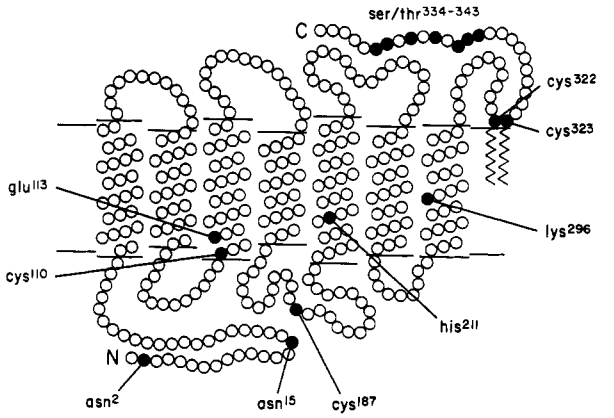

FIGURE 2: Transmembrane model of bovine rhodopsin [modified from Hargrave (1983)] with functionally important amino acids highlighted. Asparagine[2] and asparagine[15] are sites of N-linked glycosylation; glutamate[113] is the retinylidene Schiff's base counterion; cysteine[110] and cysteine[187] form an essential disulfide bond; histidine[211] modulates the MI–MII equilibrium; lysine[296] forms a Schiff's base with 11-*cis*-retinal; cysteine[322] and cysteine[323] are the sites of palmitylation; and serine[334], threonine[335], threonine[336], serine[338], threonine[340], threonine[342], and serine[343] are phosphorylated upon photoexcitation by rhodopsin kinase.

(Hayward et al., 1981; Schoenlein et al., 1991; Yan et al., 1991), the active protein conformation, metarhodopsin II, appears within 1 ms at 37 °C (Cone & Cobbs, 1969), and transducin is activated with a turnover of 1 ms^{-1} in intact rod outer segments (Voung et al., 1984). Rhodopsin is temporarily inactivated by the combined action of rhodopsin kinase, which phosphorylates multiple serine and threonine residues near the carboxy terminus, and arrestin, a protein that binds the cytoplasmic face of phosphorylated rhodopsin, thereby blocking access to transducin (Wilden et al., 1986).

The pathway of recycling rhodopsin back to the dark state is still incompletely understood. In vertebrates, *all-trans*-retinal dissociates from opsin on a time scale of seconds to minutes and is reisomerized in the adjacent pigment epithelium. The mechanism of trans-to-cis reisomerization has recently been clarified and involves a novel high-energy linkage between retinol and a phospholipid [reviewed in Rando (1990)].

At present there is no evidence for a change in rhodopsin activity during adaptation. Adaptation appears to be mediated in part or in full by a calcium feedback loop that regulates the rate of cyclic GMP synthesis (Mathews et al., 1988; Nakatani & Yau, 1988; Koch & Stryer, 1988; Dizhoor et al., 1991). However, it would seem reasonable to also look for regulation of the phototransduction cascade at its earliest step, given that this is the usual point of regulation in biochemical pathways [Neidhardt, 1987; see Barkdoll et al. (1989) for an examination of calcium effects on the early steps in phototransduction].

In the paragraphs that follow, I review recent work on vertebrate rhodopsins, primarily bovine and human. Many of the insights gained from these experiments should be applicable to the homologous vertebrate cone pigments, invertebrate visual pigments, and other G-protein coupled receptors.

Protein Structure

Bovine rhodopsin was the first G-protein coupled receptor to be sequenced (Ovchinnikov et al., 1983; Hargrave et al., 1983; Nathans & Hogness, 1983). The primary translation product of 348 amino acids is inserted into the membrane without proteolytic cleavage (Schechter et al., 1979), a characteristic that appears to apply generally to G-protein coupled receptors. The amino acid sequences of bovine, chicken (Takao et al., 1988), human (Nathans & Hogness, 1984), mouse (Baehr et al., 1988), and sheep (Findlay et al., 1984) rhodopsins are all greater than 87% identical. Cone pigments from human (Nathans et al., 1986) chicken (Kuwata et al., 1990; Tokunaga et al., 1990), and cavefish (Yokoyama & Yokoyama, 1990) are approximately 40% identical to each of the rhodopsins.

