Amino Acids (2008) 35: 1–15
DOI 10.1007/s00726-008-0637-6
Printed in The Netherlands

Amino Acids

An ion-responsive motif in the second transmembrane segment of rhodopsin-like receptors*

Review Article

M. S. Parker[1], **Y. Y. Wong**[2], and **S. L. Parker**[2]

[1] Department of Microbiology and Molecular Cell Sciences, University of Memphis, Memphis, TN, U.S.A.
[2] Department of Pharmacology, University of Tennessee Health Science Center, Memphis, TN, U.S.A.

Received June 8, 2007
Accepted November 6, 2007
Published online February 12, 2008;

Summary. A L(M)xxxD(N, E) motif (x = a non-ionic amino acid residue, most frequently A, S, L or F; small capitals indicating a minor representation) is found in the second transmembrane (tm2) segment of most G-protein coupling metazoan receptors of the rhodopsin family (Rh-GPCRs). Changes in signal transduction, agonist binding and receptor cycling are known for numerous receptors bearing evolved or experimentally introduced mutations in this tm2 motif, especially of its aspartate residue. The [Na^+] sensitivity of the receptor-agonist interaction relates to this aspartate in a number of Rh-GPCRs. Native non-conservative mutations in the tm2 motif only rarely coincide with significant changes in two other ubiquitous features of the rhodopsin family, the seventh transmembrane N(D)PxxY(F) motif and the D(E)RY(W,F) or analogous sequence at the border of the third transmembrane helix and the second intracellular loop. Native tm2 mutations with Rh-GPCRs frequently result in constitutive signaling, and with visual opsins also in shifts to short-wavelength sensitivity. Substitution of a strongly basic residue for the tm2 aspartate in Taste-2 receptors could be connected to a lack of sodium sensing by these receptors. These properties could be consistent with ionic interactions, and even of ion transfer, that involve the tm2 motif. A decrease in cation sensing by this motif is usually connected to an enhanced constitutive interaction of the mutated receptors with cognate G-proteins, and also relates to both the constitutive and the overall activity of the short-wavelength opsins.

Keywords: Ion switch – Signal transduction – Constitutive transduction – G-protein coupling – Ion transfer – Visual signaling

Introduction

Rhodopsin-like single-chain heptahelical receptors that couple to G-proteins as signal transducers (Rh-GPCRs) are the most numerous and functionally variegated group of cell membrane receptors in the vertebrata (see Fredriksson et al., 2003). In the course of evolution, these molecules were successfully adapted to a vast array of signaling functions. The canonical human Rh-GPCRs with identified selective agonists currently number more than 200, and the recent large-scale sequencing of odorant receptors has boosted the number of human Rh-GPCRs to more than 600. A recent review covers the most important details about organization of the transmembrane helices of these receptors (Nyholm et al., 2007). The signal transduction via these receptors has been amply documented to involve ionic regulation. The ion levels can be regulated through interaction with specific ion channels of the elaborate intracellular domains of many Rh-GPCRs, notably β-adrenergic (Xiong and Sperelakis, 1995) and muscarinic acetylcholine (Breitwieser, 1991) receptors.

There also is substantial evidence for a general ionic, especially cationic, regulation of signal transduction of Rh-GPCRs that involves the transmembrane segments. Ionic regulation involving the transmembrane parts could be at the level of modification in the receptor structure through a single site or segment, or via intersegment interactions, or even by a direct interaction of transducers or effectors with these structures. The regulation appears to

* This article contains electronic supplementary material, available for download from the web version under www.springerlink.com. Sequences of the transmembrane segments 2, 3 and 7 and the corresponding motifs are available as tab-delimited textfiles in the electronic supplements 1–3. These supplements can be imported into spreadsheet programs. Tables of the percentages of amino acid residues in the second transmembrane segment are in the supplement 4 (see web version of this paper).

Table 1. Commonality of three transmembrane motifs in Rh-GPCRs and opsins

Receptor group	No. of receptors	% of receptors with rhodopsin-aligned transmembrane motifs		
		tm2.7-11 motif	tm3/ic2. 24-26 motif	tm7.19-23 motif
Human canonical	203	98.1	99	98
Human olfactory	374	99.5	98.7	98.5
Human orphan	94	91.5	91.6	89.5
Metazoan opsins	429	73	99.3	97.4

Based on comparisons with bovine rhodopsin in the SSEARCH3 program (Pearson, 2000), the motifs were localized as follows: the second transmembrane, tm2.7–11, **L**(I, M, V, T)xxx**D**(E, N); the third transmembrane/second intracellular, tm3/ic2.24-26, **D**(E, N)**R**(K, H)**Y**(W, F, H); and the seventh transmembrane tm7.19-23, **N**(D, T, S, Q)**P**xx**Y**(F, W, H). The most frequent residues for each position are in *boldface*, and the variant residues for a position are indicated in *parenthesis* after the principal residue, variable residues are denoted by an *x*

be connected to three transmembrane motifs, all critically dependent on ionic or polar sidechains, and located in, or close to, the second, third and seventh transmembrane domains (the tm2, tm3 and tm7, respectively) of the receptors (Table 1).

Among these motifs, the ubiquitous D(E, N)R(K, H) Y(W, F, H) motif (see e.g. Rosenkilde et al., 2005) at the border of the tm3 and the second intracellular (ic2) segment has received most attention, and the tm7 N(D)PxxY motif is known to be indispensable for activity of the rhodopsin-like GPCRs (Gripentrog et al., 2000) as well as of the visual rhodopsins (Fritze et al., 2003). The tm2 L(M)xxxD(N, E) sequence was mainly examined with focus on its aspartate residue (e.g. Chung et al., 1988; Martin et al., 1999; Zhang et al., 2006). As will be reviewed here, the published findings about this aspect of the second transmembrane segment point to a general importance of the motif in the regulation of signal transduction, by rhodopsin-family non-visual receptors or by visual opsins.

The three segments apparently interact, and could be held together during the signal transduction. The tm2 and tm7 segments were indicated to interact in the signal transduction of the angiotensin −II AT1 receptor (Miura et al., 2003; Nikiforovich et al., 2006), and this was shown to be independent of tm3/tm7 interactions (Nikiforovich et al., 2006). The tm2 and tm7 helices also interact in the luteinizing hormone (LH) receptor (Zhou et al., 1994) and the gonadotropin-releasing hormone (GnRH) receptor (Flanagan et al., 2000). Mutations of the pivotal tm2 aspartate and of the tm7 asparagine pivot both result in defective endocytosis and signal transduction, supporting a functional coupling of these domains (Gripentrog et al., 2000). The tm3 and tm7 segments were shown to interact via asparagine residues in the AT1 receptor (Balmforth et al., 1997). A tripartite interaction of the segments could generally be important in the signal transduction. The tm2, tm3 and tm7 segments of mammalian rod opsins also interact, with participation of Asp^{83} and Gly^{90} from the tm2 segment (Rao et al., 1994; Nagata et al., 1998).

Definitions of transmembrane segments and alignment of three ubiquitous motifs in rhodopsin-like receptors

The *tmhmm* (Technical University of Denmark, Lyngby) and *tmpred* (University of Tübingen, Germany) programs were used for the initial projections of the bounds of GPCR transmembrane helices. The bovine rhodopsin (bRh; the National Library of Medicine accession NP_001014890; Swiss-Protein (s-p) accession P02699; structure in Palczewski et al. (2000)) was used as the rhodopsin standard for sequence comparisons. A vast majority of Rh-GPCRs display large overlaps with bRh, and possess tm2, tm3/ic2 and tm7 motifs that align with bRh. Also, regardless of differences in the mechanisms of agonism, the signal transduction of opsins and Rh-GPCRs is to a G-protein α-subunit (in most cases, to subunits from the Gi/o or Gq group for Rh-GPCRs, Gt for mammalian opsins, Go for protochordate opsins, and Gq for invertebrate opsins). It should also be noted that comparisons based on an aminergic receptor (the human β2-adrenergic receptor) or on a neuropeptide receptor (the human neuropeptide Y Y2 receptor) produce alignments across Rh-GPCRs and opsins similar to those obtained with bovine rhodopsin. All comparisons were performed in SSEARCH3 program (Pearson, 2000). Sequences of Rh-GPCRs of the same type frequently differ appreciably even among species in the same taxonomic class, including differences in the composition of the pivotal transmembrane motifs (e.g. the human and the rodent neurotensin-2 receptors (Mazella and Vincent, 2006)), and several types of prostaglandin receptors). For this reason, only human Rh-GPCRs were examined as a group. Opsins, however, show much less variability within taxonomic classes, and could be analyzed across the phyla.

Based on the average length of the second transmembrane domain in human rhodopsin family receptors ((Fredriksson et al., 2003); family A of the GPCRDB; http://www.gpcr.org/7tm/) predicted by the above programs, the length of this segment was fixed to 24 residues, 10 before and 13 after the D(N, E) pivot. The L(M)xxxD (N, E) motif in this segment (tm2.7–11) aligns with the

LAVAD motif in the tm2 of the bovine rhodopsin (bRh), and is present in >98% of the human Rh-GPCRs examined (Table 1). The ubiquitous D(E, N)R(K, H)Y(W, F, H) motif at the border of the third transmembrane and the second intracellular segment (see Rosenkilde et al., 2005) is variously excluded from (in about 75% of the cases) or included in the tm3 segment by the above programs. For the present evaluation, three residues containing the motif and two further residues were added to the preceding 23-residue tm3 segment (supplement S1). This motif (tm3.24-26) aligns with the ERY motif of bRh in more than 98% of the human Rh-GPCRs, or metazoan opsins (Table 1). The seventh transmembrane segment of 25 residues (18 before the N(D, T, S, Q)PxxY(F, W, H) motif; tm7.19-23) also shows alignment with the tm7 NPLIY motif of bRh in more than 98% of the Rh-GPCRs. Thus delineated, the three motifs are in a highly consistent accord with global sequence alignments, albeit sometimes out of the transmembrane segment boundaries computed by the topology programs. The corresponding accession numbers, segments and motifs are in the electronic supplement S1.

A comparison of constituents of the second transmembrane segment of Rh-GPCRs and opsins

The second transmembrane segment is known as important in signal transduction via the Rh-GPCRs at least in connection to the pivotal 2.11 aspartate residue, as will be referenced in detail later. An evaluation of sequence similarity (Fig. 1) shows a generally larger correspondence to bovine rhodopsin for the tm2 residues 1–11 in the N-terminal part of the segment for all groups. This is especially evident for the non-odorant ('canonical') and the olfactory human receptors. The drop in correspondence with bRh is especially pronounced in the C-terminal half of this segment for the canonical human receptors, but is also large for the short-wavelength cone opsins (Fig. 1 and supplements S1 and S2). This could be connected to a closer evolutionary relatedness of cone opsins and GPCRs (e.g. Teller et al., 2003).

