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Activation mechanisms of G proteins by seven-transmembrane receptors : A view from rhodopsin studies

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Signal transduction cascades consisting of seven-transmembrane receptors, heterotrimeric G proteins and effector enzymes are ubiquitous in intracellular signalling. The visual transduction cascade in rod photoreceptor cells is the one most extensively investigated, where the G protein-coupled receptor rhodopsin receives a light signal and transfers the signal to the retinal G protein transducin. Light absorption causes conformational changes in the protein moiety of rhodopsin through *cis-trans* isomerization of the 11-*cis*-retinal chromophore and finally leads to the formation of the active state. This article reviews recent spectroscopic and biochemical studies that enable us to elucidate the mechanism for activation of rhodopsin and that for the interactions between photoactivated rhodopsin and G protein (transducin). It also summarizes the features of receptor-G protein interaction surfaces on three types of G protein (Gi/o, Gs and Gq/11). The interaction surfaces are widely diversified but the mechanisms of interaction have several points similar to those between rhodopsin and transducin. Thus, the rhodopsin-transducin system is one of the model systems to provide understanding of other GPCR-G protein systems.

One of the motifs of signal transduction systems widely present in organisms is the [seven-transmembrane receptor → heterotrimeric G protein → effector enzyme (or ion channel)]. So far, more than 1000 receptors have been identified from various transduction systems, and they transfer the signals from the outer environments, such as hormones, neurotransmitters, odorants, and even photons by binding with single or multiple subtypes of G proteins[1]. The numbers of G protein subtypes are limited to about 20, and the subtype specificity of the G proteins to activate effector enzymes or channels is relatively strict. These facts indicate that a cascade system elicited by a stimulus is dependent on what subtype of G protein is activated by its cognate receptor[2]. Thus, the elucidation of the activation mechanisms of receptors by various stimuli and that of the mechanism of receptor-G protein coupling are important issues in the field of signal transduction.

Rhodopsin[#] present in retinal rod photoreceptor cells is a prototypical G protein-coupled receptor (GPCR) that receives a light signal from the outer environment. In contrast to a large variety of receptors that are activated by diffusible agonist ligands, rhodopsin has the 11-*cis*-retinal chromophore in its protein moiety as an 'inverse agonist', and light absorption causes a generation of the agonist all-*trans*-retinal through *cis-trans* photoisomerization of the chromophore. Advantages of the studies on rhodopsin in comparison with those on other GPCRs are that rhodopsin can be synchronously activated by light even at a freezing temperature. The chromophore acts as an intrinsic visible spectroscopic probe to monitor changes in global protein structure, so that each intermediate state characterized by the absorption spectrum is subjected to the analysis of detailed structural changes by various spectroscopies with excellent time and spatial resolution. Furthermore, the 'inverse agonist' nature of 11-*cis*-retinal keeps rhodopsin virtually free from spontaneous activation of G protein. This enables us to induce the process of rhodopsin to activate the G protein strictly in a light-dependent manner. Thus the rhodopsin-G protein system is one of the most suitable systems whose activation and coupling mechanisms can be elucidated at submolecular or atomic resolution. In combination with the atomic structure of various G proteins[3–7], recent success in the determination of the three-dimensional structure of rhodopsin[8] further supports the situation of the rhodopsin system as a model system among various GPCR systems.

In this article, we review studies on the mechanism of rhodopsin-G protein interaction after briefly summarizing the photochemical reactions of rhodopsin. In addition, common and different mechanisms of GPCR-G protein interactions are discussed on the basis of comparisons of the rhodopsin system with other GPCR systems.

[#]The term 'rhodopsin' represents vertebrate rhodopsin that couples with one of the G protein subtypes transducin (Gt) in rod photoreceptor cells In invertebrate photoreceptor cells, there are at least two types of different rhodopsins that couple with Gq and Go, respectively[12]

Structural motif of rhodopsin and its changes in the intermediate states

Rhodopsin is a ~40-kDa membrane protein which consists of a single polypeptide opsin and a chromophore, 11-*cis*-retinal. The primary structure (amino acid sequence) of bovine rhodopsin was first determined in 1983 by three groups based on sequencing the peptide fragments and complementary DNA (cDNA) cloning[9]. Hydrophathy analysis of the primary structure then implied that the opsin contains seven transmembrane α-helices and loop regions, which are the structural motif typical of the GPCRs (Fig. 1a). Recently, a three-dimensional crystal of bovine rhodopsin was successfully developed[10], and its atomic structure was analyzed by X-ray crystallography[8] (Fig. 1b-d). This gives a comprehensive structural basis for insight into the functional roles of rhodopsin.

