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A Family of Receptors Coupled to Guanine Nucleotide Regulatory Proteins

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Cellular systems for the transduction of external stimuli into intracellular signals are essential components of the cellular plasma membrane. An emerging paradigm is that such "transmembrane signaling systems" often involve three distinct components. First, there are the specific receptors exposed at the external surface of the cell membrane that recognize and interact with ligands such as hormones or drugs or respond to sensory stimuli such as light. Second, exposed at the cytoplasmic surface are effector enzymes such as adenylate cyclase, which generates the second messenger cAMP (Lefkowitz et al., 1983). Effector enzymes also include phospholipase C (PLC) (which hydrolyzes phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate and 1,2-diacylglycerol) (Taylor & Merritt, 1986) as well as cGMP phosphodiesterase (Klein, 1984). Other effectors may act as ion channels (Pfaffinger et al., 1985; Breitwiesser & Szabo, 1985). Third, interposed physically and functionally between many receptor and effector molecules are transducing or coupling proteins, which bind and hydrolyze GTP. These are the guanine nucleotide regulatory proteins, or "G proteins" (Gilman, 1984). Recent evidence indicates that signaling systems of this type demonstrate a degree of structural, functional, and regulatory homology that had not previously been appreciated.

To date, the most structural information has been available on the retinal light transduction system consisting of the visual pigment rhodopsin, the guanine nucleotide regulatory protein transducin, and a cGMP phosphodiesterase (Stryer, 1986; Applebury & Hargrave, 1987). The rapid progress in this area can be attributed, at least in part, to the much greater abundance and availability of the individual protein components of this system as compared with, for example, those of the hormone-responsive adenylate cyclase. However, within the past year molecular cloning techniques have revealed the primary amino acid sequences of the adenylate cyclase coupled β-adrenergic receptor from three different species as well as the porcine muscarinic cholinergic receptor. All of the receptors share sequence homology and overall topographical analogies with each other and with rhodopsin.

In this brief essay we analyze the recently elucidated structures of these hormone receptors and rhodopsin, emphasizing the points of similarity and divergence. By focusing on the common as well as the unique functional and regulatory features of such receptors, it becomes possible to generate testable hypotheses about the structural basis for their various biological actions.

There are two "subtypes" of mammalian β-adrenergic receptor coupled to stimulation of adenylate cyclase, termed β_1- and β_2-adrenergic receptors, which are distinguished by distinct pharmacological properties and which subserve different physiological functions (Stiles et al., 1984). The cloned hamster (Dixon et al., 1986) and human (Kobilka et al., 1987a) β-adrenergic receptors are clearly of the β_2 variety, based on classical drug binding affinities. The specificity of the avian β-adrenergic receptor (Yarden et al., 1986) does not neatly conform to either the β_1 or β_2 subtype, though it has generally been grouped with the "β_1 receptors" (Minneman et al., 1979). Similarly, muscarinic cholinergic receptors are of at least two pharmacologically distinguishable subtypes, M_1 and M_2, which are coupled to a variety of effectors including the inhibition of adenylate cyclase, stimulation of phosphatidylinositol hydrolysis (Klein, 1984), or activation of K^+ channels (Pfaffinger et al., 1985; Breitwiesser & Szabo, 1985). However, in this system there is no clear correlation between the pharmacologically determined subtype and the coupled functional response. Two distinct muscarinic receptor genes have been cloned and appear to be of the M_1 (Kubo et al., 1986a) and M_2 (Kubo et al., 1986b) subtypes, based on the ligand binding pharmacology of the expressed protein as well as the tissue distribution of receptor mRNA.

Figure 1 presents the optimal alignment of the deduced amino acid sequences for bovine rhodopsin, the β-adrenergic receptors from three species (hamster, human, turkey), and