The average hydrophobicities of the tm2 residues for the five groups of GPCRs match very well at positions 2.1–11, but are quite different between groups in the C-terminal part of the segment (Fig. 2A). This is in a similar way related to the average polarities and to efficacy in handling ions and ionic groups and events. Compared to other groups, the odorant receptors have a much lower hydrophobicity in the first half of the C-terminal part of

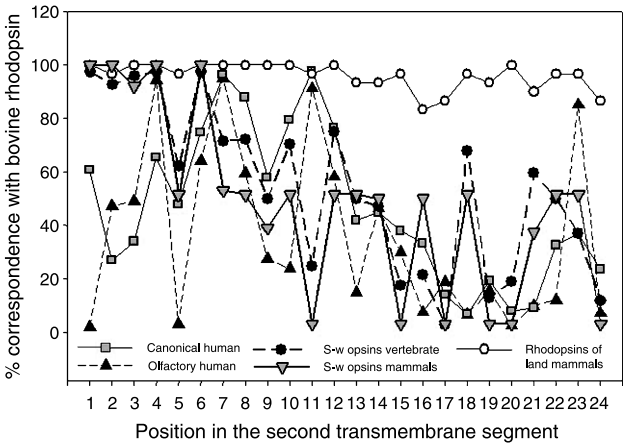

Fig. 1. Similarity to bovine rhodopsin in the second transmembrane segment for human rhodopsin-like G-protein coupling receptors and human and vertebrate opsins. The window corresponds to 24-residue segments, defined and aligned with bRh as explained in the text. Residues identical to bovine rhodopsin were summed as 100, the conservative differences (Pearson, 2000) as 50, and the sums were divided by the number of the respective residues to get the percentages shown in the graph

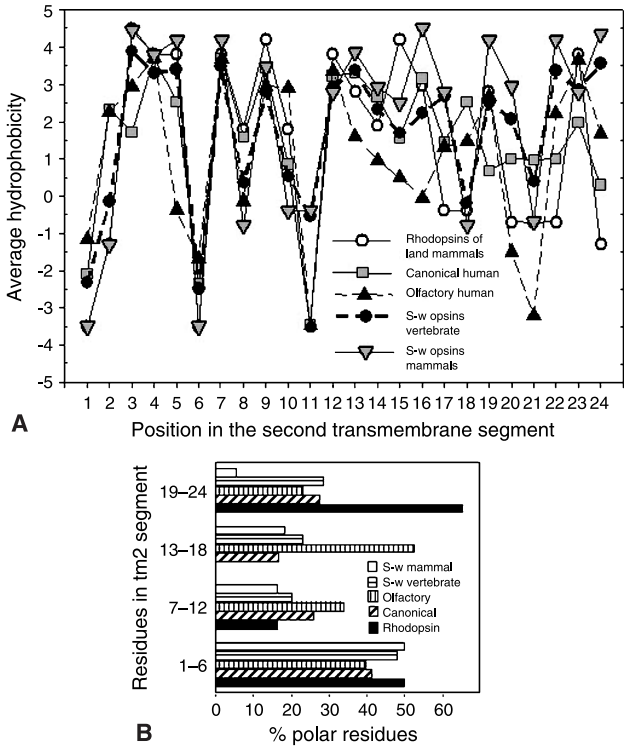

Fig. 2. Hydrophobicity and polarity of residues in the second transmembrane segment of receptor groups analyzed in Fig. 1. (**A**) Average hydrophobicity of individual residues in the segment. The hydrophobicities were estimated using the Kyte–Doolittle parameters (Kyte and Doolittle, 1982); however, similar numbers were obtained using the Roseman parameters (Roseman, 1988). (**B**) Average percent of polar residues (DEHKNQRSTWY) in four six-residue bins for the receptor groups in graph A. Note that the third bin (residues 13–18 in the segment) of the rhodopsins of land-dwelling mammals does not contain polar residues

the tm2 segment (Fig. 2). In a four-bin count of polarity (Fig. 2B), the 2.13–18 part shows no polar residues in the rod opsins, and the human canonical receptors are also quite non-polar in this stretch.

The second transmembrane LxxxD motif and similar motifs in other transmembrane segments of the rhodopsin-like receptors

As seen in Fig. 1, the most conserved positions in the second transmembrane segment (tm2) of Rh-GPCRs are 2.7 (Leu, pivot – 4) and 2.11 (the pivot Asp). This aspartate residue is ubiquitously found at the end of a tm2.7–11 L(I, V, M, T)xxxD(N, E) motif. Table 2 shows the frequencies of tm2.7 and tm2.11 residues for all examined human Rh-GPCRs. As the discovery and de-orphanization of Rh-GPCRs are still frequent, the table is an indicative, rather than exhaustive, survey of this transmembrane sequence in the human Rh-GPCRs. However, the non-olfactory human sequences with identified principal and selective agonists represent receptors that were extensively cross-verified in biochemical, pharmacological and physiological studies (''canonical'' receptors). Sequences of the human canonical and olfactory receptors have been confirmed in the genomic cloning projects, and this in most cases also applies to the examined orphan receptors. A survey of motifs in the metazoan opsins is shown in Table 4. All transmembrane segments and motifs are listed in supplements S1–S3.

Mutations of the highly conserved leucine at tm2.7 could cause constitutive activity of bovine rhodopsin (Madabushi et al., 2004), and mutations of the pivotal tm2.11 aspartate were shown to impair the normal activity of more than 30 receptors. The LxxxD tm2.7–11 motif is seldom found in other transmembrane domains of Rh-GPCRs identified with agonists, and then not in correspondence with the bRh sequence. Table 3 summarizes the occurrence of this and related motifs in all transmembrane segments of the canonical and olfactory receptors surveyed in Table 2. (Human orphan receptors have profiles quite similar to the human canonical receptors.)

As seen in Table 3, the L(M,V)xxxD(E) motifs are not frequent in other transmembrane segments. The LxxxD motif is found in transmembrane domains other than the second in, respectively, 4 and 13% of the canonical and olfactory receptors. The LxxxE motif is encountered in the tm2 segment of a single canonical receptor, the substance P receptor (supplement S1), but is well represented in that domain of the olfactory receptors. The tm3 LxxxE motif is richly represented (33% of the sequences) in the olfactory receptors. The xxx part of this motif has no cationic sidechains, but is very rich in hydroxy amino acid

Table 2. Frequencies of the second transmembrane residues 7 and 11 in human Rh-GPCRs

Type of receptor	Total	2.7 L	2.7 IMT	2.7 LIVMT	2.7 other	2.11 D	2.11 E	2.11 NGS	2.11 DNEGS	2.11 other
Known agonist	203	190	13	203	0	196	1	6	203	0
	% total	93.6	6.41	100	0	96.6	0.49	2.96	100	0
Olfactory	374	351	4	355	19	308	56	9	373	1
	% total	93.9	1.07	94.9	5.08	82.4	15.0	2.41	99.7	0.27
Orphan	94	81	8	89	5	82	1	6	89	5
	% total	86.2	8.51	94.7	5.32	87.4	1.05	6.38	94.9	4.13

Table 3. Frequency of transmembrane L(I, M, V)xxxD(N, E) motifs in human Rh-GPCRs

Found in % canonical receptors						Found in % olfactory receptors				
Segment	LxxxD	MxxxD	VxxxD	LxxxN	LxxxE	LxxxD	MxxxD	VxxxD	LxxxN	LxxxE
tm1	0	0.49	0.49	0.99	1.48	0.8	0	0	0.8	2.13
tm2	89.2	4.33	0.87	5.19	0.43	81.6	0.27	0	5.07	10.9
tm3	0.87	0.43	3.03	8.23	0	5.6	0	0.8	1.33	32.8
tm4	0.87	0.43	0.87	2.16	3.46	3.2	0.27	1.07	1.07	0.27
tm5	0	1.73	0	0.43	0.87	2.93	1.07	4.8	7.47	0.53
tm6	1.73	0	0.43	2.16	0	0	0	0.27	0.8	0
tm7	0.87	1.3	0	48.1	2.6	1.07	0.53	0.8	0.27	0

The sequences of the respective domains are shown in the supplement S1

Table 4. A survey of the transmembrane motifs in opsins

Group	No. of opsins	tm2 motifs	tm3/ic2 motifs	tm7 motifs
Rod opsins				
Rod mammalian tm2.11 D	14	LAVAD	ERY	NPV(I)IY
Rod mammalian tm2.11 N	4	LAVAN	ERY	NPVIY
Rod fish tm2.11 D	29	LxxxD	E(D)RW(Y)	NPxxY
Rod fish tm2.11 N	9	LAV(M)AN	E(D)RW(Y)	NPxxY
Long wavelength opsins				
Red mammalian	15	LxxxD	E(D)RY(W)	N(G)PxxY(F)
Red fish	57	LAI(V)AD	ERW(Y)	NPxIY
Medium wavelength opsins				
Green mammalian	7	LAV(I)AD	ERW	NPxIY
Green fish tm2.11 N	5	LAVAN	ERW	NPMIY
Green fish tm2.11 G	50	LAVAG	ERY	NPxIY
Short wavelength opsins				
S-w/blue mammalian	30	VSxGG	ERY	NPIIY
Uv bird	7	I(V)xxxG	ERY	NPxIYY
Uv fish	13	ISxxG	ERY	NPLIY
Violet bird	4	I(V)SF(A)SG	ERY	NPIIY
Blue fish 2.11 N	24	L(M)AA(IS)N	ERW	NpxIY
Blue fish 2.11 G	4	L(I)xxAG	ERW	NPxIY
Invertebrate opsins				
Rod invertebrate	3	LAxSD	DRY	NPVIY
Red invertebrate	19	LxxxD	DRY	N(D, S, T)PxxY
Blue invertebrate	23	LAxF(S)D	DRY	N(D)PxxY
Uv invertebrate	19	LAxxD	DRY(H, F)	D(NE)xVY

S-w Short wavelength; *Uv* ultraviolet. The organisms and sequences of the segment are shown in the online supplement S2. The subgroups in the table are based on the major variants in the eleventh residue of the second transmembrane segment (tm2.11), as indicated. For the tm2 positions 8–10, the residues listed in *parentheses* are the first and second variants of the preceding major residue. Occurrence of three or more variants is indicated by '*x*'. Changes in the tm3 and tm7 segments are all conservative in sequence comparisons

residues. The motif is located in the N-terminal half of the tm3 segment, starting about six residues from the exterior, and along with the tm3 LxxxD motif (present in 6% of the receptors, at the same position) could be involved in the regulation of activity of the respective receptors.