Rhodopsin forms when 11-*cis*-retinal binds to opsin via a protonated Schiff's base at lysine[296] (Bownds, 1967; Wang et al., 1980; Mullen & Akhtar, 1981; Findlay et al., 1981). Rhodopsin is also covalently modified by the addition of two N-linked oligosaccharides at asparagine[2] and asparagine[15] (Fukuda et al., 1979; Liang et al., 1979), by palmitoylation at cysteine[322] and cysteine[323] (Ovchinnikov et al., 1988), and by reversible light-dependent phosphorylation of up to seven serines and threonines near the carboxy terminus (Wilden et al., 1982; Thompson & Findlay, 1984; Figure 2). An essential disulfide bond has recently been identified between cysteine[110] and cysteine[187] (Karnik et al., 1988; Karnik & Khorana, 1990). Both wild-type rhodopsin and a mutant containing cysteines only at positions 110 and 187 react under denaturing conditions with cyanide, a reagent that cleaves disulfide bonds to form a thiocyanate. In both cases 1 mol of thiocyanate is formed per mole of protein, indicating that rhodopsin contains only a single disulfide bond. This pair of cysteines is found in all visual pigments sequenced to date and in most G-protein coupled receptors. The relevance of this disulfide bond for visual pigment function is underscored by the finding that, in individuals carrying a single red-green hybrid gene in place of the normal red and green pigment genes, substitution of cysteine[203] (the homologue of cysteine[187] in bovine rhodopsin) by arginine results in loss of the corresponding cone function (Nathans et al., 1989).

At present, no three-dimensional structure exists for rhodopsin or any other G-protein coupled receptor. However, a number of experimental constraints on the secondary and tertiary structures of rhodopsin have now made possible the construction of a reasonably accurate low-resolution model of the protein. Many of the experiments that lead to this model were made possible by a gift of nature: tens of milligrams of

rhodopsin are easily obtained from rod outer segments where it constitutes approximately 80% of the protein and is conveniently oriented within the regular array of disk membranes.

Low-angle X-ray and neutron scattering studies of rod outer segment membranes indicate that approximately one-half of rhodopsin's mass is membrane embedded (Saibil et al., 1976; Sardet et al., 1976; Osborne et al., 1978), and circular dichroism spectra indicate that rhodopsin has an α-helical content of approximately 50% (Shichi & Shelton, 1974). Intact rod outer segments orient along the lines of an external magnetic field (Chalazonitis et al., 1970; Chabre, 1978), a phenomenon that most likely reflects an orientation of rhodopsin's α-helices (and, hence, their magnetic moments) perpendicular to the plane of the disk membranes, i.e., parallel to the long axis of the outer segment (Worcester, 1978). More directly, in a magnetically oriented preparation of outer segments the infrared absorption band characteristic of carbonyl stretching within α-helices shows a dichroism indicative of an orientation of α-helices perpendicular to the disk membrane (Michel-Villaz et al., 1979). Proteolysis of intact outer segment disks has revealed points of cleavage in hydrophilic domains following the first, third, and fifth hydrophobic regions and in the carboxy-terminal tail, all of which therefore face the cytosol (Martynov et al., 1983; Mullen & Akhtar, 1983). Additional points of cleavage near the amino terminus and following the fourth hydrophobic domain are produced following freezing and thawing and therefore reside on the intradiscal face of the protein, topographically equivalent to the outside of the cell (Martynov et al., 1983). Similar studies have been performed to map sites of chemical modification (Barclay & Findlay, 1984) and of antibody (Molday & Molday, 1983) and lectin binding (Rohlich, 1976). The topography defined by these covalent modification studies agrees well with the assignment of seven putative transmembrane regions based upon hydropathy profiles (Ovchinnikov, 1983; Hargrave et al., 1983; Nathans & Hogness, 1983).

In intact rod outer segments, the extinction coefficient of rhodopsin is approximately 5-fold greater for plane polarized light with the E-field parallel to the plane of the disk membrane than for light with the E-field perpendicular to the plane of the membrane (Entine et al., 1968; Bowmaker et al., 1975). The plane of the chromophore is, therefore, oriented parallel to the plane of the disk membrane, a logical design given that the in vivo stimulus consists of unpolarized light propagating parallel to the long axis of the outer segment. The chromophore appears to sit within the rhodopsin molecule at a level near the center of the bilayer. Fluorescence energy transfer experiments show equal efficiencies of transfer to the chromophore regardless of which side of the membrane a water-soluble donor is located (Thomas & Stryer, 1982), and chemical cross-linking experiments using a photoactivatable 11-*cis*-retinal analogue [[*o*-dimethyl-*p*-[(trifluoromethyl)diazirinyl]phenyl]retinal] reveal major sites of cross-linking near the center of the third and fifth hydrophobic segments (Nakayama & Khorana, 1990).

The most recent experimental constraints on rhodopsin's structure have come from site-directed mutants. As described above, a disulfide bond brings residues 110 and 187 into proximity, and as described below, the identification of glutamate[113] as the retinylidene Schiff's base counterion places this residue near lysine[296]. Taken together, these experiments produce a remarkably consistent model in which seven membrane-embedded α-helices surround a central retinal binding pocket (Figures 1 and 2). The similarities between this model and the crystal structure of bacteriorhodopsin, a retinal-based proton pump, are striking (Henderson et al., 1990).