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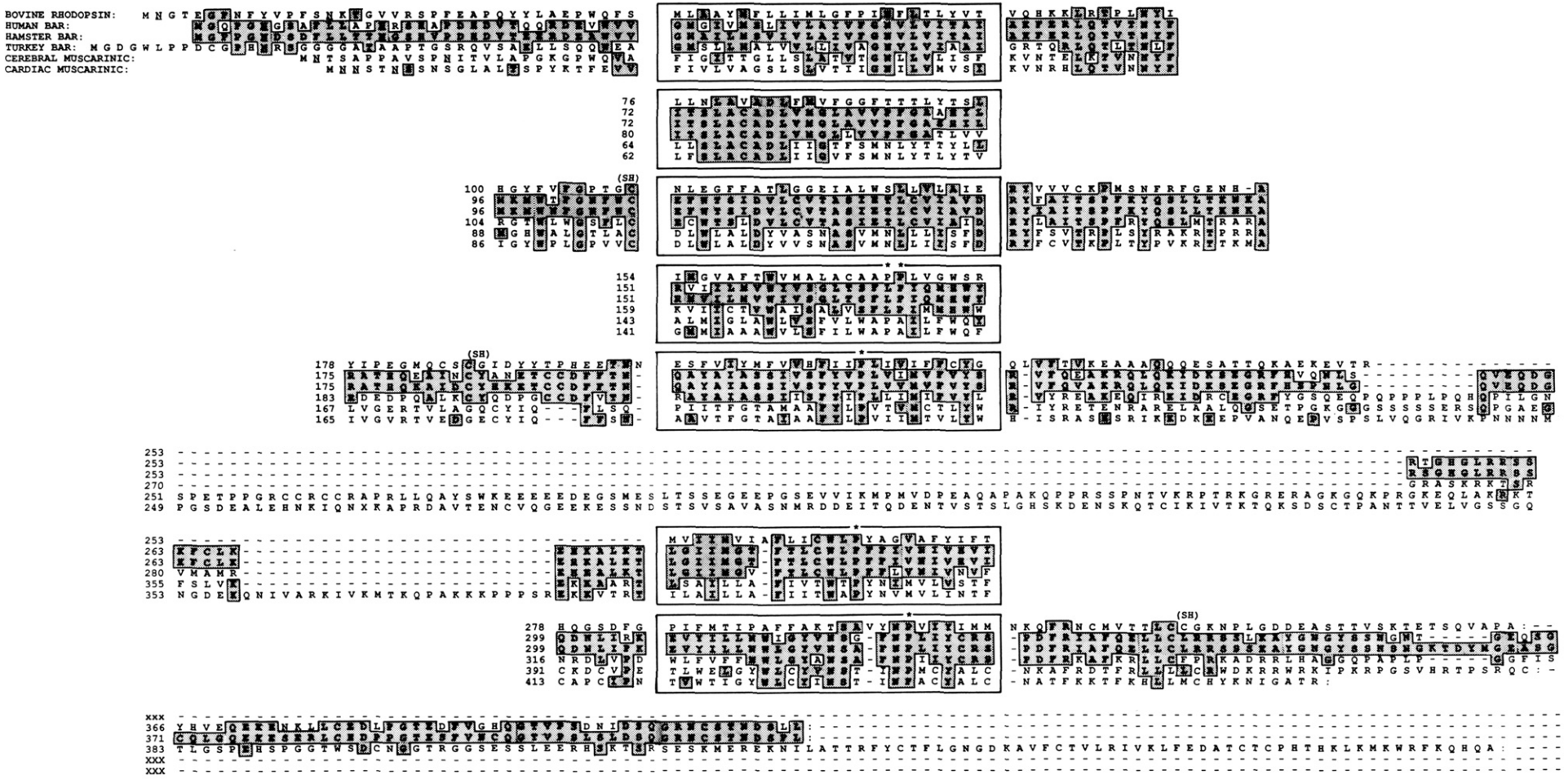

FIGURE 1: Amino acid homologies between hamster β_2-adrenergic receptor and selected G protein coupled receptors. Alignments were determined as described in Table I (except a gap penalty of −2 is used) and as presented by Dixon et al. (1986). Amino acid identity with hamster β_2AR is indicated by shading. Putative membrane spanning regions are boxed. Consensus sites for N-linked glycosylation (N-X-S or T) at the amino-terminal regions are underlined. Conserved prolines (*) in the transmembrane regions and conserved cysteines (SH) are indicated. The standard (IUPAC) single-letter code is used.

Table I: Amino Acid Homology (%) between Various Pairs of Proteins Having a Seven Membrane Spanning Domain Topology[a]

	hamster β_2AR	human β_2AR	turkey βAR	cerebral muscarinic	cardiac muscarinic	bovine opsin	human opsin	blue opsin	green opsin	red opsin	R8 opsin
human β_2AR	88 (158)										
turkey βAR	49 (77)	49 (76)									
porcine cerebral muscarinic	23 (18)	24 (19)	26 (17)								
porcine cardiac muscarinic	22 (14)	24 (12)	20 (11)	43 (72)							
bovine opsin	15 (8.6)	14 (8.4)	17 (6.8)	14 (7.4)	18 (5.1)						
human opsin	15 (8.8)	14 (9.2)	16 (7.8)	14 (7.2)	15 (4.0)	93 (151)					
human blue opsin	12 (3.4)	14 (3.6)	14 (4.6)	15 (6.1)	15 (6.2)	42 (50)	42 (55)				
human green opsin	17 (8.6)	14 (5.9)	16 (6.8)	16 (5.8)	16 (4.5)	40 (59)	41 (59)	43 (52)			
human red opsin	16 (5.8)	16 (5.9)	14 (6.6)	15 (3.9)	19 (4.8)	39 (55)	40 (46)	42 (60)	96 (153)		
Drosophila R8 opsin	15 (5.7)	16 (7.7)	15 (4.2)	17 (5.9)	15 (3.7)	22 (21)	22 (16)	18 (13)	20 (14)	20 (17)	
Drosophila R1–6 opsin	17 (4.0)	12 (4.8)	17 (6.0)	18 (8.9)	16 (4.5)	23 (20)	23 (18)	23 (17)	22 (23)	23 (20)	66 (109)

[a] Percent homologies were calculated for the optimal alignment of each pair of sequences as determined by the ALIGN computer program (Orcutt et al., 1986). The optimal alignment is based on a match score of 1, a mismatch score of 0, and a gap penalty of −8. *Z* values (given in parentheses) are in standard deviation units and refer to the difference between the optimal match score and the average maximum match score for 100 random permutations of the two sequences divided by the standard deviation of the random scores.

the porcine cerebral (M_1) and cardiac (M_2) muscarinic cholinergic receptors. Rhodopsin (Ovchinnikov, 1982; Nathans & Hogness, 1984) is representative of the class of proteins called visual pigments, which also includes the color pigments for blue, green, and red vision in humans (Nathans et al., 1986). Related proteins have been identified in most species, and the genes for two *Drosophila* opsins also have been isolated and sequenced (O'Tousa et al., 1985; Zuker et al., 1985; Cowman et al., 1986). There is no amino acid sequence homology between any eukaryotic opsin and bacteriorhodopsin, which is also a membrane-bound retinyl protein involved in light-sensitive processes (Khorana et al., 1979). Neither is there sequence homology between the nicotinic (Boulter et al., 1986) and muscarinic (Kubo et al., 1986a,b) cholinergic receptors. The amino acid homology among the different receptor and pigment proteins is presented in Table I. Although the overall homology between the β-adrenergic or muscarinic receptors and the pigments is low (12–18%), certain regional homologies are much higher, as discussed below.