The LxxxN motif is found in the second transmembrane segment of about 5% of either the canonical or the olfactory receptors. This tm2 motif is frequent in opsins of marine animals (see Table 4 and supplement S2). However, the motif is also found in the seventh transmembrane segment of 48% of the canonical GPCRs, in the extracellular (N-terminal) part of the segment. Serine and tyrosine are found, respectively, in 31 and 38% of the receptors with this tm7 motif. This motif is virtually absent from the tm7 segment of the olfactory receptors.

Residues surrounding the pivot in the second transmembrane domain show very few ionic sidechains

A survey of the percentage occurrence of individual amino acid residues for the non-olfactory receptors is presented in the supplementary Table S4A, and for olfactory receptors in Table S4B. The residue abundance by category is given in Table S4C for both groups.

The near-invariant Leu and Asp residues in the 2.7–11 motif of the tm2 segment are situated within one turn of α helix (3.6–3.7 residues; e.g. Cornette et al., 1987; Musse et al., 2006), and positions 2.12 and 2.13 also hold largely the bulky hydrophobic residues (more than 60% canonical receptors have leucine, and more than 75% have the large neutral residues at tm2.12 and 13; supplementary Table S4A). This should produce specific conformation for the motif. The Leu residue-created bulge will produce a pocket of high accessibility to Asp. As can be deduced from Tables S4A and S4B, eight amino acids represent more than 97 and 90%, respectively, of all tm2.8–14 residues in the canonical and olfactory Rh-GPCRs (see also Table S4C). Beside the ubiquitous 2.7 Leu and 2.11 Asp, these residues frequently include strong H bond formers Ser and Thr and the dynamic cation/π interactant Phe (De Wall et al., 2000). The ionic sidechain residues Asp, Glu, His, Lys and Arg, the polar amides Asn and

Gln, and the large π donors Trp and Tyr are not present in positions tm2.8-10, and are either absent, or present in a single molecule, at tm2.12-14 of the canonical receptors. In the tm2.8-14 of the olfactory receptors, most of these residues are either absent, or found in less than 2% of the receptors (supplementary Table S4B). However, 43% of the olfactory receptors have Tyr at tm2.14 (Table S4B).

The hydrophobic π electron-rich residues can act as pore formers (Oblatt-Montal et al., 1993) as well as cation sinks (Kellenberger et al., 1996). Interactions of the alkali cations with aromatic electron-rich rings occur within the van der Waals contact (Hu et al., 2002), and could be important for short-distance cation guidance (Gallivan and Dougherty, 1999), especially if in the same fold of the α-helix with Asp, as in the case of the tm2.8-10 Phe residues. Phenylalanine at tm2.8-14 should also modulate cation movements in the vicinity of the 2.11 Asp. Phenylalanine may, as in Na^+ transporters, help movement of a cationic residue (or an ion, e.g. Na^+) by changing position in the membrane (Eaholtz et al., 1994; Kellenberger et al., 1996). Also, Phe can directly π/cation-interact with Arg in a transmembrane milieu (Gromiha, 2003). Tryptophan and tyrosine, the bulky polar π interactants (Dougherty, 1996; De Wall et al., 2000; Steiner and Koellner, 2001) and strong hydrogen bond formers (e.g. Adamian and Liang, 2002) are essentially absent from the 8–10 part of the tm2 motif (Tables S4A and B). The large tryptophan and tyrosine residues seem to localize preferentially in the outer parts of the transmembrane helices (Arkin and Brunger, 1998). Tryptophan is also a very potent hydrogen/π – bonding stabilizer of helix structure (Steiner and Koellner, 2001), and Tyr is a stronger stabilizer of protein structure than Phe (Pace et al., 2001), and its presence could also limit the tm2.8-10 dynamism. The smaller and more cation/π-active phenylalanine (Fernandez-Recio et al., 1999) is found in $<7\%$ of tm2.8-9 in the canonical receptors, apparently is not present at tm2.10 in these receptors, and is very rare at tm2.10 in the olfactory receptors. This might serve to avoid cation trapping by Phe (e.g. Gapeev and Dunbar, 2001) at the N-terminal side of the 2.11 Asp. However, 15% of the canonical receptors have Phe at 2.12, and 46% olfactory receptors have Phe at 2.9, which possibly presents a cation sink (Kellenberger et al., 1996; Gapeev and Dunbar, 2001), and there are 26% non-odorant and 64% olfactory receptors with aromatic residues at tm2.14 (Tables S4A and B).

Proline is very rare in the midsection of the second transmembrane segment of Rh-GPCRs. In the human non-olfactory receptors, proline within one α-turn (four residues) of the 2.11 pivot is found only in the chemokine CXC6 receptor (see supplement S1). Four out of 374 human odorant receptors (2C3, 3A4, 4S1 and 52E1; supplement S1) have proline within four residues at either side of the 2.11 pivot. This indicates a need for conformational stability in the central portion of the tm2 helix, and is not typical of the central portion of transmembrane helices across protein families (Cordes et al., 2002). The TxP motif near the C-terminus of the tm2 segment (found in most chemokine receptors, and in neuropeptide Y receptors) can be shown to strongly bend the helix (Govaerts et al., 2001), which could be of importance in interactions with the large peptidic agonists of these receptors. Inner prolines also are not frequent in the third transmembrane segment of Rh-GPCRs. However, the seventh transmembrane domain, beside Pro 7.20 in the NpxxY motif, shows prolines in other positions (especially in the human olfactory receptor position 7.16; supplement S1), which will add to the known conformational flexibility of this segment (Sakmar et al., 2002).

Stabilization based on central tm2 cysteines (positions 8–14) is likely in multiple human receptors (all muscarinic, angiotensin II type 1, two chemotactic, bradykinin, relaxin 1 and 2, V2-vasopressin, galanin 2 and 3, and a large number of olfactory receptors; supplement S1 and Tables S4B and C). This could indicate both interactions with other receptor segments, and a conformational stabilization via intrahelical hydrogen bonding (Gray and Matthews, 1984) that can help interactions of the Asp pivot. Type 2 and 3 galanin receptors even possess tm2 Cys doublets, possibly involved in metal bridges (see Elling et al., 2000; Webster et al., 2004). There are 39% receptors with tm2.13 Cys in the olfactory group (supplementary Table S4B), possibly signifying a specific receptor sub-group.

In the canonical receptors, positions 8 and 10 are dominated by Ala (80 and 59%, respectively; supplementary Table S4A), and positions 12 and 13 by Leu (63 and 66%, respectively; Table S4A). Position 2.8 holds hydroxy sidechains in 75% of the olfactory receptors, possibly aiding in stabilization of the interhelical association by H bonding (Gray and Matthews, 1984; Senes et al., 2001). Intrahelical H bonding of these residues may facilitate cation transfer, as in the case of bacteriorhodopsin (Peralvarez-Marin et al., 2007). The tm2 serine in a short-wavelength Xenopus opsin is H-bonding with the protonated retinylidene Schiff base (Dukkipati et al., 2001).

It is of interest that the tm2.8-10 hydroxy and aromatic residues are frequent in several receptors known to be strongly sensitive to cations, including the neuropeptide Y (NPY) Y1 (Parker et al., 1996) and Y4 (Parker et al., 2002) receptors, psychosine receptor (Im et al., 2001), and

sphingosine 1-phosphate receptors (see supplement S1 for the respective sequences).

Taste-2 receptors have a LxxxR motif in the second transmembrane segment

An examination of gustatory GPCRs indicated that the Taste-2 family has a significant homology with Rh-GPCRs in the first and second transmembrane domains. More than 98% of the 211 mammalian Taste-2 receptors analyzed (including all of 25 human sequences) show a tm2 L(V,S)xxxR(K,H,T) motif aligned with the tm2 LAVAD motif in the bRh. The composition of this segment is detailed in supplements S3 and S4D.

The LxxxR motif of Taste-2 group may represent a signature indicating exclusion of salty stimuli (which are handled by epithelial Na^+ channels (DeSimone and Lyall, 2006). Similar to Rh-GPCRs, there are no Glu, His, Lys, Arg, Asn, Gln, Trp, and Tyr residues at tm2.8-10, and Asp is found at tm2.10 of a single sequence (S3 and Table S4D). Position 2.8 holds 67% Ala and 13% Ser, while the position 2.9 is all-hydrophobic (including 45% Ile). Position 2.10 has largely hydroxy amino acid side-chains, 81% Ser and 7% Thr, with possible roles in ion transfer (Thompson et al., 2000; Kinjo et al., 2005) as well as in stabilization of intrahelical and interhelical interactions (Gray and Matthews, 1984; Senes et al., 2001). Position 2.14 has Leu in 80% of the sequences.

Arginine, a bulky ionized residue, occurs rarely in the center of Rh-GPCR helices. Arginine is not found in the tm2.8-15 sequence of any of the non-olfactory human Rh-GPCRs, and is present in this part of only a few olfactory receptors (see supplements S1 and S4B). In addition to other possible roles (e.g. bridging the acidic residues in other segments (Donohue et al., 1999)), this arginine may function as a cation ''paddle'' in ion channels (Jiang et al., 2003), and is frequent in the outer portions of the helices A and D of the archaeal ion-transporting rhodopsins. It should be noted that the second transmembrane segment of the Taste-2 receptor group has a large complement of Leu (average 25%, vs. 17% in the olfactory, but 23% in the canonical Rh-GPCRs), which can serve to stabilize an expanded helical conformation (Vlassi et al., 1999).

The second transmembrane motif in opsins shows large modifications related to opsin function

A survey of opsins from 184 metazoan species indicates that the tm2.7–11 motif is much varied in connection to both phylogeny and function of these visual pigments (Table 4, Figs. 1 and 2, and the S2 supplement). Thus, the rod opsins of land mammals and the red/long-wavelength opsins of mammals or fish have similar tm2, tm3 and tm7 motifs, which also correspond to the most frequent motifs found in the canonical Rh-GPCRs (see Table 2). The red/long-wavelength opsins of fish are similar to the mammalian, and all have the tm2.11 Asp. The green mammalian opsins from land-dwelling species also have the 2.11 Asp. However, the green opsins of marine vertebrates show 2.11 Asn (a semi-conservative difference) or Gly (a non-conservative difference). All vertebrate short-wavelength opsins have Gly at 2.11, and all also are modified at 2.7. These pigments show no Asp in the tm2 helix (supplement S2). It should be noted that all fish blue- or green-classified opsins also show Asn or Gly at 2.11 (supplement S2). However, invertebrate opsins in any wavelength category show no variation at either the 2.7 (Leu) or the 2.11 (Asp) residue, and that is found for insects as well as for the marine species (supplement S2).

