PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Structure of Bacteriorhodopsin and Its Relevance to the Visual Opsins and Other Seven-Helix G-Protein Coupled Receptors

R. Henderson and G. F. X. Schertler

Phil. Trans. R. Soc. Lond. B 1990 **326**, 379-389
doi: 10.1098/rstb.1990.0019

Phil. Trans. R. Soc. Lond. B **326**, 379–389 (1990)
Printed in Great Britain

The structure of bacteriorhodopsin and its relevance to the visual opsins and other seven-helix G-protein coupled receptors

By R. Henderson, F.R.S. and G. F. X. Schertler
M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

[Plate 1]

Bacteriorhodopsin is a light-driven hydrogen-ion pump whose structure is known to about 6.0 Å† in three dimensions and 2.8 Å in projection. It consists of seven transmembrane helices surrounding the chromophore, retinal. Halorhodopsin is a second member of the same family of membrane proteins, both of them from the cell membrane of halobacteria. Halorhodopsin is a light-driven chloride-ion pump but has very close homology to bacteriorhodopsin, especially around the retinal.

In contrast, the visual opsins that are responsible for the primary step in visual transduction in all eukaryotes from *Drosophila* upwards, form a separate family with no direct sequence homology to the bacteriorhodopsin family. The visual opsin family now includes about 15 other receptor proteins, all of which activate G-protein cascades, including the β-adrenergic receptor as well as several others.

Despite the lack of clear relations at the level of amino acid sequence, there are topographical similarities between the bacteriorhodopsin and the visual opsin families in the nature and site of chromophore attachment, the number of transmembrane helices and the positions of the amino and carboxyl termini in the membrane. These suggest that if the two were at one time closely related, they have diverged too far to have sequences that are detectably similar.

1. Introduction

In 1971, a purple protein from *Halobacterium halobium* was shown to contain the chromophore retinal (Oesterhelt & Stoeckenius 1971). They named it bacteriorhodopsin (bR) because retinal is also the chromophore in rhodopsin (Rh), the visual pigment in retinal rod cells. At that time, many felt that the coining of the name bacteriorhodopsin was a poor choice because it seemed very likely that the two proteins might turn out to be completely different in structure and in function. At the time neither the function of bacteriorhodopsin, nor the mechanism of rhodopsin was known.

Subsequent work showed that bacteriorhodopsin is a light-driven proton pump (Oesterhelt & Stoeckenius 1973), whereas rhodopsin is a light-activated receptor molecule that triggers a guanine-nucleotide binding-protein (G-protein) cascade, involving the stimulation of a cyc-GMP phosphodiesterase by the α-subunit of a G-protein complex (Stryer 1986; Koutalos & Ebrey 1986). Thus, functionally, the two proteins seem to be completely different.

The conformation of the retinal has also turned out to be quite different. Bacteriorhodopsin contains all-*trans* retinal in its light-adapted state, which is thought to isomerize to 13-*cis* retinal during its photochemical cycle. Rhodopsin contains 11-*cis* retinal in the dark, which is isomerized to all-*trans* retinal by light.

† 1 Å = 10^{-10} m = 10^{-1} nm.

Typescript received – 13 *March* 1989 – *Revised* 19 *April* 1989.

However, as we will see, recent work on the structure of the polypeptide chains in each of the proteins has shown greater similarity, each molecule containing seven trans-membrane α-helices with covalent linkage to the retinal via a Schiff base to a lysine residue roughly in the centre of the seventh helix. The amino- and carboxy-termini are also arranged with respect to the cytoplasmic interior in the same way; amino-terminus outside and carboxy-terminus inside. This topographical similarity occurs despite considerable differences in the overall polypeptide length; 248 residues in bacteriorhodopsin and 348 in rhodopsin (Findlay 1986).

Very recent work on gene sequences of related proteins in each of the families is also beginning to show that there may be a closer relation than their completely different functions suggest (Findlay & Pappin 1986; Dohlman *et al.* 1987).

For example, in Halobacteria, three related proteins have been discovered (table 1, top). Halorhodopsin (hR) (Blanck & Oesterhelt 1987) is a light-driven, chloride-ion pump with approximately 31 % sequence identity with bacteriorhodopsin. Slow or sensory rhodopsin (sR) is a bacterial phototactic pigment with 26 % sequence identity with bacteriorhodopsin (Blanck *et al.* 1989). In addition, there is an ultra-violet photophobic pigment. Thus, in halobacteria, there are several related proteins that have quite different functions. The rhodopsin family has widened from the immediate family of other visual pigments with homology to rhodopsin (e.g. the red, green and blue cone pigments) to include clearly but more distantly related receptors for hormones, peptides and neurotransmitters (see table 1, bottom). Even olfactory receptors may be related (Anholt 1987).

TABLE 1. SEVEN-HELIX PROTEIN FAMILIES

	Bacteriorhodopsin family	
halobacteria	bacteriorhodopsin [proton pump]	Ovchinnikov *et al.* (1979)
	halorhodopsin [chloride pump]	Blanck & Oesterhelt (1987)
	sensory rhodopsins I & II	Blanck *et al.* (1989)
	Rhodopsin family	
mammalian	β Adrenergic	Dixon *et al.* (1986)
	α Adrenergic	Kobilka *et al.* (1987)
	Muscarinic acetylcholine	Kubo *et al.* (1986)
	5HT serotonin	Julius *et al.* (1988)
		Fargin *et al.* (1988)
	D_2-dopamine	Bunzow *et al.* (1988)
	substance K	Masu *et al.* (1987)
	MAS/angiotensin III	Young *et al.* (1986)
	Visual opsins, rod and cone	Ovchinnikov *et al.* (1982)
		Nathans *et al.* (1984, 1986)
	Opioid receptors	(not available)
Drosophila	visual opsins	O'Tousa *et al.* (1985)
		Zuker *et al.* (1985)
yeast	STE2 [α-mating factor receptor]	Nakayama *et al.* (1985)
	STE3 [*A*-mating factor receptor]	Nakayama *et al.* (1985)
Dictyostelium	cAMP receptor	Klein *et al.* (1988)

Thus, taken as a whole, the rhodopsin and bacteriorhodopsin families, though at the moment related in two separate groups at the level of amino acid sequence, may well represent an extremely favourable framework on which to build a membrane structure with many variable functions. Further work will show how similar the various members of the family are.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 1. Three-dimensional model of the structure of a single bacteriorhodopsin molecule at 6 Å resolution. The picture is a surface-shaded representation of the average density of three independently determined crystal structures. (From Tsygannik & Baldwin (1987).)