Seven Membrane Spanning Regions

Perhaps the most striking similarity in the structures of all these receptor proteins is that each contains seven stretches of 20–28 hydrophobic amino acids, which likely represent membrane spanning regions. Hydropathicity analysis of the sequences by the method of Kyte and Doolittle (1982) yields remarkably similar profiles (Dohlman et al., 1987; Kubo et al., 1986a,b). Based on such analyses, it is possible to predict how the proteins may be situated in the plasma membrane (Figure 2). Furthermore, this type of comparison highlights some common features not readily apparent from simple alignment of the amino acid sequences. For example, hamster and human β-adrenergic receptors have 88% amino acid identity overall (with one gap in their alignment). Two of the cytoplasmic loops (I–II and III–IV) and the intramembrane regions, however, are particularly well conserved with average homologies of 97% and 95%, respectively. The amino- and carboxyl-terminal regions are the least conserved with 71% and 79% identity, respectively. Between hamster β-adrenergic receptor and turkey β-adrenergic receptor, a similar pattern of conservation is observed. Overall amino acid homology is 49%, yet the intramembranous regions are 73% homologous and the first two cytoplasmic loops are 64% homologous, while the amino- and carboxyl-terminal regions are the most poorly conserved, with 18% and 20% identity, respectively. Most conserved between hamster β-adrenergic receptor and cerebral or cardiac muscarinic receptors are again the transmembrane regions (32% and 28%, respectively) and the first two cytoplasmic loops (39% and 42%); least conserved are the amino- and carboxyl-terminal regions (4–17%). Overall homology with β-adrenergic receptor is 25% for both subtypes.

When compared to one another, the porcine cardiac and cerebral muscarinic cholinergic receptors have 43% amino acid identity overall. As is the case for the β-adrenergic receptors, the identity in the transmembrane regions and in the first two cytoplasmic loops (I–II and III–IV) is much higher at 72% and 74%, respectively. The amino- and carboxyl-terminal regions, as well as cytoplasmic loop V–VI, are the most poorly conserved.

Among the opsins, a different picture emerges. Between bovine rhodopsin and each of the four human opsins (rhodopsin and the three color pigments), there is considerable homology in the cytoplasmic loops, while the amino- and carboxyl-terminal regions are remarkably divergent. In contrast to the pattern of homology seen among the ligand binding receptors, the transmembrane regions of the opsins are not particularly well conserved (Nathans et al., 1986). Yet when hamster β-adrenergic receptor is compared to bovine opsin, it is the transmembrane regions where the amino acid homology is most striking (Dixon et al., 1986).

Recently, increasing attention has focused on the possible significance of the seven membrane spanning domain topology, which has been observed for a number of integral membrane proteins. The most thoroughly documented example is bacteriorhodopsin, the purple membrane protein of *Halobacterium halobium*. The presence of a bundle of transmembrane α helices in this protein has been established by high-resolution electron diffraction (Henderson & Unwin, 1975). Similarly,