The tm2.11 modifications in the short-wavelength (s-w)/blue and uv opsins are invariably accompanied by a change of the 2.7 residue from Leu (a strong α-helix former (Chou and Fasman, 1974; Levitt, 1978)) to either Val or Ileu (Table 4) (both strong β-sheet formers (Chou and Fasman, 1974; Levitt, 1978) and good stabilizers of peptide chain packing (Chen and Stites, 2001)). These changes can lead to helix destabilization and formation of cavities (Creamer and Rose, 1995; Johansson et al., 1998; Vlassi et al., 1999). The s-w/blue mammalian opsins also have a further reduction of helix volume by the change to Gly at tm2.10. These modifications are all consonant with a large flattening of the tm2 helix and reduction of ionic interactions in the tm2.7–11 stretch. This should reflect changes in signal transduction that are necessary to support proper functioning of the various spectral classes of opsins. The tm2.11 Asp, as will be discussed later, could be the trigger of an important ionic switch supporting fast changes in intramembrane conformation of rhodopsins. A switch function would be greatly reduced by mutation to Asn, and essentially abolished by mutation to a non-polar residue (in most cases to Gly, a helix breaker that in transmembrane environments should also promote interhelix association (Javadpour et al., 1999)). The mammalian s-w/blue opsins even have glycine doublets at tm2.10-11 (Table 4). These changes may help association with transducin in the dark, equivalent to the constitutive signal transduction which is frequently observed for Rh-GPCRs that show similar changes in the second transmembrane domain. The s-w cone opsins apparently have lower quantum yields than rod opsins,

possibly linked to a lower stability of the active conformation (Starace and Knox, 1997) due to shifts in salt bridges and Schiff imine counterions (Babu et al., 2001; Kono, 2006).

Involvement of the tm2.11 aspartate in signal transduction by the rhodopsin family receptors

A significant role of the tm2.11 aspartate in signal transduction was found for many Rh-GPCRs. Mutation of the 2.11 Asp to Asn or Ala generally results in a large reduction of agonist-driven signal transduction, and often is accompanied by a decrease of affinity for the agonist. A chronological selection has the β2-adrenergic (Chung et al., 1988), α2A-adrenergic (Ceresa and Limbird, 1994; Horstman et al., 1990; Wang et al., 1991; Wilson et al., 2001), luteinizing hormone (LH) (Dhanwada et al., 1996; Ji and Ji, 1991; Quintana et al., 1993), D2 dopamine (Neve et al., 1991, 2001; see also Dahl et al., 1991 for modeling)), C5A chemoattractant (Monk et al., 1994), endothelin-1 and 2 (Rose et al., 1995), cholecystokinin-B (Jagerschmidt et al., 1995), platelet-activating factor (PAF) (Parent et al., 1996), cannabinoid CB-1 and 2 (Tao and Abood, 1998; Roche et al., 1999; Nie and Lewis, 2001), adenosine A1 (Ward and Milligan, 1999), tachykinin substance K (Donnelly et al., 1999), oxytocin (Fanelli et al., 1999), neurotensin-1 (Martin et al., 1999), muscarinic m2 (Vogel et al., 1999) and 5-HT6 (Zhang et al., 2006) receptors. A stimulatory role for 2.11 Asn was reported for the gonadotropin hormone-releasing (GnRH) receptor (Zhou et al., 1994); however, this residue is followed by Glu in the next helical fold of the tm2.7–14 LTLANLLE sequence (supplement S1). In the neurokinin-1 (substance P) receptor (with 2.7-11 LAFAE motif), substitution of Asp for Glu preserves the affinity and signal transduction, while the tm2.11 mutation to Gln abolishes the signal transduction (Brodbeck et al., 1995). Glutamate is quite frequent as the tm2.11 residue of the odorant receptors (Tables 2 and S4B). One of the most direct proofs for the importance of the tm2.11 Asp in signal transduction is the large decrease of the transduction for the $Asp^{79}Asn$ mutant of the adenosine A1 receptor (Ward and Milligan, 1999). It should be noted that the tm2.11 $Asp^{83}Asn$ mutation does not block activation of transducin by bovine rhodopsin (Fahmy et al., 1993; Breikers et al., 2001), but does reduce the overall opsin activity.

Other mutations at tm2.7–2.11 also support functioning of this segment in signal transduction, possibly in a switch-like manner. Thus, in thyrotropin hormone-releasing receptor, substitution of Ala for Asp at tm2.11 strongly reduces the affinity of agonist binding, and the stimulation of phosphoinositide production by the releasing hormone (Perlman et al., 1997). In the rodent neurotensin-2 receptor, which has tm2.11 Ala in the wildtype, and shows no sensitivity to Na^+ in the agonist binding, mutation to 2.11 Asp induces a large Na^+ sensitivity, similar to that shown by neurotensin-1 receptor, which has 2.11 Asp (Martin et al., 1999). With the 5-HT6 receptor, a triple tm2 mutation in positions 8, 9, and 11, $F^{69}L$, $T^{70}I$, $D^{72}A$, eliminates stimulation of adenylate cyclase by the agonist, while the agonist binding affinity is only reduced (Zhang et al., 2006). The mutation of the tm2.7 Leu to either a neutral (Ala) or a polar (Ser) residue was shown to result in constitutive activation of the bovine rhodopsin (Madabushi et al., 2004).

The tm2.7–11 motif in many mutated, viral and orphan rhodopsin-like receptors and opsins shows large modifications that can relate to constitutive signal transduction

An examination of the three transmembrane motifs in several Rh-GPCRs that show constitutive signal transduction is presented in Table 5. The Rh-GPCR part of the table, in addition to the functional canonical α1A-adrenergic receptor, contains three receptors known for a low activation of fast-loading G-proteins (Mazella and Vincent, 2006), and two promiscuous binding proteins that lost the ability to transduce, the Duffy antigen/Plasmodium receptor (Du et al., 2002), and the chemokine binding protein 2 (Nibbs et al., 1997).

The viral receptor section presents motifs in four mammalian herpesvirus receptors that show constitutive transduction. The receptors are all heavily mutated, and there is a large reduction in sequence overlap with the bovine rhodopsin. While there are changes in all three transmembrane motifs, the second transmembrane motif is arguably the most affected. The Epstein-Barr BILF1 receptor is the most modified (Table 5), but its tm3/ic2 motif still is preserved, and the receptor shows a strong constitutive transduction (Paulsen et al., 2005). Both the human herpesvirus 6 and the saimiri herpesvirus 11 support the chemokine (agonist)-specific as well as the constitutive transduction (Rosenkilde et al., 2004; Fitzsimons et al., 2006). The Kaposi herpesvirus-8 receptor is known to activate phospholipase C beyond normal utilization of the enzyme, with a detrimental over-production of second messengers (Arvanitakis et al., 1997).

The orphan part of the table contains three receptors already shown to have constitutive signal transduction. In

Table 5. Transmembrane motifs of Rh-GPCRs that show constitutive signal transduction

Receptor*	Accession No.	tm2 motif	tm3/ic2 motif	tm7 motif	Functionality**	References
Canonical receptors						
α1A-adrenergic human	SP-P35348	LAVAD	DRY	NPIIY	Fully functional	
Neurotensin type 2 rat	SP_Q63384	LALSA	ERC	TPVLY	Loss of cation sensitivity; CST	(Martin et al., 1999; Mazella and Vincent, 2006; Vita et al., 1998)
Neurotensin type 2 human	SP-O95665	LAVAG	ERC	TPLLY	Loss of cation sensitivity; CST ?	(Martin et al., 1999; Mazella and Vincent, 2006; Vita et al., 1998)
Plasmodium/chemokine	SP-Q16570	LAVGS	HRL	TPLLL	Loss of signal transduction	(Du et al., 2002)
Chemokine binding protein 2	SP_O00590	LAISN	HRL	SPILY	Loss of signal transduction	(Bonini et al., 1997; Nibbs et al., 1997)
Viral receptors						
Epstein-Barr BILF1	NC_007605	LLIEL	EKT	GPAAC	CST	(Paulsen et al., 2005)
Human herpesvirus 6 U51 var A	SP-P52382	FAGMS	ERI	IPVMA	CST	(Fitzsimons et al., 2006)
Saimiri herpesvirus strain 11	SP-Q01035	GFCLN	TRL	LPLMF	CST	(Rosenkilde et al., 2004)
Human Kaposi herpesvirus 8	SP-Q98146	ICLNS	VRY	VPLIY	CST	(Arvanitakis et al., 1997)
Orphan receptors						
GPR26	SP_Q8NDV2	LTCGN	DRW	DPFVY	CST	(Jones et al., 2007)
GPR33	SP_Q49SQ1	HLILS	DRY	SPTLY	CST	(Rompler et al., 2006)
GPR78	SP-Q96P69	LSLGH	DQW	DPFTY	CST	(Jones et al., 2007)
Opsins						
Rhodopsin bovine	SP-P02699	LAVAD	ERY	NPVIY	Rod opsin	
Blue opsin, eel	NM-AAA99297	LAVAN	ERW	NPVIY	Cone opsin – deep sea form	(Archer et al., 1995)
Green opsin, eel	NM-AAA99200	LAVAD	ERW	NPVIY	Cone opsin – freshwater form	(Archer et al., 1995)
Violet Xenopus frog	SP-P51473	ITVGG	ERY	NPIIY	Cone opsin	(Starace and Knox, 1997)
Violet chicken	SP-P28684	ISASG	ERY	NPIIY	Cone opsin	(Okano et al., 1992)
Uv goldfish	SP-Q90309	ISLGG	ERY	NPLIY	Cone opsin	(Hisatomi et al., 1996)
S-w blue bovine***	NM-U92557	VSLGG	ERY	NPIIY	Cone opsin	
S-w blue human***	NM_001708	VSFGG	ERY	NPIIY	Cone opsin	(Kono, 2006)

* The receptors without a species identification are human

** *CST* Constitutive signal transduction

*** Short-wavelength mammalian blue visual pigments have λ_{max} in the uv to blue range (Babu et al., 2001)

all cases, there are large changes in the tm2 motif, while the mutations in the tm3 and tm7 motifs are essentially conservative. The asparagine or histidine residues in the 2.7–11 stretch (both accepted as alternatives for aspartate) may complement changes in the tm7 pivot.