Facing p. 381

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

2. Bacteriorhodopsin

The structure of bR has been solved to 6 Å resolution in three-dimensions (figure 1, plate 1); Tsygannik & Baldwin 1987) and 2.8 Å in projection (figure 2; Baldwin *et al.* 1988). The structure is the same in three crystal forms. Figure 3 shows an outline of the projected structure of the molecule in the native (Unwin & Henderson 1975), the orthorhombic (Michel *et al.* 1980) and the deoxycholate-treated form (Glaeser *et al.* 1985).

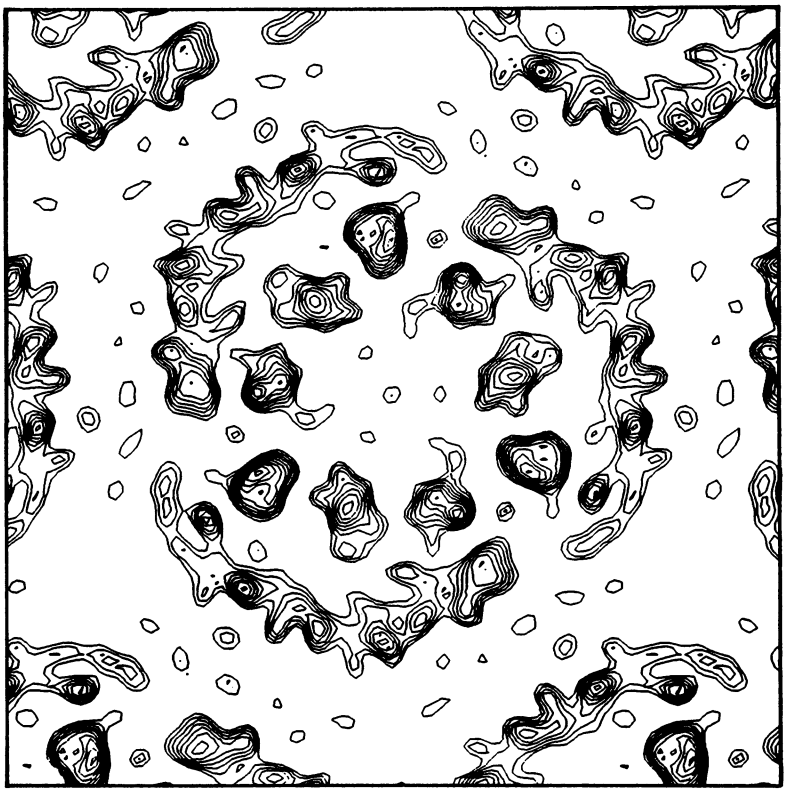

Figure 2. Projection structure of purple membrane at 2.8 Å resolution. (From Baldwin *et al.* (1988).)

The amino acid sequence (Ovchinnikov *et al.* 1979) of the protein can be arranged into seven trans-membrane helical segments (figure 4*a*), which must correspond to the seven rod-shaped densities in the three-dimensional structure shown in figure 1. A tentative assignment of sequence to structure has been made (Engelman *et al.* 1980). Neutron diffraction has determined the projected position and orientation of the retinal (Jubb *et al.* 1984; Heyn *et al.* 1988). Site-directed mutagenesis (Khorana 1988) has shown that many amino acids can be changed with no effect on either the absorption spectrum or on proton pumping. However, replacement of Asp85, Asp96 or Asp212 with asparagine (Mogi *et al.* 1988) abolished proton pumping completely or substantially. The changes of several other residues, particularly tryptophans and tyrosines into phenylalanine (Mogi *et al.* 1987) have substantial effects on the absorption spectrum and are therefore most probably involved in the binding site for the retinal. Khorana (1988) has summarized much of these data in a helical wheel model shown in figure 5, where residues thought to interact with retinal are shown in bold ovals and the three aspartic acids whose substitution affects proton pumping are shown shaded.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

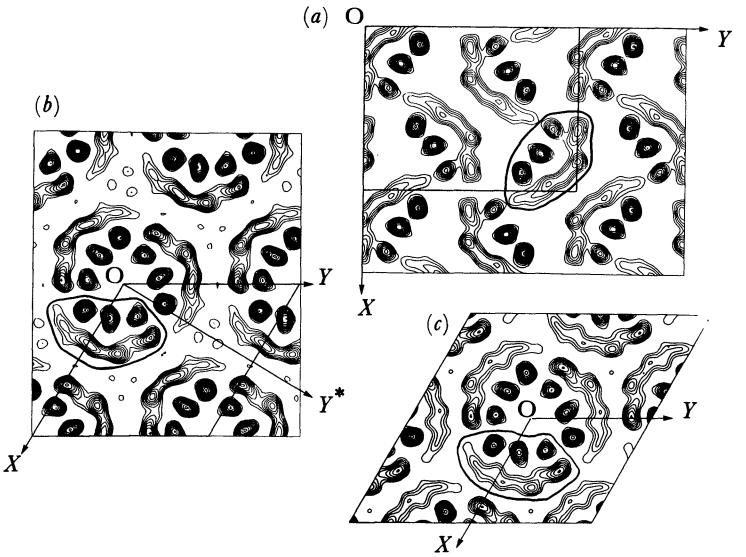

FIGURE 3. Projection maps (6 Å) of the different crystal forms studied. An envelope is drawn round the bR monomer in each structure. The trimers of bR molecules are packed more closely together in the DOC p3 (*c*) form than in the native p3 (*b*) form because the shell of lipid molecules surrounding the trimer in the native has been removed. In the orthorhombic $p22_12_1$ (*a*) form, no trimer is present.