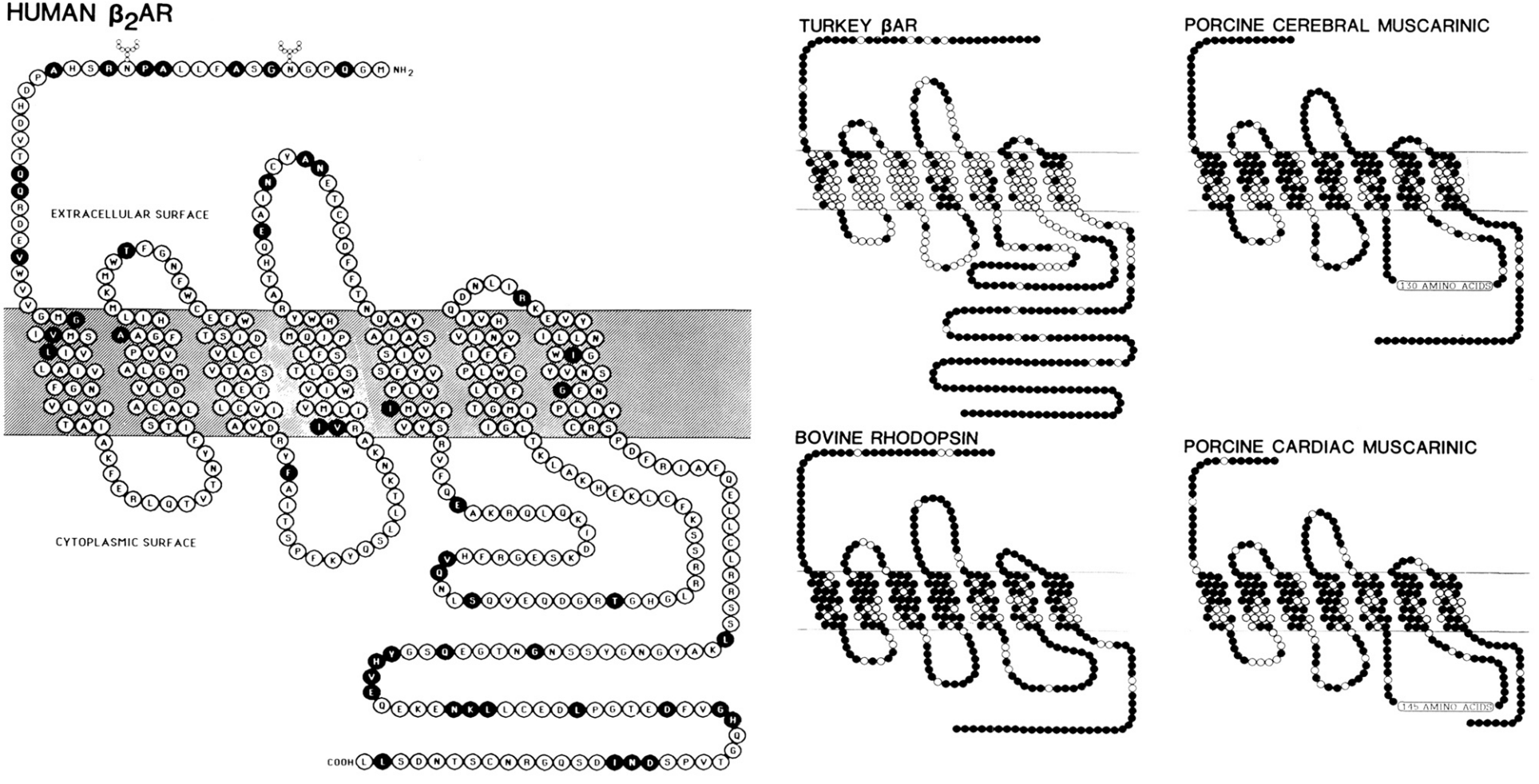

FIGURE 2: Structures of human β_2-adrenergic receptor, turkey β-adrenergic receptor, porcine cerebral muscarinic cholinergic receptor, porcine cardiac muscarinic cholinergic receptor, and bovine rhodopsin, as they may be organized within the membrane. Open circles represent amino acids that are identical with those of hamster β_2-adrenergic receptor, as presented in Figure 1.

the presence in the vertebrate photoreceptor pigment rhodopsin of seven such helices analogous to bacteriorhodopsin is supported by circular dichroism, infrared dichroism, X-ray, and neutron diffraction studies, as well as by limited proteolysis of exposed regions and chemical modifications of hydrophobic regions [reviewed by Applebury and Hargrave (1987)]. The depictions shown in Figure 2, however, are based solely on hydropathicity analyses and analogy with rhodopsin. For the ligand binding receptors the presence of these topological features are only now being subjected to testing by various chemical, spectroscopic, or immunological techniques. Moreover, several other proteins whose structures have recently been elucidated by molecular cloning techniques have been suggested to also share the seven membrane spanning domain organization. These include the α and *a* factor receptors of the yeast *Saccharomyces cerevisiae* (Nakayama et al., 1985; Burkholder & Hartwell, 1985), the human *mas* oncogene (Young et al., 1986), and the enzyme HMG-CoA reductase (Chin et al., 1984).

An intriguing, but as yet unanswered, question is what unique properties this particular membrane organization may possess and what evolutionary pressures have led to its occurrence in each of these proteins? By analogy with the light-sensitive proteins and their chromophores the seven-helix bundle may in some way accommodate the binding of specific ligands or substrates (Dixon et al., 1986). Perhaps it is uniquely suited for transmitting a signal from the external surface via a ligand-induced conformational change to the internal surface of the plasma membrane. One common feature of many of these proteins is that they function as plasma membrane receptors coupled to guanine nucleotide regulatory proteins.

Consistent with the notion that the region bounded by the seven α helices may be important for hormone binding is the unusually high degree of sequence homology in these helices among all the receptors (including mammalian opsins) examined. Two unexpected features that appear to be conserved among the opsins and the β-adrenergic and muscarinic cholinergic receptors are several prolines and charged residues in the membrane α helices. The retinaldehyde of each of the mammalian opsins is attached via a Schiff base to the ϵ-amino group of Lys-296, in the middle of the seventh transmembrane helix. The Schiff base of rhodopsin has been shown to be protonated (Oseroff & Callender, 1974), which would be energetically unfavorable in this hydrophobic environment without a counterion. Indeed, helix II contains a unique aspartic acid and helix III a glutamic acid that are both well positioned to serve as the Schiff base counterion, and these residues are conserved in each of the mammalian opsins. While a lysine is absent from the seventh membrane spanning segment of the muscarinic and β-adrenergic receptors, an aspartic acid in helix II and in helix III is present in each of the ligand binding receptors (Figure 1). Applebury and Hargrave (1987) have suggested that these negatively charged residues may serve instead as counterions for the positively charged amine of ligands such as epinephrine (or acetylcholine).

Proline, with its rigid backbone angles and the lack of an H-bond forming amide proton, is not commonly found within α helices. Yet prolines are found within at least five of the seven membrane spanning domains of all the mammalian opsins and receptors, and the positioning is well conserved (Figure 1). In a survey of published sequences, Brandle and Deber (1986) observed proline residues in the membrane spanning regions of 8 of 9 transport proteins, but only 3 of 16 nontransport proteins. They proposed that cis–trans isomerization of membrane-buried X–Pro peptide bonds might be responsible for conformational changes that regulate events such as the opening and closing of membrane channels. Likewise, ligand binding may lead to conformational changes in membrane-bound receptors analogous to those proposed for ion transport proteins.

The electron diffraction pattern of bacteriorhodopsin shows that some of the transmembrane helices are kinked (Henderson & Unwin, 1975), possibly due to the presence of five prolines present in these regions. These might be important in creating a binding pocket internal to the bundle of seven α helices that could accommodate the bound chromophore of bacteriorhodopsin. In the case of the β-adrenergic receptor, a similar pocket may sterically accommodate hormone or drug binding.