Bovine rhodopsin motifs are included for comparison in the opsin section of the table. The following two opsins from the eel *Anguilla* present an interesting quasi-ontogenetic recapitulation with the change in the cone opsin expression from green to blue type during the adaptation from shallow to deep water that occurs within the lifetime of the European eel (*Anguilla anguilla*). The blue opsin has the 2.11 Asn, as well as two serines in place of nonpolar residues in the tm3 and tm7 segments (Archer et al., 1995; see Table 7 and supplement S2), with an overall effect of increased rigidity for the affected transmembrane segments. The tm2.11 Asn – containing opsins are expressed in many marine vertebrates (Table 4 and supplement S2) and invariably respond to photic stimuli of shorter wavelength (and higher energy) than the tm2.11 Asp opsins, and especially rhodopsins. The tm2 motifs of all short-wavelength opsins are quite different from those in rod opsins, while there is a complete conservation of the tm3 and the tm7 motifs, indicating that the main thrust of sequence evolution for these motifs was toward a lower cation sensitivity at tm2.7–11.

Evidence for involvement of the tm2.7–11 motif in the cationic regulation of agonist binding

Studies on the regulation of Rh-GPCR function via the second transmembrane segment thus far have largely been confined to the tm2.11 Asp residue. The allosteric modulation by sodium of agonist binding to the α2A adrenergic

receptor is lost upon changing tm2.11 Asp to Asn (Ceresa and Limbird, 1994). Somatostatin receptors (SSRs) 1–4 all have glutamate after the tm2.7–11 LAVAD motif (supplement S1), and all are sensitive to sodium in agonist binding, while SSR5, which lacks this acidic tm2 doublet, is less affected by Na^+ (Raynor et al., 1993; Williams et al., 1997). The tm2.11 mutation of Asp to Asn reduces SSR1 sensitivity to sodium (Kong et al., 1993). The dopamine D2 receptor is also exceptionally sensitive to Na^+ in agonist binding; the regulation by sodium with a participation of the tm2.11 Asp was modeled by Neve et al. (2001). Amiloride and derivatives affect the attachment of agonists to receptors that also are highly sensitive to Na^+ in this binding (Neve et al., 1991; Parker et al., 2002). The neuropeptide Y (NPY) Y1 subgroup receptors (the Y1, with 2.7–11 LSFSD, the Y4 and Y5 with 2.7–11 LAFSD motifs) are sensitive to cations in the attachment of their peptidic agonists (Parker et al., 1996; Parker et al., 2002). The proton-sensing glycosphingosine and sphingosine phosphate receptors (Ludwig et al., 2003) are quite likely to have participation of residues and motifs of the second transmembrane segment in signal transduction. As seen in the supplement S1, these receptors, in addition to the presence of serine and/or threonine at tm2.8-10 also have a number of other polar residues in the motif, increasing the interactions with charged ions or groups. Transmembrane serine is known to support the constitutive signal transduction of β-adrenergic receptors (Ambrosio et al., 2000). The conversion of bovine rhodopsin to constitutive signaling by the $Leu^{79}Ala$ mutation (Madabushi et al., 2004) should also signal involvement of the tm2 motif in the cation path.

The question of a possibly direct regulation of signal transduction by interaction of transmembrane residues or motifs of GPCRs, including the tm2.7–11 motif, is difficult to answer, as it currently is not clear if any inner transmembrane residues of Rh-GPCRs do physically associate with G-protein transducers, as can be shown for a juxtacytoplasmic aspect of the sixth transmembrane segment (Abell et al., 1998). However, cationic aspects of proteins are likely to penetrate the phospholipid layers (Zhang et al., 2001). Phosphatidylinositol-hydrolyzing phospholipase C activity is very sensitive to bilayer membrane curvature, indicating an extensive direct interaction (Ahyayauch et al., 2005). The C2 domains (targeting many enzymes to membrane in response to Ca^{2+} (Nalefski et al., 2001) in phospholipase A2 are electrostatically rather than conformationally regulated (Frazier et al., 2002; Malmberg et al., 2003). Similar is found for phospholipase Cβ2 (Sutton and Sprang, 1998). Transmembrane anionic switches could be exposed to cations by filament proteins, as e.g. penetration of F-actin into bilayers boosts the cation entry (Grigoriev et al., 2000).

The large presence of alanine and serine at tm2.8 and 10 could reflect a selective assistance in cation handling, as also observed in some Na^+ transporters (Kellenberger et al., 1999) and K^+ channels (Thompson et al., 2000). There is a decrease in polar amino acid fraction in the tm2.13-18 stretch in both opsins and the canonical Rh-GPCRs (Fig. 2B). This could be consistent with a mainly lateral cation transfer, as documented for the mutant tm2 Asp^{90} of rhodopsin in accepting proton from the retinylidene chromophore (Zvyaga et al., 1996).

General discussion

The constitutive signal transduction apparently is, to a varying degree, detected with normal opsins (Zvyaga et al., 1996) as well as with wildtype Rh-GPCRs (Alewijnse et al., 2000; Parker et al., 2007a). In the absence of agonist-induced reconformation, the constitutively activated receptors may exist as oligomers loosely associated with G-protein heterotrimers or subunits (Baneres and Parello, 2003; Parker et al., 2007b). Especially with rod opsins, a physiologic constitutive activation should be helped by the organization in large oligomeric matrices (Fotiadis et al., 2006).

The tm2 motif could be a more sensitive predictor of constitutive association with transducers than the tm3/ic2 and the tm7 motifs, which apparently are more critical to the signal transduction, and in many cases are not changed even in receptors that display a predominant constitutive activity. In bovine rhodopsin, tm2.11 Asp^{83} interacts with tm3.11 Gly^{121} (Nagata et al., 1998), and might only marginally support interactions in normal visual signal transduction (Fahmy et al., 1993; Breikers et al., 2001). In the night blindness-inducing mutation tm2.19 $G^{90}D$ there is a constitutive rhodopsin activation (Zvyaga et al., 1996; Kim et al., 2004). In addition to the principal Glu^{113} switch (tm3.13), other anionic switches (Glu^{122} (Beck et al., 1998) and Glu^{134} (Cohen et al., 1993)) and the mutant tm7.10 $A^{292}E$ switch (Jin et al., 2003) could be involved in the proton path and in transmembrane conformational equilibria. Even a polar-to-neutral tm2.22 mutation $T^{94}I$ of rhodopsin (Gross et al., 2003; Kim et al., 2004) is producing a constitutive activation.

Change of Asp to Asn or Gly in the second transmembrane segment of Rh-GPCRs was amply documented to reduce the rate of signal transduction, and also to occur in parallel to constitutive coupling to G-proteins. The large

non-polar mutations of the tm2 motif in many of these opsins may reflect the need for a longer-lasting opsin-transducin interaction in conditions of low illumination. Indeed, this is strongly supported by activity of the violet opsin of Xenopus (Starace and Knox, 1997; Vought et al., 1999; Babu et al., 2001; Dukkipati et al., 2001). An extended signaling due to this type of sequence modification could be equivalent to the mass-action-related amplification of the signal via e.g. an increase in the transducin component of the visual signal transduction (Langlois et al., 1996). It should be noted that some deep-sea fishes may express just the s-w/blue opsin variant (Partridge et al., 1989). A decrease in cation-linked switching in these opsins could principally translate into a larger signal amplification per photoexcitation event (Stryer, 1986)

Glutamate and aspartate switches linked to cation transfer are the indispensable components of the visual signal transduction. In mammalian rod opsins, the most important of these switches is Glu^{113}, which reversibly accepts proton from the activated retinylidene Schiff imine. Neutral mutations of Glu^{113} dramatically blue-shift the absorbance of bovine rhodopsin (Sakmar et al., 1989; Nathans, 1990a). Several other acidic residues are minimally involved in the spectral properties of bRh (Nathans, 1990b), but could matter in the proton path. A large number of Asp and Glu mutations to amide or hydrophobic sidechains produce blue shifts and decrease in activity of opsins, comparable to reduction of non-visual signal transduction by substitution of Asn or Ala for Asp in the second transmembrane segment of Rh-GPCRs. Also, proton release in the phoborhodopsin cycle can be enhanced by replacement of neutral Pro^{193} by Asp (Iwamoto et al., 2004). The sub-sensitivity of this archaeal rhodopsin compared to other bacteriorhodopsins should be linked to Asp^{204} (Ren et al., 2001). The substitution of Asn for Asp in the tm3/ic2 DRY motif of the β2-adrenergic receptors stimulates the constitutive G-protein coupling (Rasmussen et al., 1999). However, in several other receptors mutations to non-ionic residues in this critical switch cause a loss of coupling to G-proteins, constitutive or agonist-stimulated, and destabilization of the receptors (Alewijnse et al., 2000; Wilbanks et al., 2002; Capra et al., 2004; Gruijthuijsen et al., 2004; Lagane et al., 2005).

The very high frequency of the LxxxD motif in human receptors with known specific agonists could indicate an important functionality linked to cation movement within the bilayer. Ca^{2+} and Na^{+} have already been shown to strongly affect the agonist binding to many GPCRs, and calcium at submicromolar levels is known to regulate the activity of phospholipases and kinases. The polarity barrier past the LxxxD motif of the tm2 segment (Fig. 2B) could help in limiting the access of cations from extracellular sources. Cations interacting with the tm2.11 Asp (or Asn) could be inorganic ions passing laterally (similar to the proton path in opsins), or from the cytosol, or cationic residues from other transmembrane segments establishing short-term bridges at tm2.11.

By analogy with ion transporters, functions of the tm2 LxxxD motif might be significantly regulated by hydroxy or aromatic amino acid residues. Some peptide receptors known to be sensitive to cations in the attachment of agonists, including pancreatic polypeptide Y4 receptors, neuropeptide Y, Y1, and Y5 receptors, neurokinin-3 receptor, and luteinizing hormone receptor, contain a LxFSD tm2 motif. Other cation-sensitive Rh-GPCRs also show at least one hydroxy amino acid or aromatic (π) anion (Phe) residue in the 2.7–11 motif. In some Rh-GPCRs highly sensitive to cations (including GnRH and somatostatin SSR1-SSR4 receptors), cation effects should be augmented by an additional acidic residue in the next helical fold. In Taste-2 receptors, the second transmembrane segment contains a basic pivot, which may serve as cation sensor as well as deflector. The regulation via polar residues in this segment could also be important in heptahelical receptors of the GPCRDB family C (glutamate subfamily of (Fredriksson et al., 2003)), especially the Ca^{2+} -sensing and the metabotropic glutamate receptors.