Protein–Chromophore Interactions

A long-standing question in visual pigment research is the mechanism by which different visual pigments tune the absorption spectrum of 11-*cis*-retinal. It is a remarkable fact that all of the colors we see rely upon the differential absorption of light by three cone pigments, each containing an 11-*cis*-retinal chromophore. The absorption maxima of the three human cone pigments are 426, 530, and 552 or 557 nm, the latter depending upon which one of two polymorphic variants of the red pigment one possesses (Oprian et al., 1991; Merbs & Nathans, 1992). Human rhodopsin, which plays little or no role in color vision, absorbs maximally at 493 nm (Wald & Brown, 1958). By way of comparison, the chloride salt of a protonated retinylidene Schiff's base in methanol absorbs maximally at 440 nm. This solvent and counterion arrangement, in which the chromophore is minimally perturbed by its environment, is taken as the reference to which each visual pigment is compared.

Over the past 30 years, a number of plausible models of spectral tuning have been proposed. All start with the observation that retinal undergoes a significant change in dipole moment upon photoexcitation. The positive charge that is localized primarily to the Schiff's base nitrogen in the ground state is distributed more evenly throughout the π-electron system in the photoexcited state (Kropf & Hubbard, 1958; Mathies & Stryer, 1976). Various perturbations could favor or disfavor this charge delocalization and thereby produce respectively a smaller or larger energy gap between ground and excited states, i.e., a red or blue shift. Placing a negative charge, presumably a glutamate or aspartate, along the polyene chain would favor charge delocalization (the point charge model; Kropf & Hubbard, 1958; Honig et al., 1976); polar groups along the polyene chain would favor or disfavor charge delocalization depending upon their orientation (Hays et al., 1980): polarizable groups along the polyene chain would stabilize the excited state by compensatory electronic movement (Irving et al., 1969, 1970); twisting about single and double bonds would produce respectively a decrease or increase in charge delocalization (Blatz & Liebman, 1973); and moving the retinylidene Schiff's base counterion further from the chromophore would induce a red shift (Blatz et al., 1972).

One elegant experimental approach to this problem has involved reconstituting rhodopsin with a series of 11-*cis*-retinal derivatives in which one-by-one each double bond has been saturated. By interrupting the conjugated π-electron system at different locations, one can map the point(s) at which opsin perturbs the chromophore. If, for a given dehydroretinal, the point(s) is (are) separated from the retinylidene Schiff's base by the saturated bond, then there will be little or no effect of that interaction on the primary absorption band of the chromophore. In practice, the absorption spectrum of the reconstituted pigment is compared to that of the corresponding protonated Schiff's base of the dehydroretinal in methanol to determine the difference in energy of photoexcitation. In bovine rhodopsin the major perturbation appears to be near C-13 (Koutalos et al., 1989), whereas in the chicken red pigment iodopsin there is a significant interaction near the β-ionone ring (Chen et al., 1989).

A second approach to the protein–chromophore question has been via site-directed mutants of rhodopsin. The major result to date has been the identification of glutamate[113] as the retinylidene Schiff's base counterion (Zhukovsky & Oprian, 1989; Sakmar et al., 1989; Nathans, 1990; Sakmar et al., 1991). The assignment rests on the following observations:

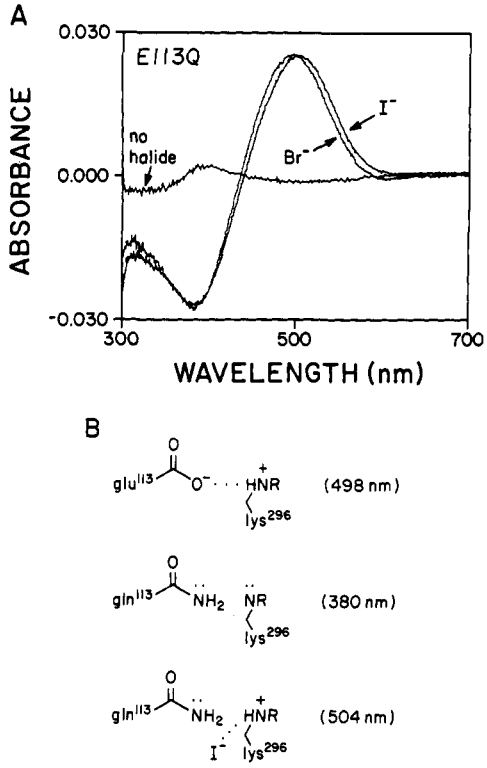

FIGURE 3: Effect of solution anions on a bovine rhodopsin mutant in which the retinylidene Schiff's base counterion, glutamate[113], has been replaced by glutamine. (A) Photobleaching absorption difference spectra. Samples were maintained at 20 °C in 0.1 M sodium phosphate, pH 6.0, 5% digitonin, and either 0.1 M sodium iodide, 0.1 M sodium bromide, or no halide. To obtain the photobleaching difference spectra, an absorption spectrum recorded after exposure to light of greater than 495 nm was subtracted from a spectrum obtained prior to light exposure. The difference spectrum shows the rhodopsin absorption band as a positive peak in the visible region and the product of photobleaching, *all-trans*-retinal, as a negative peak in the ultraviolet region. The absorption maximum in the presence of iodide is 504.5 ± 2.5 nm (n = 5) and in the presence of bromide 498.5 ± 1.5 (n = 5). In the absence of halide, the chromophore is not protonated and the absorption maximum lies in the ultraviolet near that of free *all-trans*-retinal. Halides have no effect on the absorption spectrum of wild-type rhodopsin. [From Nathans (1990b).] (B) Model of the retinylidene Schiff's base and its counterion, glutamate[113], in bovine rhodopsin: top, wild-type rhodopsin; middle, mutant glutamate[113] to glutamine in the absence of solution counterions; bottom, mutant glutamate[113] to glutamine in the presence of iodide.

first, replacement of glutamate[113] by glutamine leads to a decrease in the pK_a of the retinylidene Schiff's base from >10 to 6.0 when measured in 0.1 M NaCl; second, under conditions where the retinylidene Schiff's base in the glutamate[113]-to-glutamine mutant is deprotonated, small solution anions can promote reprotonation by serving as surrogate counterions, with each species of anion producing a distinctive absorption spectrum in the neighborhood of the wild-type spectrum (Figure 3); and third, substitutions in which a neutral residue replaces each of the remaining 21 aspartate and glutamate residues lead to pigments with wild-type or nearly wild-type absorption properties. The last observation also eliminates all carboxylates as candidates for a point charge along the polyene chain, suggesting that the 60-nm red shift that distinguishes 11-*cis*-retinal bound to bovine rhodopsin from the reference protonated retinylidene Schiff's base in methanol arises from interactions with neutral residues in the binding pocket.

A partial answer to the spectral tuning problem has recently come from studies of long-wavelength cone pigments in primates (Neitz et al., 1991). A comparison of amino acid sequences with the corresponding action spectra determined by electroretinography suggests that three variable amino acids—serine/threonine[180], tyrosine/phenylalanine[277], and threonine/alanine[285]—are the primary determinants of differential chromophore absorption in the 530–560-nm region of the spectrum. Interestingly, in each case the hydroxyl-bearing residue leads to a red shift. The recent success in producing recombinant cone pigments and their derivatives will greatly facilitate a detailed analysis of spectral tuning by this class of visual pigments (Oprian et al., 1991; Merbs & Nathans, 1992).

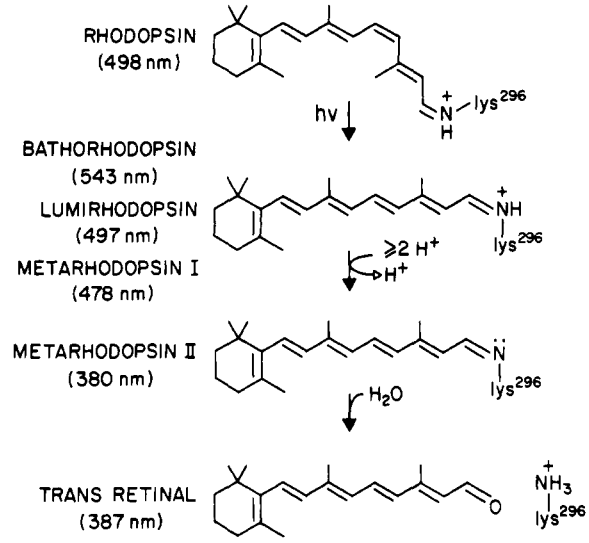

FIGURE 4: Changes in chromophore structure following photoactivation of rhodopsin. The absorption maximum of each photointermediate is indicated in parentheses. [From Yoshizawa and Wald (1963).]