An alternate role of the proline residues may be to stabilize ligand binding by an electrostatic effect. The X–Pro linkage is via a tertiary amide peptide bond. This would result in increased basicity at the carbonyl oxygen (Veis & Nawrot, 1970) and promote H-bond formation to any positively charged species in the binding site including the amine-containing ligands.

Extracellular Regions

Rhodopsin and the β-adrenergic receptor each contain two asparagine-linked oligosaccharides in the amino-terminal sequence, exposed at the extracellular surface of the cell membrane. Avian β-adrenergic receptor, porcine cerebral muscarinic receptor, and the human color pigments, as well as the two *Drosophila* opsins, each have one consensus site for N-linked glycosylation at the amino terminus; the cardiac muscarinic receptor has three such sites. The purified β-adrenergic receptor can be completely deglycosylated enzymatically without alteration of ligand binding or biological activating functions (Benovic et al., 1987). Neither bovine rhodopsin nor the hamster β-adrenergic receptor have N-terminal signal sequences. Of the other extracellularly exposed regions, loop II–III and loop IV–V contain cysteines that are perfectly conserved among all of the vertebrate and invertebrate pigments as well as all five ligand binding receptors (Figure 1). This suggests the possibility of a disulfide link between loop II–III and IV–V. However, while reduction of purified avian β-adrenergic receptor by dithiothreitol was found to promote receptor–G_s coupling in a reconstituted system (Pedersen & Ross, 1985), exhaustive modification of cysteines in rhodopsin has no effect on rhodopsin–transducin coupling (Hofmann & Reichert, 1985).

Cytoplasmic Regions

The carboxyl-terminal region of bovine rhodopsin is exposed at the cytoplasmic surface. The last 12 residues include 7 which are either serine or threonine and which in the presence of light become substrates for phosphorylation by the specific enzyme rhodopsin kinase (Thompson & Findlay, 1984). Phosphorylation of rhodopsin is thought to result in accelerated deactivation of the phosphodiesterase (Kuhn et al., 1973; Liebman & Pugh, 1980). Despite having sequences that are poorly conserved in this region, mammalian and avian β-adrenergic receptors contain a similar serine- and threonine-rich region at or near the carboxyl terminus. A β-adrenergic receptor kinase has been identified in mammals that will phosphorylate agonist-occupied β-adrenergic receptors (as well as light-bleached rhodopsin), presumably at the carboxyl terminus (Benovic et al., 1986). Serine- and threonine-rich carboxyl termini are also seen in human rhodopsin (6 of 15 are serine or threonine), the *Drosophila* opsins (6/13 and

6/17), and human red (10/20), green (10/20), and blue (11/25) color pigments. Fewer serines and threonines are present in the terminal residues of the cerebral (3/10) and cardiac (3/21) muscarinic receptors. If this is a common site for regulatory phosphorylation, why is the precise positioning of substrate amino acids, as well as the sequence of nearby residues, not conserved? Perhaps determinants for recognition by receptor kinases lie in the other exposed regions of the receptors which are well conserved.

The purified mammalian β-adrenergic receptor has also been shown to be a substrate in vitro for the cAMP-dependent protein kinase (Benovic et al., 1985) and protein kinase C (Bouvier et al., 1987), and there is considerable evidence that phosphorylation of agonist-occupied β-adrenergic receptor constitutes a regulatory (desensitization) mechanism by which subsequent response to agonist is attenuated in vivo [reviewed by Sibley and Lefkowitz (1985)]. In a similar fashion, prolonged exposure to cholinergic agonists leads to desensitization of muscarinic cholinergic receptor function (Masters et al., 1985; Green & Clark, 1982). This is accompanied by phosphorylation of the receptor in cardiac tissue, though the kinase(s) involved is (are) not known (Kwatra & Hosey, 1986).

The cAMP-dependent protein kinase phosphorylates hydroxyl amino acids carboxyl terminal to two basic residues (K or R-K or R-X-S or T; Glass & Krebs, 1980). There are a number of such potential sites of phosphorylation localized in the cytoplasmic regions of the hamster (S^{262} and S^{346}), human (S^{262} and S^{346}), and turkey (S^{278}) β-adrenergic receptors, as well as the cerebral (T^{330} and T^{354}) and cardiac (T^{137} and T^{386}) muscarinic cholinergic receptors. Although less well characterized, sites of phosphorylation by protein kinase C also appear to require proximal basic residues (Kishimoto et al., 1985) and there is an abundance of potential sites at the cytoplasmic surface of all the receptors.

Besides being a substrate for phosphorylation by regulatory kinases, the cytoplasmic surfaces of these receptor proteins may also be involved in recognition and coupling to regulatory G proteins. Cytoplasmic loops I–II and III–IV are the most conserved cytoplasmic sequences among all of the mammalian visual pigments, and the homology extends to invertebrate pigments in loop I–II. Among the ligand binding receptors, the same regions are well conserved with each other, but not as a group with the opsins. Loop V–VI is the only connecting loop that varies appreciably in length among all the ligand binding receptors and visual pigments. Rhodopsin loop V–VI contains a serine at position 240, which is phosphorylated by rhodopsin kinase (McDowell et al., 1985). This loop has been directly implicated in rhodopsin–transducin coupling by proteolysis studies. Removal of the entire carboxyl terminus and part of the rhodopsin cytoplasmic loop V–VI destroys transducin coupling while removal of the last 12 residues has no effect on G-protein coupling (Kuhn & Hargrave, 1981; Kuhn, 1984). The great variability in the size of this loop between rhodopsin and β-adrenergic and muscarinic cholinergic receptors (all of which couple to different G proteins) may suggest an important role for this region in determining the specificity of this coupling. It is worth noting that while they do not have carboxyl termini that are serine and threonine rich, several such regions are found in the cytoplasmic loop V–VI of both muscarinic receptors. So perhaps, like rhodopsin, the muscarinic receptors are phosphorylated by a specific kinase at both the cytoplasmic loop V–VI and near the carboxyl terminus.