References

Abell AN, McCormick DJ, Segaloff DL (1998) Certain activating mutations within helix 6 of the human luteinizing hormone receptor may be explained by alterations that allow transmembrane regions to activate Gs. Mol Endocrinol 12: 1857–1869

Adamian L, Liang J (2002) Interhelical hydrogen bonds and spatial motifs in membrane proteins: polar clamps and serine zippers. Proteins 47: 209–218

Ahyayauch H, Villar AV, Alonso A, Goni FM (2005) Modulation of PI-specific phospholipase C by membrane curvature and molecular order. Biochemistry 44: 11592–11600

Alewijnse AE, Timmerman H, Jacobs EH, Smit MJ, Roovers E, Cotecchia S, et al. (2000) The effect of mutations in the DRY motif on the constitutive activity and structural instability of the histamine H(2) receptor. Mol Pharmacol 57: 890–898

Ambrosio C, Molinari P, Cotecchia S, Costa T (2000) Catechol-binding serines of beta(2)-adrenergic receptors control the equilibrium between active and inactive receptor states. Mol Pharmacol 57: 198–210

Archer S, Hope A, Partridge JC (1995) The molecular basis for the green–blue sensitivity shift in the rod visual pigments of the European eel. Proc Biol Sci 262: 289–295

Arkin IT, Brunger AT (1998) Statistical analysis of predicted transmembrane alpha-helices. Biochim Biophys Acta 1429: 113–128

Arvanitakis L, Geras-Raaka E, Varma A, Gershengorn MC, Cesarman E (1997) Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. Nature 385: 347–350

Babu KR, Dukkipati A, Birge RR, Knox BE (2001) Regulation of phototransduction in short-wavelength cone visual pigments via the retinylidene Schiff base counterion. Biochemistry 40: 13760–13766

Balmforth AJ, Lee AJ, Warburton P, Donnelly D, Ball SG (1997) The conformational change responsible for AT1 receptor activation is dependent upon two juxtaposed asparagine residues on transmembrane helices III and VII. J Biol Chem 272: 4245–4251

Baneres JL, Parello J (2003) Structure-based analysis of GPCR function: evidence for a novel pentameric assembly between the dimeric leukotriene B4 receptor BLT1 and the G-protein. J Mol Biol 329: 815–829

Beck M, Sakmar TP, Siebert F (1998) Spectroscopic evidence for interaction between transmembrane helices 3 and 5 in rhodopsin. Biochemistry 37: 7630–7639

Bonini JA, Martin SK, Dralyuk F, Roe MW, Philipson LH, Steiner DF (1997) Cloning, expression, and chromosomal mapping of a novel human CC-chemokine receptor (CCR10) that displays high-affinity binding for MCP-1 and MCP-3. DNA Cell Biol 16: 1249–1256

Breikers G, Bovee-Geurts PH, DeCaluwe GL, DeGrip WJ (2001) A structural role for Asp83 in the photoactivation of rhodopsin. Biol Chem 382: 1263–1270

Breitwieser GE (1991) G protein-mediated ion channel activation. Hypertension 17: 684–692

Brodbeck RM, Sachais BS, Krause JE (1995) Residue 78 in the second transmembrane domain of the neurokinin-1 receptor is important in coupling high affinity agonist binding to multiple second messenger responses. Mol Pharmacol 47: 1065–1071

Capra V, Veltri A, Foglia C, Crimaldi L, Habib A, Parenti M, et al. (2004) Mutational analysis of the highly conserved ERY motif of the thromboxane A2 receptor: alternative role in G protein-coupled receptor signaling. Mol Pharmacol 66: 880–889

Ceresa BP, Limbird LE (1994) Mutation of an aspartate residue highly conserved among G-protein-coupled receptors results in nonreciprocal disruption of alpha 2-adrenergic receptor-G-protein interactions. A negative charge at amino acid residue 79 forecasts alpha 2A-adrenergic receptor sensitivity to allosteric modulation by monovalent cations and fully effective receptor/G-protein coupling. J Biol Chem 269: 29557–29564

Chen J, Stites WE (2001) Packing is a key selection factor in the evolution of protein hydrophobic cores. Biochemistry 40: 15280–15289

Chou PY, Fasman GD (1974) Prediction of protein conformation. Biochemistry 13: 222–245

Chung FZ, Wang CD, Potter PC, Venter JC, Fraser CM (1988) Site-directed mutagenesis and continuous expression of human beta-adrenergic receptors. Identification of a conserved aspartate residue involved in agonist binding and receptor activation. J Biol Chem 263: 4052–4055

Cohen GB, Yang T, Robinson PR, Oprian DD (1993) Constitutive activation of opsin: influence of charge at position 134 and size at position 296. Biochemistry 32: 6111–6115

Cordes FS, Bright JN, Sansom MS (2002) Proline-induced distortions of transmembrane helices. J Mol Biol 323: 951–960

Cornette JL, Cease KB, Margalit H, Spouge JL, Berzofsky JA, DeLisi C (1987) Hydrophobicity scales and computational techniques for detecting amphipathic structures in proteins. J Mol Biol 195: 659–685

Creamer TP, Rose GD (1995) Interactions between hydrophobic side chains within alpha-helices. Protein Sci 4: 1305–1314

Dahl SG, Edvardsen O, Sylte I (1991) Molecular dynamics of dopamine at the D2 receptor. Proc Natl Acad Sci USA 88: 8111–8115

De Wall SL, Meadows ES, Barbour LJ, Gokel GW (2000) Synthetic receptors as models for alkali metal cation-pi binding sites in proteins. Proc Natl Acad Sci USA 97: 6271–6276

DeSimone JA, Lyall V (2006) Taste receptors in the gastrointestinal tract III. Salty and sour taste: sensing of sodium and protons by the tongue. Am J Physiol Gastrointest Liver Physiol 291: G1005–G1010

Dhanwada KR, Vijapurkar U, Ascoli M (1996) Two mutations of the lutropin/choriogonadotropin receptor that impair signal transduction also interfere with receptor-mediated endocytosis. Mol Endocrinol 10: 544–554

Donnelly D, Maudsley S, Gent JP, Moser RN, Hurrell CR, Findlay JB (1999) Conserved polar residues in the transmembrane domain of the human tachykinin NK2 receptor: functional roles and structural implications. Biochem J 339: 55–61

Donohue PJ, Sainz E, Akeson M, Kroog GS, Mantey SA, Battey JF, et al. (1999) An aspartate residue at the extracellular boundary of TMII and an arginine residue in TMVII of the gastrin-releasing peptide receptor interact to facilitate heterotrimeric G protein coupling. Biochemistry 38: 9366–9372

Dougherty DA (1996) Cation-pi interactions in chemistry and biology: a new view of benzene, Phe, Tyr, and Trp. Science 271: 163–168

Du J, Luan J, Liu H, Daniel TO, Peiper S, Chen TS, et al. (2002) Potential role for Duffy antigen chemokine-binding protein in angiogenesis and maintenance of homeostasis in response to stress. J Leukoc Biol 71: 141–153

Dukkipati A, Vought BW, Singh D, Birge RR, Knox BE (2001) Serine 85 in transmembrane helix 2 of short-wavelength visual pigments interacts with the retinylidene Schiff base counterion. Biochemistry 40: 15098–15108

Eaholtz G, Scheuer T, Catterall WA (1994) Restoration of inactivation and block of open sodium channels by an inactivation gate peptide. Neuron 12: 1041–1048

Elling CE, Raffetseder U, Nielsen SM, Schwartz TW (2000) Disulfide bridge engineering in the tachykinin NK1 receptor. Biochemistry 39: 667–675

Fahmy K, Jager F, Beck M, Zvyaga TA, Sakmar TP, Siebert F (1993) Protonation states of membrane-embedded carboxylic acid groups in rhodopsin and metarhodopsin II: a Fourier-transform infrared spectroscopy study of site-directed mutants. Proc Natl Acad Sci USA 90: 10206–10210

Fanelli F, Barbier P, Zanchetta D, de Benedetti PG, Chini B (1999) Activation mechanism of human oxytocin receptor: a combined study of experimental and computer-simulated mutagenesis. Mol Pharmacol 56: 214–225

Fernandez-Recio J, Romero A, Sancho J (1999) Energetics of a hydrogen bond (charged and neutral) and of a cation-pi interaction in apoflavodoxin. J Mol Biol 290: 319–330

Fitzsimons CP, Gompels UA, Verzijl D, Vischer HF, Mattick C, Leurs R, et al. (2006) Chemokine-directed trafficking of receptor stimulus to different g proteins: selective inducible and constitutive signaling by human herpesvirus 6-encoded chemokine receptor U51. Mol Pharmacol 69: 888–898

Flanagan CA, Rodic V, Konvicka K, Yuen T, Chi L, Rivier JE, et al. (2000) Multiple interactions of the Asp(2.61(98)) side chain of the gonadotropin-releasing hormone receptor contribute differentially to ligand interaction. Biochemistry 39: 8133–8141

Fotiadis D, Jastrzebska B, Philippsen A, Muller DJ, Palczewski K, Engel A (2006) Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. Curr Opin Struct Biol 16: 252–259

Frazier AA, Wisner MA, Malmberg NJ, Victor KG, Fanucci GE, Nalefski EA, et al. (2002) Membrane orientation and position of the C2 domain from cPLA2 by site-directed spin labeling. Biochemistry 41: 6282–6292

Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol 63: 1256–1272

Fritze O, Filipek S, Kuksa V, Palczewski K, Hofmann KP, Ernst OP (2003) Role of the conserved NPxxY(x)5, 6F motif in the rhodopsin ground state and during activation. Proc Natl Acad Sci USA 100: 2290–2295

Gallivan JP, Dougherty DA (1999) Cation-pi interactions in structural biology. Proc Natl Acad Sci USA 96: 9459–9464

Gapeev A, Dunbar RC (2001) Cation-pi interactions and the gas-phase thermochemistry of the $Na^{(+)}$/phenylalanine complex. J Am Chem Soc 123: 8360–8365

Govaerts C, Blanpain C, Deupi X, Ballet S, Ballesteros JA, Wodak SJ, et al. (2001) The TXP motif in the second transmembrane helix of CCR5. A structural determinant of chemokine-induced activation. J Biol Chem 276: 13217–13225