3. OTHER HALOBACTERIAL PROTEINS RELATED TO BACTERIORHODOPSIN

In addition to bacteriorhodopsin, halobacteria have a light-driven chloride-ion pump, halorhodopsin (hR), and two light sensing pigments, sensory rhodopsins I and II (sRI and sRII). The gene sequence of hR is shown in figure 4*b* (from Blanck & Oesterhelt 1987) where it has been drawn with identical residues in identical positions to those for bR in figure 4*a*.

Chloride ions are pumped into the cell by hR, whereas bR pumps protons out. The retinal undergoes a similar photochemical cycle (Oesterhelt & Tittor 1989). There is a substantial degree of sequence homology, but differences in the sequence must include those amino acid residues responsible for the different function.

In particular, we can study the corresponding residues in helices C, F and G, which are thought to surround the retinal, as shown in figure 5. Helix G contains Lys216, which forms a Schiff base with the retinal in both bR and hR. Asp212, probably situated very close to the Schiff base as it is precisely one turn of the helix down from Lys216, is also present in both bR and hR. Similarly, the two tryptophans and Tyr185 in helix F are all present in the highly conserved sequence W--YP--W (Oesterhelt & Tittor 1989). It can be seen from figure 5 that these three bulky hydrophobic side chains must be virtually above one another, on one side of helix F and that, because they are conserved, are likely to form part of the binding site for the retinal. A likely arrangement would sandwich the retinal between the side chains of the two tryptophans with Tyr185 alongside. Similar possible arrangements have been discussed (Oesterhelt & Tittor 1989; Khorana 1988).

In helix C, however, the two aspartic acid residues (85 and 96), which are essential for proton pumping (Mogi *et al.* 1988) in bR are replaced in hR by threonine and alanine respectively. This is most satisfying as these two residues must be involved in the proton transfer in bR, but would not be necessary for chloride ion transport in hR because Cl^- and H^+ have

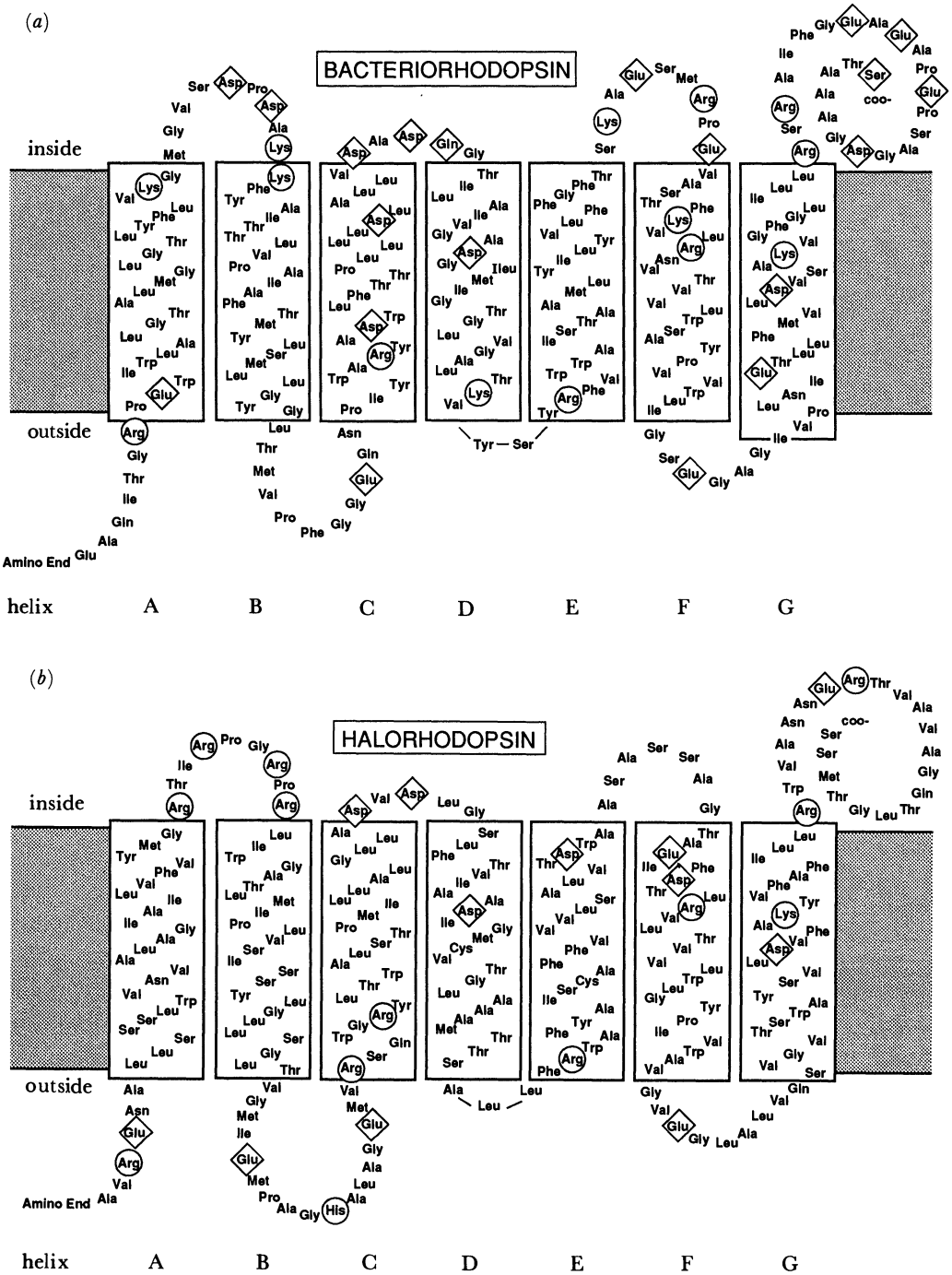

FIGURE 4. Schematic diagram showing an arrangement of the amino acid sequence of (*a*) bacteriorhodopsin and (*b*) halorhodopsin in the form of seven transmembrane helices.