Intermediates in Photoexcitation

A central question in receptor chemistry is the mechanism by which agonist or antagonist binding changes the conformation of a receptor. In the case of rhodopsin, the antagonist, 11-*cis*-retinal, is photochemically converted into an agonist, *all-trans*-retinal. Unlike other G-protein coupled receptors, conformational transitions in rhodopsin can be conveniently monitored by using the chromophore as a spectroscopic reporter of changes in the surrounding protein. Photoisomerization induces a series of conformational transitions, first defined by low-temperature spectroscopy (Yoshizawa & Wald, 1963) and more recently by time-resolved room temperature spectroscopy [Figure 4; reviewed in Shichida (1986)]. Bathorhodopsin, the first *all-trans*-retinal intermediate, stores 35 kcal/mol out of a total photon energy of 57 kcal/mol at 500 nm, as determined by microcalorimetry (Cooper, 1979). The mechanism of energy storage, for example, charge separation or conformational strain, is not known [reviewed in Birge (1990)].

Within 1 ms of photoisomerization, rhodopsin arrives at an equilibrium between two conformations, metarhodopsin I (MI) and metarhodopsin II (MII). This equilibrium mixture is stable for several seconds at room temperature and for many minutes near 0 °C, terminating in both cases by release of *all-trans*-retinal. MII is the form that interacts with transducin. In the absence of GTP, addition of stoichiometric quantities of the transducin drives the equilibrium toward MII, an effect that is abolished upon GTP addition (Bennett et al., 1982; Emeis et al., 1982). Circumstantial evidence suggests that the MI and MII conformations differ significantly. In MI the retinylidene Schiff's base is protonated whereas in MII it is not, a difference that accounts in large part for the 100-nm

difference in their absorption spectra (Doukas et al., 1978). MI and the preceding intermediates, bathorhodopsin and lumirhodopsin, can form in ice (Yoshizawa & Wald, 1963), in dehydrated samples (Rothschild et al., 1980), or below the phase transition temperature of the surrounding lipid (O'Brien et al., 1977; Mitchell et al., 1990). These conditions block the formation of MII, which only subsequently appears upon thawing, hydrating, or increasing lipid fluidity, respectively. Direct evidence that MI is more compact than MII comes from the observation that high pressure drives the MI–MII equilibrium toward MI (Lamola et al., 1974).

Rando and colleagues have shown that a neutral retinylidene Schiff's base is obligatory for the formation of MII, implying an important role for electrostatic interactions in the MI–MII transition (Longstaff & Rando, 1985; Longstaff et al., 1986; Seckler & Rando, 1989). Permethylated rhodopsin was prepared under conditions in which only those molecules containing a monomethylated retinylidene Schiff's base would survive. Photolysis of the permethylated rhodopsin produced a stable MI-like species that was unable to activate transducin and unable to serve as a substrate for phosphorylation by rhodopsin kinase. Interestingly, the covalent attachment of retinal to opsin appears not to be essential for photoactivation (Zhukovsky et al., 1991). Reconstitution of opsin carrying a lysine^{296}-to-alanine substitution (the site of attachment of 11-*cis*-retinal) with a butylamine retinylidene Schiff's base results in the formation of a photolabile pigment that can activate transducin. This experiment renders unlikely any model of photoactivation that envisions a movement of the seventh transmembrane segment by virtue of its covalent attachment to retinal.

Deprotonation of the retinylidene Schiff's base during the MI to MII transition might lead one to suppose that high pH favors MII. Surprisingly, high pH has the opposite effect (Mathews et al., 1963), and indeed, photolysis is accompanied by a net uptake of protons from solution (Radding & Wald, 1956). The midpoint of the MI–MII pH titration curve is 6.4, an observation that led Wald and his colleagues to propose that MII formation is coupled both to deprotonation of the retinylidene Schiff's base and to protonation of two or more histidines. This hypothesis has recently been tested by an analysis of site-directed mutants in which each of rhodopsin's six histidines was substituted one-by-one with phenylalanine (Weitz & Nathans, 1992). While all of the mutants could bind 11-*cis*-retinal to produce a photolabile pigment, three of the mutants differ from wild type in the behavior of the MI–MII equilibrium. Histidine^{65} to phenylalanine and histidine^{152} to phenylalanine show a small excess of MII compared to wild type. By contrast, histidine^{211} to phenylalanine, as well as histidine^{211} to cysteine, appears to form exclusively MI independent of pH. Addition of either octyl β-glucoside (Konig et al., 1989) or a peptide corresponding to the carboxy terminus of transducin α (Hamm et al., 1989), both of which drive the MI–MII equilibrium toward MII, had respectively little and no effect on the MI–MII equilibria of the histidine^{211} mutants (Figure 5).