Gray TM, Matthews BW (1984) Intrahelical hydrogen bonding of serine, threonine and cysteine residues within alpha-helices and its relevance to membrane-bound proteins. J Mol Biol 175: 75–81

Grigoriev PA, Tarahovsky YS, Pavlik LL, Udaltsov SN, Moshkov DA (2000) Study of F-actin interaction with planar and liposomal bilayer phospholipid membranes. IUBMB Life 50: 227–233

Gripentrog JM, Jesaitis AJ, Miettinen HM (2000) A single amino acid substitution (N297A) in the conserved NPXXY sequence of the human N-formyl peptide receptor results in inhibition of desensitization and endocytosis, and a dose-dependent shift in p42/44 mitogen-activated protein kinase activation and chemotaxis. Biochem J 352: 399–407

Gromiha MM (2003) Influence of cation-pi interactions in different folding types of membrane proteins. Biophys Chem 103: 251–258

Gross AK, Rao VR, Oprian DD (2003) Characterization of rhodopsin congenital night blindness mutant T94I. Biochemistry 42: 2009–2015

Gruijthuijsen YK, Beuken EV, Smit MJ, Leurs R, Bruggeman CA, Vink C (2004) Mutational analysis of the R33-encoded G protein-coupled receptor of rat cytomegalovirus: identification of amino acid residues critical for cellular localization and ligand-independent signalling. J Gen Virol 85: 897–909

Hisatomi O, Satoh T, Barthel LK, Stenkamp DL, Raymond PA, Tokunaga F (1996) Molecular cloning and characterization of the putative ultraviolet-sensitive visual pigment of goldfish. Vision Res 36: 933–939

Horstman DA, Brandon S, Wilson AL, Guyer CA, Cragoe EJ Jr, Limbird LE (1990) An aspartate conserved among G-protein receptors confers allosteric regulation of alpha 2-adrenergic receptors by sodium. J Biol Chem 265: 21590–21595

Hu J, Barbour LJ, Gokel GW (2002) Probing alkali metal-pi interactions with the side chain residue of tryptophan. Proc Natl Acad Sci USA 99: 5121–5126

Im DS, Heise CE, Nguyen T, O'Dowd BF, Lynch KR (2001) Identification of a molecular target of psychosine and its role in globoid cell formation. J Cell Biol 153: 429–434

Iwamoto M, Hasegawa C, Sudo Y, Shimono K, Araiso T, Kamo N (2004) Proton release and uptake of pharaonis phoborhodopsin (sensory rhodopsin II) reconstituted into phospholipids. Biochemistry 43: 3195–3203

Jagerschmidt A, Guillaume N, Goudreau N, Maigret B, Roques BP (1995) Mutation of Asp100 in the second transmembrane domain of the cholecystokinin B receptor increases antagonist binding and reduces signal transduction. Mol Pharmacol 48: 783–789

Javadpour MM, Eilers M, Groesbeek M, Smith SO (1999) Helix packing in polytopic membrane proteins: role of glycine in transmembrane helix association. Biophys J 77: 1609–1618

Ji I, Ji TH (1991) Asp383 in the second transmembrane domain of the lutropin receptor is important for high affinity hormone binding and cAMP production. J Biol Chem 266: 14953–14957

Jiang Y, Ruta V, Chen J, Lee A, MacKinnon R (2003) The principle of gating charge movement in a voltage-dependent K^+ channel. Nature 423: 42–48

Jin S, Cornwall MC, Oprian DD (2003) Opsin activation as a cause of congenital night blindness. Nat Neurosci 6: 731–735

Johansson JS, Gibney BR, Rabanal F, Reddy KS, Dutton PL (1998) A designed cavity in the hydrophobic core of a four-alpha-helix bundle improves volatile anesthetic binding affinity. Biochemistry 37: 1421–1429

Jones PG, Nawoschik SP, Sreekumar K, Uveges AJ, Tseng E, Zhang L, et al. (2007) Tissue distribution and functional analyses of the constitutively active orphan G protein coupled receptors, GPR26 and GPR78. Biochim Biophys Acta 1770: 890–901

Kellenberger S, Gautschi I, Schild L (1999) A single point mutation in the pore region of the epithelial Na^+ channel changes ion selectivity by modifying molecular sieving. Proc Natl Acad Sci USA 96: 4170–4175

Kellenberger S, Scheuer T, Catterall WA (1996) Movement of the Na^+ channel inactivation gate during inactivation. J Biol Chem 271: 30971–30979

Kim JM, Altenbach C, Kono M, Oprian DD, Hubbell WL, Khorana HG (2004) Structural origins of constitutive activation in rhodopsin: role of the K296/E113 salt bridge. Proc Natl Acad Sci USA 101: 12508–12513

Kinjo TG, Kang K, Szerencsei RT, Winkfein RJ, Schnetkamp PP (2005) Site-directed disulfide mapping of residues contributing to the Ca^{2+} and K^+ binding pocket of the NCKX2 Na^+/Ca^{2+}-K^+ exchanger. Biochemistry 44: 7787–7795

Kong H, Raynor K, Yasuda K, Bell GI, Reisine T (1993) Mutation of an aspartate at residue 89 in somatostatin receptor subtype 2 prevents Na+ regulation of agonist binding but does not alter receptor-G protein association. Mol Pharmacol 44: 380–384

Kono M (2006) Constitutive activity of a UV cone opsin. FEBS Lett 580: 229–232

Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157: 105–132

Lagane B, Ballet S, Planchenault T, Balabanian K, Le Poul E, Blanpain C, et al. (2005) Mutation of the DRY motif reveals different structural requirements for the CC chemokine receptor 5-mediated signaling and receptor endocytosis. Mol Pharmacol 67: 1966–1976

Langlois G, Chen CK, Palczewski K, Hurley JB, Vuong TM (1996) Responses of the phototransduction cascade to dim light. Proc Natl Acad Sci USA 93: 4677–4682

Levitt M (1978) Conformational preferences of amino acids in globular proteins. Biochemistry 17: 4277–4285

Ludwig MG, Vanek M, Guerini D, Gasser JA, Jones CE, Junker U, et al. (2003) Proton-sensing G-protein-coupled receptors. Nature 425: 93–98

Madabushi S, Gross AK, Philippi A, Meng EC, Wensel TG, Lichtarge O (2004) Evolutionary trace of G protein-coupled receptors reveals clusters of residues that determine global and class-specific functions. J Biol Chem 279: 8126–8132

Malmberg NJ, Van Buskirk DR, Falke JJ (2003) Membrane-docking loops of the cPLA2 C2 domain: detailed structural analysis of the protein-membrane interface via site-directed spin-labeling. Biochemistry 42: 13227–13240

Martin S, Botto JM, Vincent JP, Mazella J (1999) Pivotal role of an aspartate residue in sodium sensitivity and coupling to G proteins of neurotensin receptors. Mol Pharmacol 55: 210–215

Mazella J, Vincent JP (2006) Functional roles of the NTS2 and NTS3 receptors. Peptides 27: 2469–2475

Miura S, Zhang J, Boros J, Karnik SS (2003) TM2–TM7 interaction in coupling movement of transmembrane helices to activation of the angiotensin II type-1 receptor. J Biol Chem 278: 3720–3725

Monk PN, Pease JE, Marland G, Barker MD (1994) Mutation of aspartate 82 of the human C5a receptor abolishes the secretory response to human C5a in transfected rat basophilic leukemia cells. Eur J Immunol 24: 2922–2925

Musse AA, Wang J, Deleon GP, Prentice GA, London E, Merrill AR (2006) Scanning the membrane-bound conformation of helix 1 in the colicin E1 channel domain by site-directed fluorescence labeling. J Biol Chem 281: 885–895

Nagata T, Terakita A, Kandori H, Shichida Y, Maeda A (1998) The hydrogen-bonding network of water molecules and the peptide backbone in the region connecting Asp83, Gly120, and Glu113 in bovine rhodopsin. Biochemistry 37: 17216–17222

Nalefski EA, Wisner MA, Chen JZ, Sprang SR, Fukuda M, Mikoshiba K, et al. (2001) C2 domains from different Ca^{2+} signaling pathways

display functional and mechanistic diversity. Biochemistry 40: 3089–3100

Nathans J (1990a) Determinants of visual pigment absorbance: identification of the retinylidene Schiff's base counterion in bovine rhodopsin. Biochemistry 29: 9746–9752

Nathans J (1990b) Determinants of visual pigment absorbance: role of charged amino acids in the putative transmembrane segments. Biochemistry 29: 937–942

Neve KA, Cox BA, Henningsen RA, Spanoyannis A, Neve RL (1991) Pivotal role for aspartate-80 in the regulation of dopamine D2 receptor affinity for drugs and inhibition of adenylyl cyclase. Mol Pharmacol 39: 733–739

Neve KA, Cumbay MG, Thompson KR, Yang R, Buck DC, Watts VJ, et al. (2001) Modeling and mutational analysis of a putative sodium-binding pocket on the dopamine D2 receptor. Mol Pharmacol 60: 373–381

Nibbs RJ, Wylie SM, Yang J, Landau NR, Graham GJ (1997) Cloning and characterization of a novel promiscuous human beta-chemokine receptor D6. J Biol Chem 272: 32078–32083

Nie J, Lewis DL (2001) Structural domains of the CB1 cannabinoid receptor that contribute to constitutive activity and G-protein sequestration. J Neurosci 21: 8758–8764

Nikiforovich GV, Zhang M, Yang Q, Jagadeesh G, Chen HC, Hunyady L, et al. (2006) Interactions between conserved residues in transmembrane helices 2 and 7 during angiotensin AT1 receptor activation. Chem Biol Drug Des 68: 239–249

Nyholm TK, Ozdirekcan S, Killian JA (2007) How protein transmembrane segments sense the lipid environment. Biochemistry 46: 1457–1465

Oblatt-Montal M, Buhler LK, Iwamoto T, Tomich JM, Montal M (1993) Synthetic peptides and four-helix bundle proteins as model systems for the pore-forming structure of channel proteins. I. Transmembrane segment M2 of the nicotinic cholinergic receptor channel is a key pore-lining structure. J Biol Chem 268: 14601–14607

Okano T, Kojima D, Fukada Y, Shichida Y, Yoshizawa T (1992) Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc Natl Acad Sci USA 89: 5932–5936

Pace CN, Horn G, Hebert EJ, Bechert J, Shaw K, Urbanikova L, et al. (2001) Tyrosine hydrogen bonds make a large contribution to protein stability. J Mol Biol 312: 393–404