There appears to be some interchangeability of the components of the visual transduction system and adenylate cyclase coupled receptors. This has been revealed by reconstitution experiments in which receptor proteins are incorporated into lipid vesicles together with various guanine nucleotide regulatory proteins such as transducin, G_s, G_i (mediates hormonal inhibition of adenylate cyclase), and G_o (a G protein not involved in adenylate cyclase regulation closely related to G_i in structure). In such experiments, light-bleached rhodopsin will activate the GTPase activity of transducin as well as G_i (Cerione et al., 1985; Kanaho et al., 1984; Fung, 1983) and G_o (Sternweis & Robishaw 1984; Florio & Sternweis, 1985) but not that of G_s (Cerione et al., 1985). Vertebrate transducin can be activated by either vertebrate or invertebrate (*Drosophila*) rhodopsin (Vandenberg & Montal, 1984; Ebrey et al., 1980). Agonist-occupied β-adrenergic receptors will promote activation of G_s and to a much lesser extent G_i, while transducin is hardly activated at all (Cerione et al., 1985; Asano et al., 1984). Finally, the adenylate cyclase inhibitory α_2-adrenergic receptor is quite effective in activating G_i and G_o, less so with regard to transducin, and is virtually inactive with G_s (Cerione et al., 1986). It remains to be determined which structural features may be responsible for determining the differential abilities of these receptor proteins to interact with the various G proteins, but as mentioned above the variability in the cytoplasmic loop V–VI makes this domain of the receptors a particularly attractive one to dictate this function.

The Genes

Several interesting features about the genes for these receptors are worthy of comment and have implications for understanding the evolutionary origin of these proteins. We have suggested that the mammalian β_2-adrenergic receptor may have arisen as a processed gene for what is now a related but functionally distinct gene. This is based on the unusual lack of introns within the coding region of both the hamster (Dixon et al., 1986) and human gene (Kobilka et al., 1987b) for the β_2-adrenergic receptor and on the presence of direct repeats bordering the genes. While the human and hamster β-adrenergic receptor genes contain no introns, introns are present in conserved positions among the mammalian opsin genes (Baehr & Applebury, 1986). Three of the same four intron positions are preserved in bovine and human rhodopsin, as well as the three human color pigment genes and the rhabdomere 1–6 opsin of *Drosophila*. The coding regions of human green and red pigment genes each contain an additional intron. The gene structures of the muscarinic cholinergic receptors and of the avian β-adrenergic receptor are not known. Interestingly, the recently identified *mas* oncogene also lacks introns and is predicted to code for a protein with seven membrane spanning domains, analogous to the β-adrenergic receptor (Young et al., 1986).

Future Directions

The premise that most proteins evolved from a small number of archetypal proteins is based on the notion that it is simpler to duplicate, modify, and even combine genes than to assemble them de novo (Doolittle, 1981). That the family of related proteins discussed here probably arose from gene duplication events from some common ancestral receptor gene is evidenced by conservation of protein sequence, as well as the organization of structural domains within the membrane. Each receptor has evolved and adapted to new and distinct roles, yet retains many preexisting features. The challenge is to determine which features are conserved because of constraints imposed by evolution of function and which divergent features represent structural adaptations to new functions. It will be of great interest to learn whether the genes for other G protein coupled

receptors also lack introns. In terms of the proteins, the overall pattern of conservation of structure for such receptors includes seven hydrophobic transmembrane domains, a glycosylated amino terminus with no signal sequence, and the greatest variability in length and the weakest sequence homology at the amino- and carboxyl-terminal regions and at the third cytoplasmic loop (loop V–VI). The generality and importance of the seven membrane spanning domains will become known only by virtue of a detailed knowledge of the function of a number of such proteins, for example, the color pigments and the *mas* oncogene. Furthermore, the assignment of structural domains of the β-adrenergic receptor as being involved with specific functions such as membrane insertion, ligand binding, coupling to G proteins, and interaction with regulatory kinases will have to await further biochemical and molecular genetic (mutagenesis) studies.

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REFERENCES

Applebury, M. L., & Hargrave, P. A. (1987) *Vision Res. 26*, 1881–1895.

Asano, T., Katada, T., Gilman, A. G., & Ross, E. M. (1984) *J. Biol. Chem. 259*, 9351–9354.

Baehr, W., & Applebury, M. L. (1986) *Trends NeuroSci. (Pers. Ed.) 9*, 198–203.

Benovic, J. L., Pike, L. J., Cerione, R. A., Staniszewski, C., Yoshimasa, T., Codina, J., Birnbaumer, L., Caron, M. G., & Lefkowitz, R. J. (1985) *J. Biol. Chem. 260*, 7094–7101.

Benovic, J. L., Mayor, F., Jr., Somers, R. L., Caron, M. G., & Lefkowitz, R. J. (1986) *Nature (London) 322*, 869–872.

Benovic, J. L., Staniszewski, C., Cerione, R. A., Codina, J., Lefkowitz, R. J., & Caron, M. G. (1987) *J. Recept. Res.* (in press).

Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., Heinemann, S., & Patrick, J. (1986) *Nature (London) 319*, 368–374.

Bouvier, M., Leeb-Lundberg, L. M., Benovic, J. L., Caron, M. G., & Lefkowitz, R. J. (1987) *J. Biol. Chem. 262*, 3106–3113.