Although a change in the protonation state of these histidines has not been directly demonstrated, the simplest interpretation of these experiments is that histidine^{211} is a site of protonation that is strongly coupled to the transition to MII, whereas histidine^{65} and histidine^{152} are sites at which protonation weakly favors MI. This model of negative cooperativity between histidines explains in a simple way the long-standing observation that the slope of the MI–MII pH titration curve is shallower than would be predicted for a single titratable group (Mathews et al., 1963; Parkes & Liebman, 1984). How might protonation of histidine^{211} drive the MI–MII equilibrium toward MII? One possibility is that in its protonated form histidine^{211} interacts directly with the retinylidene Schiff's base and/or its counterion, with a resulting decrease in the pK_a of the retinylidene Schiff's base.

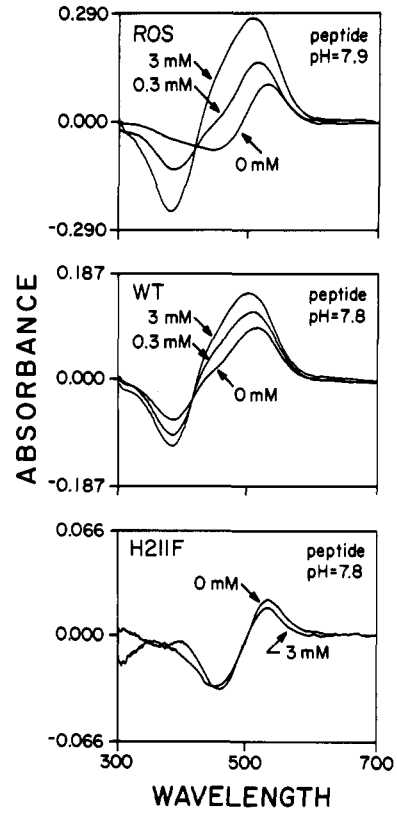

FIGURE 5: Photobleaching absorption difference spectra showing the effect on the MI–MII equilibrium of a synthetic peptide corresponding to the carboxy-terminal 11 amino acids of transducin α (IKENLKDCGLF; Hamm et al., 1989). Each difference spectrum was obtained by subtracting an absorption spectrum obtained after exposure to light greater than 580 nm from an absorption spectrum obtained prior to light exposure. The samples were solubilized in 2% digitonin and maintained at 2 °C in a water-chilled cuvette holder. The positive peak at 498 nm is derived from rhodopsin prior to photobleaching. The two products of photoactivation observed at this temperature, MI and MII, absorb maximally at 478 and 380 nm, respectively. The concentration of peptide added—0, 0.3, or 3 mM—is indicated for each spectrum. Top: rhodopsin from rod outer segments. At pH = 7.9, most of the photoproduct is in MI in the absence of the transducin peptide; increasing concentrations of peptide shift the equilibrium toward MII. Middle: wild-type rhodopsin produced in tissue culture. Bottom: rhodopsin mutant histidine^{211} to phenylalanine produced in tissue culture. [From Weitz and Nathans (1992).]

Interactions with Transducin, Arrestin, and Rhodopsin Kinase

Rhodopsin interacts with three proteins—transducin, rhodopsin kinase, and arrestin—each of which selectively recognizes the photoactivated conformation. Of great interest is the mechanism by which these proteins discriminate between inactive and photoactivated rhodopsin. The sequences of each of these proteins are now known from molecular cloning experiments [transducin α, β, and γ reviewed in Simon et al. (1991) rhodopsin kinase in Lorenz et al. (1991), and arrestin in Wistow et al. (1986)]. As described above, an 11 amino acid peptide corresponding to the carboxy terminus of transducin α shifts the MI–MII equilibrium toward MII with a half-maximal effect at 200 μM peptide (Hamm et al., 1989). Transducin α and arrestin share significant sequence homology

near their carboxy termini. Like transducin, arrestin binding shifts the MI–MII equilibrium toward MII, although the arrestin-dependent shift differs in requiring phosphorylated rhodopsin (Schleicher et al., 1989).

Which regions of rhodopsin interact with transducin, arrestin, and rhodopsin kinase? Two complementary approaches, both based upon modulation of the MI–MII equilibrium, have begun to answer this question for the case of transducin. In one approach, synthetic peptides corresponding to different surface loops of rhodopsin were assayed for their ability to compete with MII for binding to transducin (Konig et al., 1989). Three peptides, corresponding to the second, third, and fourth cytosolic loops, competed for transducin binding with K_d values of approximately 200 μM. (The fourth loop connects transmembrane segment 7 and the pair of palmitylated cysteines at positions 322 and 323.) Peptides derived from the first cytosolic loop, the amino-terminal domain and three extracellular loops, and the carboxy-terminal tail distal to cysteine[322] were ineffective as competitors. Interestingly, combining any two of the three active peptides resulted in an approximately 30-fold enhancement in effectiveness of competition. Simultaneous addition of all three active peptides produced only a small, nonsynergistic increase in effectiveness beyond that produced by two peptides. In a second approach, site-directed mutants of bovine rhodopsin have been examined for their ability to bind and activate transducin (Franke et al., 1988, 1990). Two mutants in which most of the second cytosolic loop was replaced with irrelevant amino acids, or in which one-half of the third cytosolic loop was deleted, bind transducin but appear to be defective in transducin release as inferred from the persistent surplus of MII in the presence of GTP. These mutants were also defective in light-dependent transducin activation as determined by GTP hydrolysis.