opposite charge, and chloride ions are present at much higher concentration than hydrogen ions in the halobacterial environment.

It will be interesting to see how the sequence of the two sensory rhodopsins compares to bR and hR.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

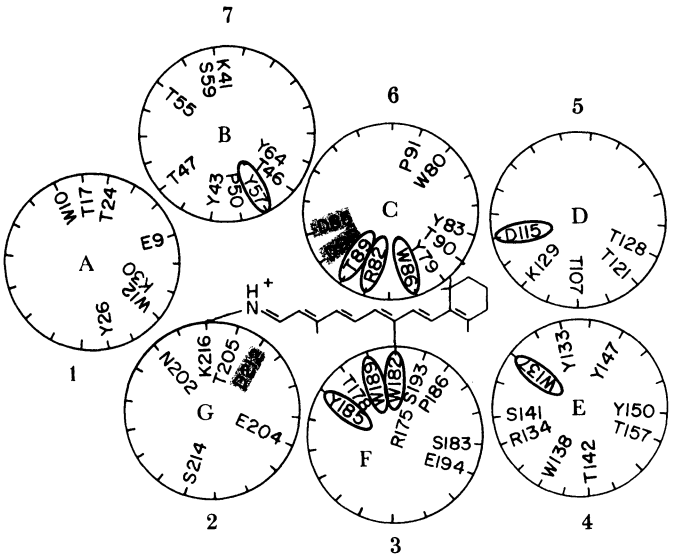

FIGURE 5. A helical wheel model of bacteriorhodopsin showing the relations (*a*) between the aspartic acid mutations affecting proton pumpting (shaded) and (*b*) the amino acids that are believed to interact with the chromophore (bold ovals). (Reproduced from Khorana (1988) with permission.)

4. RHODOPSIN

As discussed in the introduction, there are similarities between bR and Rh in the number of transmembrane helices, the topography of the polypeptide chain arrangement across the lipid bilayer, and in the position of the lysine side chain at which retinal is attached. Figure 6 (from Hargrave *et al.* (1983)) presents a similar schematic diagram for the rhodopsin (Rh) structure to those for bR and hR shown in figure 4.

Although there is no convincing relation at the sequence level between the bR family and rhodopsin, these topographical similarities suggest that the polypeptide chain of the two families may have the same fold. That is, in rhodopsin, the positions of the seven helices may have the same relation to each other and to the retinal as suggested in figure 5 for bR.

At the present time, a comparison of amino sequences within the rhodopsin family may be informative. Figure 7 (from Nathans *et al.* 1986) shows comparisons of amino acid sequences of the human rod and cone visual pigments. We note that, among the three more distantly related pigments (blue and green cone pigments, and rod rhodopsin) the sequences of helices C, F and G seem to be more conserved than the others. This would be consistent with these helices forming the main part of the retinal binding pocket, and supporting the idea that the helices might be arranged in the same way as in bR.

However, between the red and green pigments, there are only 15 amino acid changes, which must cause the shift in their absorption maxima. Of these only seven are residues in helices C, F or G. Of these seven residues, only three involve a change in polarity. The others involve a change in the size of a hydrophobic residue. No tryptophans are involved. The three remaining residues are changed from Phe to Tyr in helix G; Phe to Tyr and Ala to Thr in helix F, when green pigment is changed to red pigment. These are shown underlined in figure 6. Thus one or more of these three extra hydroxyl groups in helices F and G may be responsible for the spectral shift of the green cone pigment to form the red cone pigment. Juxtaposition of the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

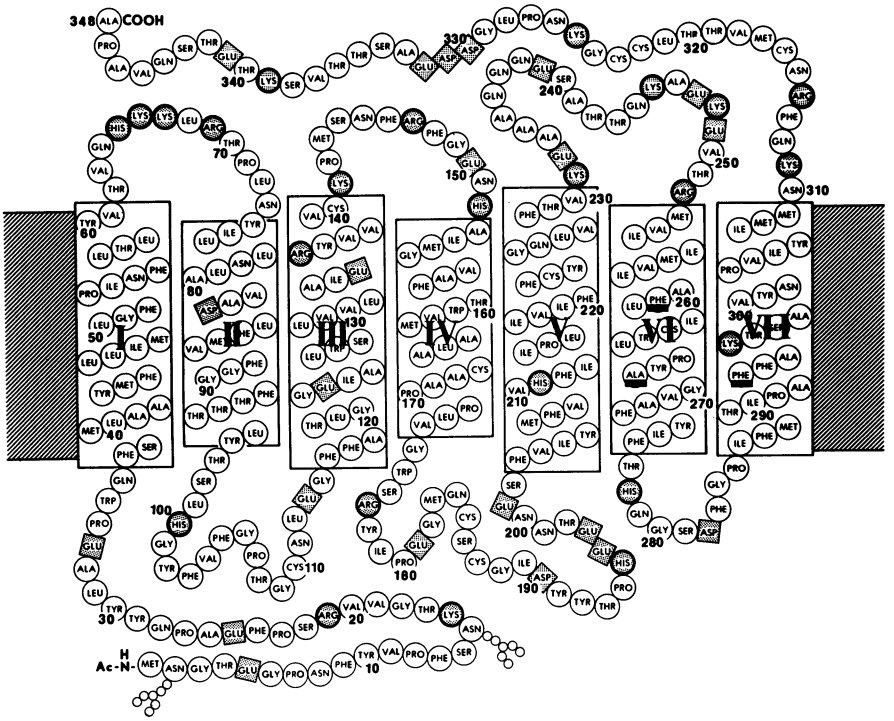

FIGURE 6. Sequence of bovine rod rhodopsin drawn as seven trans-membrane helices (from Hargrave *et al.* (1983)). The three residues mentioned in the text that change their polarity between the red and green cone pigments are shown underlined.