Brandl, C. J., & Deber, C. M. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 917–921.

Breitwiesser, G., & Szabo, G. (1985) *Nature (London) 317*, 538–540.

Burkholder, A. C., & Hartwell, L. H. (1985) *Nucleic Acids Res. 13*, 8463–8475.

Cerione, R. A., Staniszewski, C., Benovic, J. L., Lefkowitz, R. J., Caron, M. G., Gierschik, P., Somers, R., Spiegel, A. M., Codina, J., & Birnbaumer, L. (1985) *J. Biol. Chem. 260*, 1493–1500.

Cerione, R. A., Regan, J. W., Nakata, H., Codina, J., Benovic, J. L., Gierschik, P., Somers, R. L., Spiegel, A. M., Birnbaumer, L., Lefkowitz, R. J., & Caron, M. G. (1986) *J. Biol. Chem. 261*, 3901–3909.

Chin, D. J., Gil, G., Russel, D. W., Liscum, L., Luskey, K. L., Basu, S. K., Okayama, H., Berg, P., Goldstein, J. L., & Brown, M. S. (1984) *Nature (London) 308*, 613–617.

Cowman, A. C., Zuker, C. S., & Rubin, G. M. (1986) *Cell (Cambridge, Mass.) 44*, 705–710.

Dixon, R. A. F., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. G., Frielle, T., Bolanowski, M. A., Bennett, C. D., Rands, E., Diehl, R. E., Mumford, R. A., Slater, E. E., Sigal, I. S., Caron, M. G., Lefkowitz, R. J., & Strader, C. D. (1986) *Nature (London) 321*, 75–79.

Dohlman, H. G., Caron, M. G., & Lefkowitz, R. J. (1987) *Kidney Int.* (in press).

Doolittle, R. E. (1981) *Science (Washington, D.C.) 214*, 149–158.

Ebrey, T., Tsuda, M., Sassenrath, G., West, J. L., & Waddell, W. H. (1980) *FEBS Lett. 116*, 217–219.

Florio, V. A., & Sternweis, P. C. (1985) *J. Biol. Chem. 260*, 3477–3483.

Fung, B. K.-K. (1983) *J. Biol. Chem. 258*, 10495–10502.

Gilman, A. G. (1984) *Cell (Cambridge, Mass.) 36*, 577–579.

Glass, D. B., & Krebs, E. G. (1980) *Annu. Rev. Pharmacol. Toxicol. 20*, 363–388.

Green, D. A., & Clark, R. B. (1982) *J. Neurochem. 39*, 1125–1131.

Henderson, R., & Unwin, P. N. T. (1975) *Nature (London) 257*, 28–32.

Hofmann, K. P., & Reichert, J. (1985) *J. Biol. Chem. 260*, 7990–7995.

Kanaho, Y., Tsai, S. C., Adamik, R., Hewlett, E. L., Moss, J., & Vaughan, M. (1984) *J. Biol. Chem. 259*, 7378–7381.

Khorana, H. G., Gerber, G. E., Herlihy, W. C., Gray, C. P., Anderegg, R. J., Nihei, K., & Biemann, K. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5046–5050.

Kishimoto, A., Nishiyama, K., Nakanishi, H., Uratsuji, Y., Nomura, H., Takeyama, Y., & Nishizuka, Y. (1985) *J. Biol. Chem. 260*, 12492–12499.

Klein, W. (1984) *Curr. Top. Cell. Regul. 24*, 129–144.

Kobilka, B. K., Dixon, R. A. F., Frielle, T., Dohlman, H. G., Bolanowski, M. A., Sigal, I. S., Yang-Feng, T. L., Francke, U., Caron, M. G., & Lefkowitz, R. J. (1987a) *Proc. Natl. Acad. Sci. U.S.A. 84*, 46–50.

Kobilka, B. K., Frielle, T., Dohlman, H. G., Bolanowski, M. A., Dixon, R. A. F., Keller, P., Caron, M. G., & Lefkowitz, R. J. (1987b) *J. Biol. Chem.* (in press).

Kubo, T., Fukuda, K., Mikami, A., Maeda, A., Takahashi, H., Mishina, M., Haga, T., Haga, K., Ichiyama, A., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., & Numa, S. (1986a) *Nature (London) 232*, 411–416.

Kubo, T., Maeda, A., Sugimoto, K., Akiba, I., Mikami, A., Takahashi, H., Haga, T., Haga, K., Ichiyama, A., Kangawa, K., Matsuo, H., Hirose, T., & Numa, S. (1986b) *FEBS Lett. 209*, 367–372.

Kuhn, H. (1984) *Prog. Retinal Res. 3*, 123–156.

Kuhn, H., & Hargrave, P. A. (1981) *Biochemistry 20*, 2410–2417.

Kuhn, H., Cook, J. H., & Dreyer, W. J. (1973) *Biochemistry 12*, 2495–2502.

Kwatra, M. M., & Hosey, M. M. (1986) *J. Biol. Chem. 261*, 12429–12432.

Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105–132.

Lefkowitz, R. J., Stadel, J. M., & Caron, M. G. (1983) *Annu. Rev. Biochem. 52*, 159–186.

Liebman, P. A., & Pugh, E. N. (1980) *Nature (London) 287*, 734–736.

Masters, S. B., Quinn, M. T., & Brown, J. H. (1985) *Mol. Pharmacol. 27*, 325–332.

McDowell, J. H., Curtis, D. R., Baker, V. A., & Hargrave, P. A. (1985) *Invest. Ophthalmol. Visual Sci. 26*, 291.