The original description of catalytic GDP–GTP exchange by photoactivated rhodopsin demonstrated a release of GDP in the absence of GTP at high fractional photobleaches (Fung & Stryer, 1980). This release appears to derive from stabilization by rhodopsin of a transducin conformation that lacks guanine nucleotide. The high concentration of intracellular GTP (approximately 2 mM) ensures that the reaction proceeds along the pathway of GTP binding and dissociation of transducin from rhodopsin. The structural basis for the rhodopsin-mediated stabilization of transducin in a guanine nucleotide-free state is unknown.

Rhodopsin Mutations in Autosomal Dominant Retinitis Pigmentosa

Given rhodopsin's central role in vision, it would be reasonable to suppose that alterations in its structure might be deleterious to the retina. This general notion has recently been borne out by the finding of rhodopsin mutations in one-quarter of patients with autosomal dominant retinitis pigmentosa (ADRP; Dryja et al., 1990a,b; Inglehearn et al., 1991; Sung et al., 1991a; Sheffield et al., 1991; Gal et al., 1991; Dryja et al., 1991; Keen et al., 1991; Inglehearn et al., 1992). Retinitis pigmentosa (RP) is a group of inherited disorders that affect approximately one person in 4000 regardless of ethnic background. The hallmarks of RP are an early loss of rod function followed by a progressive degeneration of the retina. Visual field loss typically begins in the rod-rich peripheral retina and proceeds toward the cone-rich central retina. The search for rhodopsin mutations in ADRP was stimulated by the observation of McWilliam et al. (1989) that in one large Irish family with ADRP the disease cosegregates with the region of chromosome 3 to which the rhodopsin gene maps (Nathans et al., 1986b). Thus far, 32 different rhodopsin

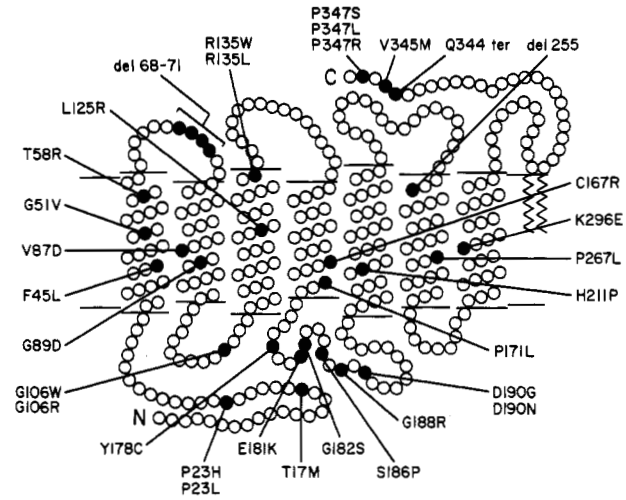

FIGURE 6: Mutations in human rhodopsin responsible for autosomal dominant retinitis pigmentosa. The substitutions are threonine[17] to methionine, proline[23] to histidine, proline[23] to leucine, phenylalanine[45] to leucine, glycine[51] to valine, threonine[58] to arginine, valine[87] to aspartate, glycine[89] to aspartate, glycine[106] to arginine, glycine[106] to tryptophan, leucine[125] to arginine, arginine[135] to leucine, arginine[135] to tryptophan, cysteine[167] to arginine, proline[171] to leucine, tyrosine[178] to cysteine, glutamate[181] to lysine, glycine[182] to serine, serine[186] to proline, glycine[188] to arginine, aspartate[190] to glycine, aspartate[190] to asparagine, histidine[211] to proline, proline[267] to leucine, lysine[296] to glutamate, valine[345] to methionine, proline[347] to leucine, proline[347] to serine, and proline[347] to arginine. Three mutations have been identified that are not amino acid substitutions: deletion of codons 68–71, deletion of codon 255, and glutamine[344] to stop. See text for references.

mutations have been identified, of which 29 are single amino acid substitutions (Figure 6).