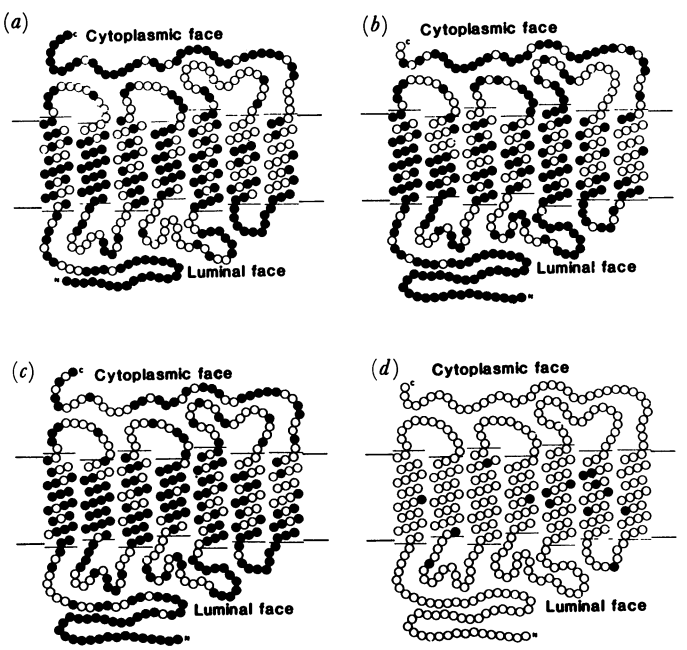

FIGURE 7. Pair-wise comparisons of human visual pigments showing amino acid identities (white) and differences (black): (*a*) blue vs rhodopsin; (*b*) green vs. rhodopson; (*c*) green vs. blue, (*d*) red vs. green. (From Nathans *et al.* (1986).)

partial negative charge on one of the hydroxyls with one of the double bonds in the retinal would tend to cause a red-shift in the absorption maximum. Examination of helical wheel plots like that shown in figure 5, suggest the tyrosine in helix G is too far from the retinal to affect its spectrum directly (figure 8). The residues W––Y in helix F of rhodopsin suggest a possible homology with the conserved sequence W––YP––W in the bR family that would place the Ala to Thr changed in helix F adjacent to the retinal ring. Thus, taking the speculation to its logical end point, it may be that the threonine in the sequence W––PYT of the red pigment is directly responsible for the red-shift in human colour vision. Similar but different speculations are presented by Applebury & Hargrave (1987), and by Nathans *et al.* (1986). Further work by using site-directed mutagenesis should determine the contributions of the different amino acid side chains.

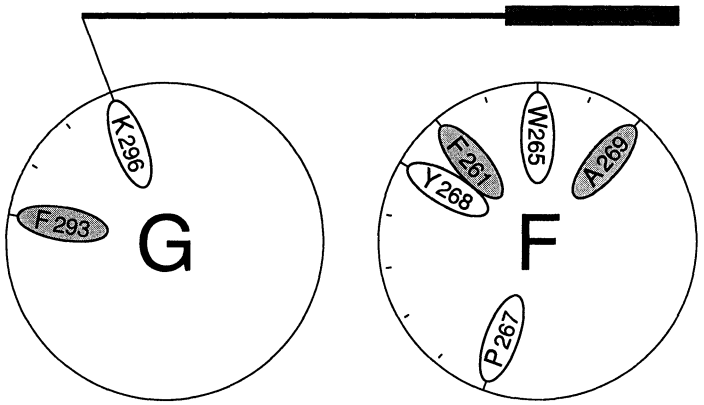

FIGURE 8. Suggested positions for helices F and G relative to retinal in rhodopsin. Residues F293, F261 and A269 in the green cone pigment are changed to the more polar Y, Y and T, respectively, in the red cone pigment. The numbering of amino acids is from the homologous bovine rhodopsin (Hargrave *et al.* 1983). The horizontal black line represents the polyene chain and the thick bar represents the β-ionone ring of the retinylidene moiety

5. OTHER EUKARYOTIC PROTEINS RELATED TO RHODOPSIN

Rhodopsin represents one member of a family of receptors that transduce a signal across the membrane and take part in the amplification of this signal by activating several trimeric (α.β.γ) guanine nucleotide binding proteins that in turn activate several effector molecules (in the case of rhodopsin, cGMP-specific phosphodiesterase). A similar transduction and amplification scheme holds for several amino acid derived hormones (adrenaline, acetylcholine, dopamine, serotonin), neuropeptides (substance K, opioids) and the pheromone peptides *a* and α from yeast. The target of the signalling cascade might be such different enzymes as adenylate cyclase, cGMP-specific phosphodiesterase, phospholipase *C* or phospholipase *A*2. Some potassium or calcium channels may also be the final target of similar signalling cascades. The overall similarity within the family of membrane signal transduction pathways is well reflected in the amino acid sequence homology of the receptor proteins. Figure 9 shows an example of an alignment of the amino acid sequence of several representatives of this family of seven helix receptors that regulate through coupling to guanine-nucleotide binding-proteins. The overall homology between the β-adrenergic or muscarinic receptor and the visual pigments is low (12–18%) but the homology in certain local regions is much higher (Dohlman *et al.* 1987). The topographic similarity is reflected in seven stretches of 20–28 hydrophobic amino acids, which