Minneman, K. P., Weiland, G. A., & Molinoff, P. B. (1979) *Mol. Pharmacol. 17*, 1–7.

Nakayama, N., Miyajima, A., & Arai, K. (1985) *EMBO J. 4*, 2643–2648.

Nathans, J., & Hogness, D. S. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4851–4855.

Nathans, J., Thomas, D., & Hogness, D. S. (1986) *Science (Washington, D.C.) 232*, 193–202.
Orcutt, B. C., Dayhoff, M. O., George, D. G., & Baker, W. C. (1986) PIR Report ALI-1284, National Biomedical Research Foundation, Washington, D.C.
Oseroff, A. R., & Callender, R. H. (1974) *Biochemistry 13*, 4243–4248.
O'Tousa, J. E., Baehr, W., Martin, R. L., Hirsh, J., Pak, W. L., & Applebury, M. L. (1985) *Cell (Cambridge, Mass.) 40*, 839–850.
Ovchinnikov, Y. A. (1982) *FEBS Lett. 148*, 179–191.
Pedersen, S. E., & Ross, E. M. (1985) *J. Biol. Chem. 260*, 14150–14157.
Pfaffinger, P., Martin, J., Hunter, D., Nathanson, N., & Hille, B. (1985) *Nature (London) 317*, 536–538.
Sibley, D. R., & Lefkowitz, R. J. (1985) *Nature (London) 317*, 124–129.
Sternweis, P. C., & Robishaw, J. D. (1984) *J. Biol. Chem. 259*, 13806–13813.
Stiles, G. L., Caron, M. G., & Lefkowitz, R. J. (1984) *Physiol. Rev. 64*, 661–742.
Stryer, L. (1986) *Annu. Rev. Neurosci. 9*, 87–119.
Taylor, C. W., & Merritt, J. E. (1986) *Trends Pharmacol. Sci. 7*, 238–242.
Thompson, P., & Findlay, S. B. C. (1984) *Biochem. J. 221*, 773–780.
Vandenberg, C. A., & Montal, M. (1984) *Biochemistry 23*, 2339–2347.
Veis, A., & Nawrot, C. F. (1970) *J. Am. Chem. Soc. 92*, 3910–3914.
Yarden, Y., Rodriguez, H., Wong, S. K.-F., Brandt, D. R., May, D. C., Burnier, J., Harkins, R. N., Chen, E. Y., Ramachandran, J., Ullrich, A., & Ross, E. M. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6795–6799.
Young, D., Waitches, G., Birchmeier, C., Fasano, O., & Wigler, M. (1986) *Cell (Cambridge, Mass.) 45*, 711–719.
Zuker, C. S., Cowman, A. F., & Rubin, G. M. (1985) *Cell (Cambridge, Mass.) 40*, 851–858.

Accelerated Publications

2′,3′-Dideoxythymidine 5′-Triphosphate Inhibition of DNA Replication and Ultraviolet-Induced DNA Repair Synthesis in Human Cells: Evidence for Involvement of DNA Polymerase δ†

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ABSTRACT: It is well established that DNA replication and ultraviolet-induced DNA repair synthesis in mammalian cells are aphidicolin-sensitive and thus are mediated by one or both of the aphidicolin-sensitive DNA polymerases, α and/or δ. Recently, it has been shown that DNA polymerase δ is much more sensitive to inhibition by the nucleotide analogue 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP) than DNA polymerase α but is less sensitive than DNA polymerase β [Wahl, A. F., Crute, J. J., Sabatino, R. D., Bodner, J. B., Marraccino, R. L., Harwell, L. W., Lord, E. M., & Bambara, R. A. (1986) *Biochemistry 25*, 7821–7827]. We find that DNA replication and ultraviolet-induced DNA repair synthesis in permeable human fibroblasts are also more sensitive to inhibition by ddTTP than polymerase α and less sensitive than polymerase β. The K_i for ddTTP of replication is about 40 μM and that of repair synthesis is about 25 μM. These are both much less than the K_i of polymerase α (which is greater than 200 μM) but greater than the K_i of polymerase β (which is less than 2 μM). These data suggest that DNA polymerase δ participates in DNA replication and ultraviolet-induced DNA repair synthesis in human cells.

The identity of the DNA polymerase(s) involved in DNA replication and ultraviolet- (UV)[1] induced DNA repair synthesis in mammalian cells has again become a subject of great interest. It is well established that replication and UV-induced repair synthesis are inhibited by aphidicolin (Huberman, 1981; Dresler, 1984), and this fact has been regarded as evidence for the involvement of DNA polymerase α in these two processes. The recent finding that a second aphidicolin-sensitive DNA polymerase, δ, is also present in abundance in mammalian cell extracts (Byrnes, 1985; Crute et al., 1986) indicates, however, that δ should also be regarded as a candidate for participation in DNA replication and repair. The fact that polymerase δ has a 3′–5′ ("proofreading") exonuclease activity has led to suggestions that δ is particularly well suited for high-fidelity cellular DNA synthesis (Crute et al., 1986;

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[1] Abbreviations: UV, ultraviolet; BuPh-dGTP, N^2-(*p*-*n*-butylphenyl)-2′-deoxyguanosine 5′-triphosphate; ddTTP, 2′,3′-dideoxythymidine 5′-triphosphate; dThd, thymidine; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid; dATP, 2′-deoxyadenosine 5′-triphosphate; dCTP, 2′-deoxycytidine 5′-triphosphate; dGTP, 2′-deoxyguanosine 5′-triphosphate; dTTP, thymidine 5′-triphosphate; K_m, Michaelis constant; K_i, inhibitor constant.

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