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, et al. (2000) Crystal structure of rhodopsin: A G protein-coupled receptor. Science 289: 739–745

Parent JL, Le Gouill C, Rola-Pleszczynski M, Stankova J (1996) Mutation of an aspartate at position 63 in the human platelet-activating factor receptor augments binding affinity but abolishes G-protein-coupling and inositol phosphate production. Biochem Biophys Res Commun 219: 968–975

Parker MS, Crowley WR, Parker SL (1996) Differences in cation sensitivity of ligand binding to Y_1 and Y_2 subtype of neuropeptide Y receptor of rat brain. Eur J Pharmacol 318: 193–200

Parker MS, Lundell I, Parker SL (2002) Pancreatic polypeptide receptors: affinity, sodium sensitivity and stability of agonist binding. Peptides 23: 291–302

Parker SL, Parker MS, Sah R, Sallee FR, Balasubramaniam A (2007a) Parallel inactivation of Y2 receptor and G-proteins in CHO cells by pertussis toxin. Regul Pept 139: 128–135

Parker SL, Parker MS, Sallee FR, Balasubramaniam A (2007b) Oligomerization of neuropeptide Y (NPY) Y2 receptors in CHO cells depends on functional pertussis toxin-sensitive G-proteins. Regul Pept (in press)

Partridge JC, Shand J, Archer SN, Lythgoe JN, van Groningen-Luyben WA (1989) Interspecific variation in the visual pigments of deep-sea fishes. J Comp Physiol [A] 164: 513–529

Paulsen SJ, Rosenkilde MM, Eugen-Olsen J, Kledal TN (2005) Epstein-Barr virus-encoded BILF1 is a constitutively active G protein-coupled receptor. J Virol 79: 536–546

Pearson WR (2000) Flexible sequence similarity searching with the FASTA3 program package. Methods Mol Biol 132: 185–219

Peralvarez-Marin A, Lorenz-Fonfria VA, Bourdelande JL, Querol E, Kandori H, Padros E (2007) Inter-helical hydrogen bonds are essential elements for intra-protein signal transduction: the role of Asp115 in bacteriorhodopsin transport function. J Mol Biol 368: 666–676

Perlman JH, Colson AO, Wang W, Bence K, Osman R, Gershengorn MC (1997) Interactions between conserved residues in transmembrane helices 1, 2, and 7 of the thyrotropin-releasing hormone receptor. J Biol Chem 272: 11937–11942

Quintana J, Wang H, Ascoli M (1993) The regulation of the binding affinity of the luteinizing hormone/choriogonadotropin receptor by sodium ions is mediated by a highly conserved aspartate located in the second transmembrane domain of G protein-coupled receptors. Mol Endocrinol 7: 767–775

Rao VR, Cohen GB, Oprian DD (1994) Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness. Nature 367: 639–642

Rasmussen SG, Jensen AD, Liapakis G, Ghanouni P, Javitch JA, Gether U (1999) Mutation of a highly conserved aspartic acid in the beta2 adrenergic receptor: constitutive activation, structural instability, and conformational rearrangement of transmembrane segment 6. Mol Pharmacol 56: 175–184

Raynor K, O'Carroll AM, Kong H, Yasuda K, Mahan LC, Bell GI, et al. (1993) Characterization of cloned somatostatin receptors SSTR4 and SSTR5. Mol Pharmacol 44: 385–392

Ren L, Martin CH, Wise KJ, Gillespie NB, Luecke H, Lanyi JK, et al. (2001) Molecular mechanism of spectral tuning in sensory rhodopsin II. Biochemistry 40: 13906–13914

Roche JP, Bounds S, Brown S, Mackie K (1999) A mutation in the second transmembrane region of the CB1 receptor selectively disrupts G protein signaling and prevents receptor internalization. Mol Pharmacol 56: 611–618

Rompler H, Yu HT, Arnold A, Orth A, Schoneberg T (2006) Functional consequences of naturally occurring DRY motif variants in the mammalian chemoattractant receptor GPR33. Genomics 87: 724–732

Rose PM, Krystek SR Jr, Patel PS, Liu EC, Lynch JS, Lach DA, et al. (1995) Aspartate mutation distinguishes ETA but not ETB receptor subtype-selective ligand binding while abolishing phospholipase C activation in both receptors. FEBS Lett 361: 243–249

Roseman MA (1988) Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. J Mol Biol 200: 513–522

Rosenkilde MM, Kledal TN, Schwartz TW (2005) High constitutive activity of a virus-encoded seven transmembrane receptor in the absence of the conserved DRY motif (Asp-Arg-Tyr) in transmembrane helix 3. Mol Pharmacol 68: 11–19

Rosenkilde MM, McLean KA, Holst PJ, Schwartz TW (2004) The CXC chemokine receptor encoded by herpesvirus saimiri, ECRF3, shows ligand-regulated signaling through Gi, Gq, and G12/13 proteins but constitutive signaling only through Gi and G12/13 proteins. J Biol Chem 279: 32524–32533

Sakmar TP, Franke RR, Khorana HG (1989) Glutamic acid-113 serves as the retinylidene Schiff base counterion in bovine rhodopsin. Proc Natl Acad Sci USA 86: 8309–8313

Sakmar TP, Menon ST, Marin EP, Awad ES (2002) Rhodopsin: insights from recent structural studies. Annu Rev Biophys Biomol Struct 31: 443–484

Senes A, Ubarretxena-Belandia I, Engelman DM (2001) The Calpha –H. . .O hydrogen bond: a determinant of stability and specificity in transmembrane helix interactions. Proc Natl Acad Sci USA 98: 9056–9061

Starace DM, Knox BE (1997) Activation of transducin by a Xenopus short wavelength visual pigment. J Biol Chem 272: 1095–1100

Steiner T, Koellner G (2001) Hydrogen bonds with pi-acceptors in proteins: frequencies and role in stabilizing local 3D structures. J Mol Biol 305: 535–557

Stryer L (1986) Cyclic GMP cascade of vision. Annu Rev Neurosci 9: 87–119

Sutton RB, Sprang SR (1998) Structure of the protein kinase Cbeta phospholipid-binding C2 domain complexed with Ca^{2+}. Structure 6: 1395–1405

Tao Q, Abood ME (1998) Mutation of a highly conserved aspartate residue in the second transmembrane domain of the cannabinoid receptors, CB1 and CB2, disrupts G-protein coupling. J Pharmacol Exp Ther 285: 651–658

Teller DC, Stenkamp RE, Palczewski K (2003) Evolutionary analysis of rhodopsin and cone pigments: connecting the three-dimensional structure with spectral tuning and signal transfer. FEBS Lett 555: 151–159

Thompson GA, Leyland ML, Ashmole I, Sutcliffe MJ, Stanfield PR (2000) Residues beyond the selectivity filter of the K+ channel kir2.1 regulate permeation and block by external Rb+ and Cs+. J Physiol 526 Pt 2: 231–240

Vita N, Oury-Donat F, Chalon P, Guillemot M, Kaghad M, Bachy A, et al. (1998) Neurotensin is an antagonist of the human neurotensin NT2 receptor expressed in Chinese hamster ovary cells. Eur J Pharmacol 360: 265–272

Vlassi M, Cesareni G, Kokkinidis M (1999) A correlation between the loss of hydrophobic core packing interactions and protein stability. J Mol Biol 285: 817–827

Vogel WK, Peterson GL, Broderick DJ, Mosser VA, Schimerlik MI (1999) Double mutant cycle analysis of aspartate 69, 97, and 103 to asparagine mutants in the m2 muscarinic acetylcholine receptor. Arch Biochem Biophys 361: 283–294

Vought BW, Dukkipatti A, Max M, Knox BE, Birge RR (1999) Photochemistry of the primary event in short-wavelength visual opsins at low temperature. Biochemistry 38: 11287–11297

Wang CD, Buck MA, Fraser CM (1991) Site-directed mutagenesis of alpha 2A-adrenergic receptors: identification of amino acids involved in ligand binding and receptor activation by agonists. Mol Pharmacol 40: 168–179

Ward RJ, Milligan G (1999) An Asp79Asn mutation of the alpha2A-adrenoceptor interferes equally with agonist activation of individual Gialpha-family G protein subtypes. FEBS Lett 462: 459–463

Webster SM, Del Camino D, Dekker JP, Yellen G (2004) Intracellular gate opening in Shaker K+ channels defined by high-affinity metal bridges. Nature 428: 864–868

Wilbanks AM, Laporte SA, Bohn LM, Barak LS, Caron MG (2002) Apparent loss-of-function mutant GPCRs revealed as constitutively desensitized receptors. Biochemistry 41: 11981–11989

Williams AJ, Michel AD, Feniuk W, Humphrey PP (1997) Somatostatin5 receptor-mediated [35S]guanosine-5′-O-(3-thio)triphosphate binding: agonist potencies and the influence of sodium chloride on intrinsic activity. Mol Pharmacol 51: 1060–1069

Wilson MH, Highfield HA, Limbird LE (2001) The role of a conserved inter-transmembrane domain interface in regulating alpha(2a)-adrenergic receptor conformational stability and cell-surface turnover. Mol Pharmacol 59: 929–938

Xiong Z, Sperelakis N (1995) Regulation of L-type calcium channels of vascular smooth muscle cells. J Mol Cell Cardiol 27: 75–91

Zhang J, Shen CP, Xiao JC, Lanza TJ, Lin LS, Francis BE, et al. (2006) Effects of mutations at conserved TM II residues on ligand binding and activation of mouse 5-HT6 receptor. Eur J Pharmacol 534: 77–82

Zhang L, Rozek A, Hancock RE (2001) Interaction of cationic antimicrobial peptides with model membranes. J Biol Chem 276: 35714–35722

Zhou W, Flanagan C, Ballesteros JA, Konvicka K, Davidson JS, Weinstein H, et al. (1994) A reciprocal mutation supports helix 2 and helix 7 proximity in the gonadotropin-releasing hormone receptor. Mol Pharmacol 45: 165–170

Zvyaga TA, Fahmy K, Siebert F, Sakmar TP (1996) Characterization of the mutant visual pigment responsible for congenital night blindness: a biochemical and Fourier-transform infrared spectroscopy study. Biochemistry 35: 7536–7545

Authors' address: Steven L. Parker, Ph.D., Department of Pharmacology, University of Tennessee Health Science Center, Memphis, TN 38163, U.S.A.,
Fax: +1-901-448-7206, E-mail: slparker@utmem.edu