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

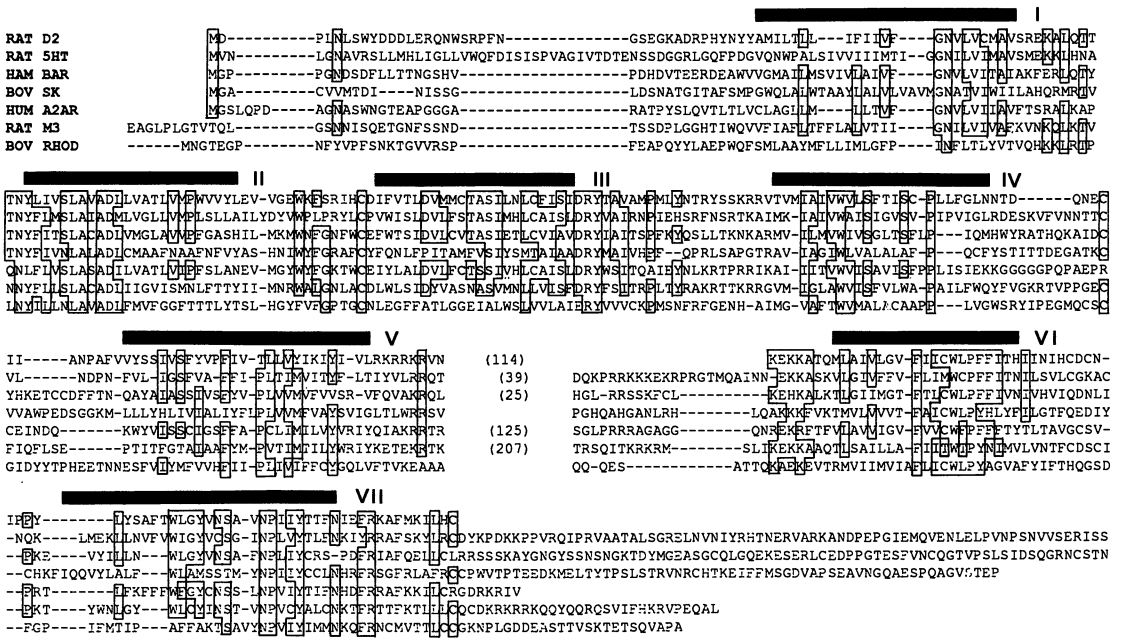

FIGURE 9. Sequence alignment of a selection of seven-helix, G-protein coupled receptors, with homologous residues boxed. The dopamine sequence has been added to the sequences examined by Julius *et al.* (1988). The labels I–VII correspond to helices A–G.

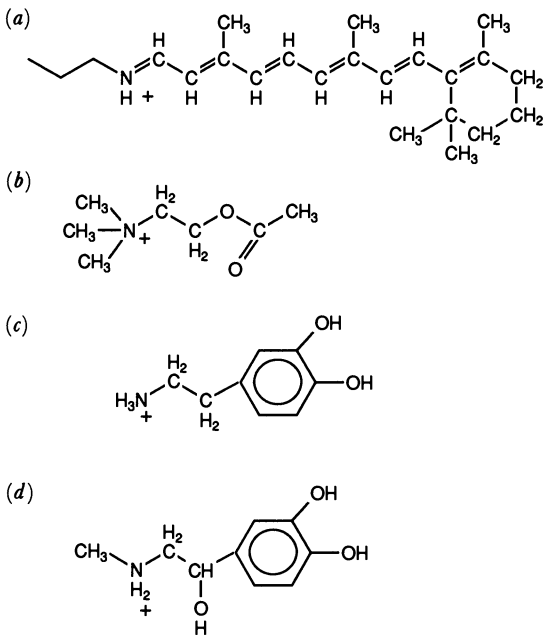

FIGURE 10. Chemical structures of a few effector molecules for seven helix, G-protein coupled receptors; (*a*) retinal; (*b*) acetylcholine; (*c*) dopamine; (*d*) epinephrine.

might represent membrane-spanning helices. These regions contain several conserved prolines, which might therefore be of structural relevance to the formation of the binding pocket of the hormones. Several charged residues are also conserved in the putative membrane helix regions. One of the negatively charged groups, for example in helix B, might serve as counter ion in the process of hormone binding as acetylcholine, epinephrine and dopamine all contain a positively

charged nitrogen atom possibly corresponding to the protonated Schiff base of the bound retinal in the visual pigments (figure 10). In the case of the *Dictyostelium* receptor for the negatively charged cAMP chemoattractant, helices B and G contain additional lysine and histidine residues which might be involved in ligand binding. Some conserved residues on the extracellular side correspond to the N-glycosylation sites. The conserved loops on the cytoplasmic side might participate in the receptor G-protein interaction or in the phosphorylation by a receptor-specific kinase that might be involved in turning off the signal and in the adaptation (desensitization) of the receptor.

References

Anholt, R. R. H. 1987 Primary events in olfactory reception. *Trends biochem. Sci.* **12**, 58–62.

Applebury, M. L. & Hargrave, P. A. 1987 Molecular biology of the visual pigments. *Vision Res.* **26**, 1881–1895.

Baldwin, J. M., Henderson, R., Beckmann, E. & Zemlin, F. 1988 Images of purple membrane at 2.8 Å resolution obtained by cryo-electron microscopy. *J. molec. Biol.* **202**, 585–591.