Thirteen of the 32 mutant opsins have been produced by transfection of tissue culture cells with the corresponding cDNAs, and these fall into two phenotypic classes (Sung et al., 1991b). Three mutants (class I) resemble the wild type in yield, regenerability with 11-*cis*-retinal, and plasma membrane localization. Ten mutants (class II) accumulate to levels far below that of the wild type, regenerate variably or not at all with 11-*cis*-retinal, and are retained partially or completely in the endoplasmic reticulum. The class II mutants appear to be unstable and/or misfolded. In this regard they resemble a large number of site-directed mutants in the extracellular domains of bovine opsin (Doi et al., 1990). Doi et al. hypothesized that the extracellular domain may form a structure which organizes protein folding or serves to maintain a stably folded protein. It is reasonable to suppose that in the photoreceptor cell large quantities of an aberrantly folded or unstable opsin may interfere with intracellular trafficking and/or phototransduction.

To date, five mutations have been identified that map within five residues of opsin's carboxy terminus (Figure 6). Two of these, glutamine[344] to stop and proline[347] to leucine, have been produced by transfection, and both fall into class I; i.e., they resemble the wild type. This clustering suggests that opsin's carboxy terminus plays an important role in the photoreceptor that may not be apparent when the protein is expressed in a heterologous cell type.

Conclusion

The last decade has witnessed exciting advances in our understanding of rhodopsin. Many questions can now be posed at a mechanistic level. However, answering them precisely will require a high-resolution three-dimensional structure. Attempts to crystallize rhodopsin are now in progress in a number of laboratories—may luck favor them.

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Accelerated Publications

RNA Tetraloops as Minimalist Substrates for Aminoacylation†

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ABSTRACT: Previous work established that seven-base-pair hairpin microhelices with sequences based on the acceptor stems of alanine, glycine, methionine, and histidine tRNAs can be aminoacylated specifically with their cognate amino acids. To obtain "minimalist" substrates with fewer base pairs, we took advantage of the high thermodynamic stability of RNA tetraloop motifs that are found in ribosomal RNAs. We show here that rationally designed RNA tetraloops with as few as four base pairs are substrates for aminoacylation. Major nucleotide determinants for recognition by the class II synthetases were incorporated into each of the respective tetraloop substrates, resulting in specific aminoacylation by the alanine, glycine, and histidine tRNA synthetases. An analysis of the kinetics of aminoacylation shows that, for the alanine system, the majority of the transition-state stabilization provided by the synthetase–tRNA interaction is reproduced by the interaction of the synthetase with nucleotides in its minimalist tetraloop substrate. In an extension of this work, we also observed specific aminoacylation with the class I methionine tRNA synthetase of RNA tetraloops based on sequences in the acceptor stem of methionine tRNA. Thus, the results demonstrate four different examples where specific aminoacylation is directed by sequences/structures contained in less than half of a turn of an RNA helix.

Nucleotide sequence elements in the acceptor stems of several tRNAs are recognition signals for aminoacylation by the cognate aminoacyl-tRNA synthetases (aaRSs)[1] (Normanly & Abelson, 1989; Pütz et al., 1991; Jahn et al., 1991; Rould et al., 1989, 1991; Ruff et al., 1991; Schimmel, 1989, 1991). These sequence elements have been incorporated into seven-base-pair hairpin helices that are joined at the 3′-end through the bridging "discriminator" base N73 to the single stranded CCA_{76} terminus that is common to all tRNAs[2] (Francklyn & Schimmel, 1989; 1990; Francklyn et al., 1992; Martinis & Schimmel, 1992). In this way, microhelices that are aminoacylated specifically by the alanine, histidine, or glycine tRNA synthetase have been obtained. For these three examples from the class II tRNA synthetase family, the nucleotide sequence elements that determine the specificity and efficiency of aminoacylation are concentrated within the span from N73 through the first three base pairs near the acceptor end of the helix. These nucleotide sequence determinants overlap, so that the introduction of a determinant for one aaRS simultaneously removes a determinant for another aaRS (Francklyn et al., 1992). Thus, for at least these three amino acids, there is a unique relationship between a specific sequence/structure in an acceptor helix and the particular amino acid which is directed to that helix.

In an effort to define the minimal RNA structure required for specific aminoacylation, we synthesized short complementary single strands based on the acceptor helix of $tRNA^{Ala}$ (Musier-Forsyth et al., 1991a). Although the single strands are not aminoacylated, when hybridized together they yield a duplex with a 3′-ACCA single-stranded tetranucleotide

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[1] Abbreviations: aaRS, aminoacyl-tRNA synthetase; EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ATP, adenosine 5′-triphosphate; HPLC, high-pressure liquid chromatography.
[2] The standard 76-nucleotide numbering system for a tRNA sequence has been used.

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