Blanck, A. & Oesterhelt, D. 1987 The Halo–Opsin gene. II. Sequence, primary structure of halorhodopsin and comparison with bacteriorhodopsin. *EMBO J.* **6**, 265–273.

Blanck, A., Oesterhelt, D., Ferrando, E., Schegk, E. S. & Lottspeich, F. 1989 Primary structure of sensory rhodopsin I, a procaryotic photoreceptor. *EMBO J.* (in the Press.)

Bunzow, J. R., Van Tol, H. H. M., Grandy, D. K., Albert, P., Salon, J., Christie, M., Machida, C. A., Neve, K. A. & Civelli, O. 1988 Cloning and expression of a rat D_2 dopamine receptor cDNA. *Nature, Lond.* **336**, 783–787.

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. L. & Strader, C. D. 1986 Cloning of the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin. *Nature, Lond.* **321**, 75–79.

Dohlman, H. G., Caron, M. G. & Lefkowitz, R. J. 1987 A family of receptors coupled to guanine nucleotide regulatory proteins. *Biochemistry* **26**, 2657–2664.

Engelman, D. M., Henderson, R., McLachlan, A. D. & Wallace, B. A. 1980 The path of the polypeptide in bacteriorhodopsin. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2023–2027.

Findlay, J. B. C. 1986 The structure of rhodopsin. *Photochem. Photophys.* **13**, 213–218.

Findlay, J. B. C. & Pappin, D. J. C. 1986 The opsin family of proteins *Biochem. J.* **238**, 625–642.

Glaeser, R. M., Jubb, J. S. & Henderson R. 1985 Structural comparison of native and deoxycholate-treated purple membrane. *Biophys. J.* **48**, 775–780.

Hargrave, P. A., McDowell, J. H., Curtis, D. R., Wang, J. K., Juszczak, E., Fong, S.-L., Rao, J. K. M. & Smith, D. P. 1983 The structure of bovine rhodopsin. *Biophys. struct. Mech.* **9**, 235–244.

Heyn, M. P., Westerhausen, J., Wallat, I. & Sieff, F. 1988 High sensitivity neutron diffraction of membranes: location of the Schiff base end of the chromophore of bacteriorhodopsin. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2146–2150.

Jubb, J. S., Worcester, D. L., Crespi, H. L. & Zaccai, G. 1984 Retinal location in purple membrane of *Halobacterium halobium*: a neutron diffraction study of membranes labelled *in vivo* with deuterated retinal. *EMBO J.* **3**, 1455–1461.

Julius, D., MacDermott, A., Axel, R. & Jessell, T. 1988 Molecular characterization of a functional cDNA encoding the serotonin lc receptor. *Science, Wash.* **241**, 558–564.

Khorana, H. G. 1988 Bacteriorhodopsin, a membrane protein that uses light to translocate protons. *J. biol. Chem.* **263**, 7439–7442.

Klein, P. S., Sun, T. J., Saxe, C. L., Kimmel, A. R., Johnson, R. L. & Devreotes, P. N. 1988 A chemo-attractant receptor controls development in *Dictyostelium discoideum*. *Science, Wash.* **241**, 11467–11472.

Kobilka, B. K., Matsui, H., Kolbilka, T. S., Yang-Feng, T. L., Francke, U., Caron, M. G., Lefkowitz, R. J. & Regan, J. W. 1987 Cloning, sequencing and expression of the gene coding for the human platelet α_2-adrenergic receptor. *Science, Wash.* **238**, 650–656.

Koutalos, Y. & Ebrey, T. G. 1986 Recent progress in vertebrate photoreception. *Photochem. Photobiol.* **44**, 809–817.

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. 1986 Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. *Nature, Lond.* **323**, 411–416.

Masu, Y., Nakayama, K., Tamaki, H., Harada, Y., Kuno, M. & Nakanishi, S. 1987 cDNA cloning of bovine substance-K receptor through oocyte expression system. *Nature, Lond.* **329**, 836–838.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Michel, H., Oesterhelt, D. & Henderson, R. 1980 An orthorhombic two-dimensional crystal form of purple membrane. *Proc. natn. Acad. Sci. U.S.A.* **77**, 338–342.

Mogi, T., Stern, L. J., Hackett, N. R. & Khorana, H. G. 1987 Bacteriorhodopsin mutants containing single tyrosine to phenylalanine substitutions are all active in proton translocation. *Proc. natn. Acad. Sci. U.S.A.* **84**, 5595–5599.

Mogi, T., Stern, L. J., Marti, T., Chao, B. H. & Khorana, H. G. 1988 Aspartic acid substitutions affect proton translocation by bacteriorhodopsin. *Proc. natn. Acad. Sci. U.S.A.* **85**, 4148–4152.

Nakayama, N., Miyajima, A. & Arai, K. 1985 Nucleotide sequences of STE2 and STE3, cell type specific sterile genes from *Saccharomyces cerevisiae*. *EMBO J.* **4**, 2643–2648.

Nathans, J. & Hogness, D. S. 1984 Isolation and nucleotide sequence of the gene encoding human rhodopsin. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4851–4855.

Nathans, J., Thomas, D. & Hogness, D. S. 1986 Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. *Science, Wash.* **232**, 193–202.

Oesterhelt, D. & Stoeckenius, W. 1971 Rhodopsin-like protein from purple membrane of *Halobacterium halobium*. *Nature, Lond.* **233**, 149–152.

Oesterhelt, D. & Stoeckenius, W. 1973 Functions of a new photoreceptor membrane. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2853–2857.

Oesterhelt, D. & Tittor, J. 1989 Two pumps, one principle: light-driven ion transport in Halobacteria. *Trends biochem. Sci.* **14**, 57–61.

O'Tousa, J. E., Baehr, W., Martin, R. L., Hirsh, I., Pak, W. L. & Applebury, M. L. 1983 The *Drosophila nina* E gene encodes an opsin. *Cell* **40**, 839–850.

Ovchinnikov, Y. A., Adbulaev, N. G., Feigina, M. Y., Kiselev, A. V. & Lobanov, N. A. 1979 The structural basis of the functioning of bacteriorhodopsin: an overview. *FEBS Lett.* **100**, 219–224.

Ovchinnikov, Y. A., Abdulaev, N. G., Feigina, M. Y., Artomonov, I. D., Zolotarev, A. S., Moroshnikov, A. I., Martynow, V. I., Kostina, M. B., Kudelin, A. G. & Bogachuk, A. S. 1982 The complete amino acid sequence of visual rhodopsin. *Biorg. Khim.* **8**, 1424–1427.

Stryer, L. 1986 Cyclic GMP cascade of vision. *A. Rev. Neurosci.* **9**, 87–119.

Tsygannik, I. N. & Baldwin, J. M. 1987 Three-dimensional structure of deoxycholate-treated purple membrane at 6 Å resolution and molecular averaging of three crystal forms of bacteriorhodopsin. *Eur. biophys. J.* **14**, 263–272.

Unwin, P. N. T. & Henderson, R. 1975 Molecular structure determination by electron microscopy of unstained crystalline specimens. *J. molec. Biol.* **94**, 425–440.

Young, D., Waitches, G., Birchmeier, C., Fasano, G. & Wigler, M. 1986 Isolation and characterization of a new cellular oncogene encoding a protein with multiple potential transmembrane domains. *Cell* **45**, 711–719.

Zuker, C. S., Cowman, A. F. & Rubin, G. M. 1985 Isolation and structure of a rhodopsin gene from *D. melanogaster*. *Cell* **40**, 851–858.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

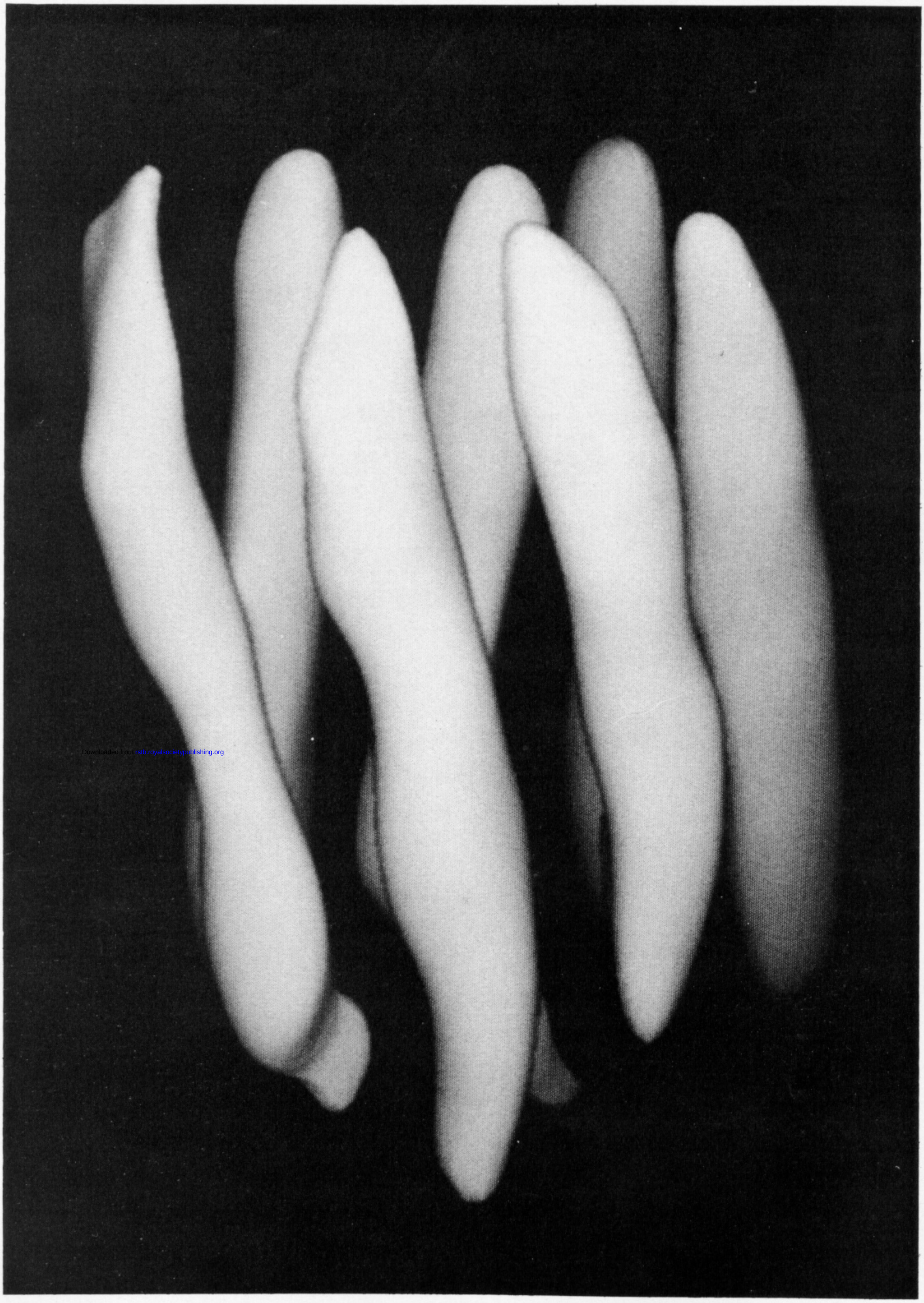

FIGURE 1. Three-dimensional model of the structure of a single bacteriorhodopsin molecule at 6 Å resolution. The picture is a surface-shaded representation of the average density of three independently determined crystal structures. (From Tsygannik & Baldwin (1987).)