The primary role of rhodopsin is to receive a photon signal from the outer environment and to transfer the signal to the retinal G protein transducin (Gt); therefore, the amino acid residues responsible for the chromophore binding and activation of Gt are important. The crystal structure of rhodopsin has now confirmed that the 11-*cis*-retinal chromophore is bound to Lys296 via a Schiff base. The Schiff base has been identified to be protonated and the positive charge on it is stabilized by the negatively charged Glu113 (Ref. 11). The crystal structure of rhodopsin also confirmed that the amino acid residues responsible for the spectral tuning of rhodopsin, which had been identified from various techniques[12], are situated near the chromophore. The new finding from the crystal structure is that the second extracellular loop forms an antiparallel β-sheet and that one of the strands (β4) is in the proximity of the chromophore (Fig. 1a). This indicates that a part of the second extracellular loop forms a chromophore binding site. Furthermore, one of the oxygen atoms of Glu181 is located close (about 4.5 Å) to the C_{12} of retinal, which affects the absorption characteristics of the chromophore[13] (Fig. 1b and c).

On the absorption of light, rhodopsin is converted to an enzymatically active intermediate metarhodopsin II (meta II) through several thermolabile intermediates (Fig. 2). The initial event occurring in the light-absorbed rhodopsin is the *cis-trans* isomerization of the chromophore which starts at about 60 fs[14] and is completed within 200 fs in the excited state of rhodopsin[15,16]. Such an ultra-fast isomerization is the molecular basis of the high quantum yield of rhodopsin, because the high quantum yield (0.67) originates from the high-rate isomerization, which in turn competes with other relaxation processes in the excited state of rhodopsin.

Because *cis-trans* isomerization causes an extension of the longitudinal length of the chromophore, the chromophore should be in a highly twisted conformation in the restricted chromophore binding site. This causes an elevation of the potential energy (~30 kcal)[17] that induces conformational changes in the protein to its active state meta II. Several lines of evidence have suggested that changes in conformation of the protein moiety proceed in a stepwise manner. The first rearrangement of the amino acid residues occurs near the protonated Schiff base in the chromophore, because isomerization of the chromophore proceeds by movement of the half of the polyene chain containing the Schiff base[18]. To release a part of the strain of the chromophore, the β-ionone ring of the chromophore then changes its interaction with a nearby protein during the batho-to-lumi transition[19]. The subsequent changes occur near the 9-methyl group of the chromophore and its surrounding amino acid residue during the lumi-to-meta I transition[20]. Finally, the relaxation of the whole protein into a thermodynamically stable state occurs concurrently with the deprotonation of the Schiff base during the metal I-to-metal II transition.

Recently, important findings on the conformational changes of rhodopsin in relation to the activation mechanism of the G protein were obtained from the cross-linking studies using a retinal analog having a photo-affinity substituent in its β-ionone ring[21]. The results showed that the cross-linking group is attached to Trp265 in helix VI in unactivated rhodopsin, as the crystal structure presumes, and is still attached in the photoisomerized bathorhodopsin. However, the β-ionone ring becomes cross-linked to Ala169 in helix IV when bathorhodopsin converts to lumirhodopsin. Thus, during the batho-to-lumi transition, the β-ionone ring of the chromophore flips over in the helical bundle using the energy stored as a highly strained chromophore structure. The crystal structure indicates that the β-ionone ring is hardly accessible to Ala169 in the ground state of rhodopsin, because helix III is situated between the chromophore and Ala169. Moreover, Ala169 is located on the lipid face of the helix IV. Thus, the cross-linking experiments strongly suggest that substantial movement of helix III and/or the concerted motions of the helix VI and IV occur during the batho-to-lumi transition. In fact, previous approaches by use of site-directed spin labels and cross-linking methods have suggested that rhodopsin activation causes the cytoplasmic ends of helices III and VI to separate from one another[22,23]. Thus the cross-linking studies[21] provide a experimental evidence that elucidates how the light energy absorbed by the chromophore is utilized for conformational changes in the protein moiety of rhodopsin.

Interaction between photoactivated rhodopsin (Rh*) and transducin (Gt)

Sites involved in the activation of Gt by Rh :*

Biochemical studies in combination with the methods of site-directed mutagenesis, peptide competition and antibody competition have revealed the interaction sites of photoactivated rhodopsin (Rh*) and Gt (24-28; Fig. 1). The interaction sites of Rh* with Gt are at the second, third and fourth intracellular loops (IL2, 3 and 4), the cytoplasmic surface of helix III, VI and VII, and the cytoplasmic *C*-terminal tail. On the other hand, those of Gt with Rh* are the *C*-terminal (Ile340-Phe350) and the α4-β6-α5 (Asp311-Lys329) regions of the α-subunit and the *C*-terminal region of γ-subunit with its farnesylated substituent. The crystal structure of the G protein $\alpha\beta\gamma$ complex indicates that the putative binding sites in the α-subunit are located apart from the site in the γ-subunit[6] (Fig. 3). Thus it is reasonable that the interaction sites in Rh* are distributed in almost all of the cytoplasmic domain of Rh*. Although rhodopsin in the resting state should have a conformation different from that

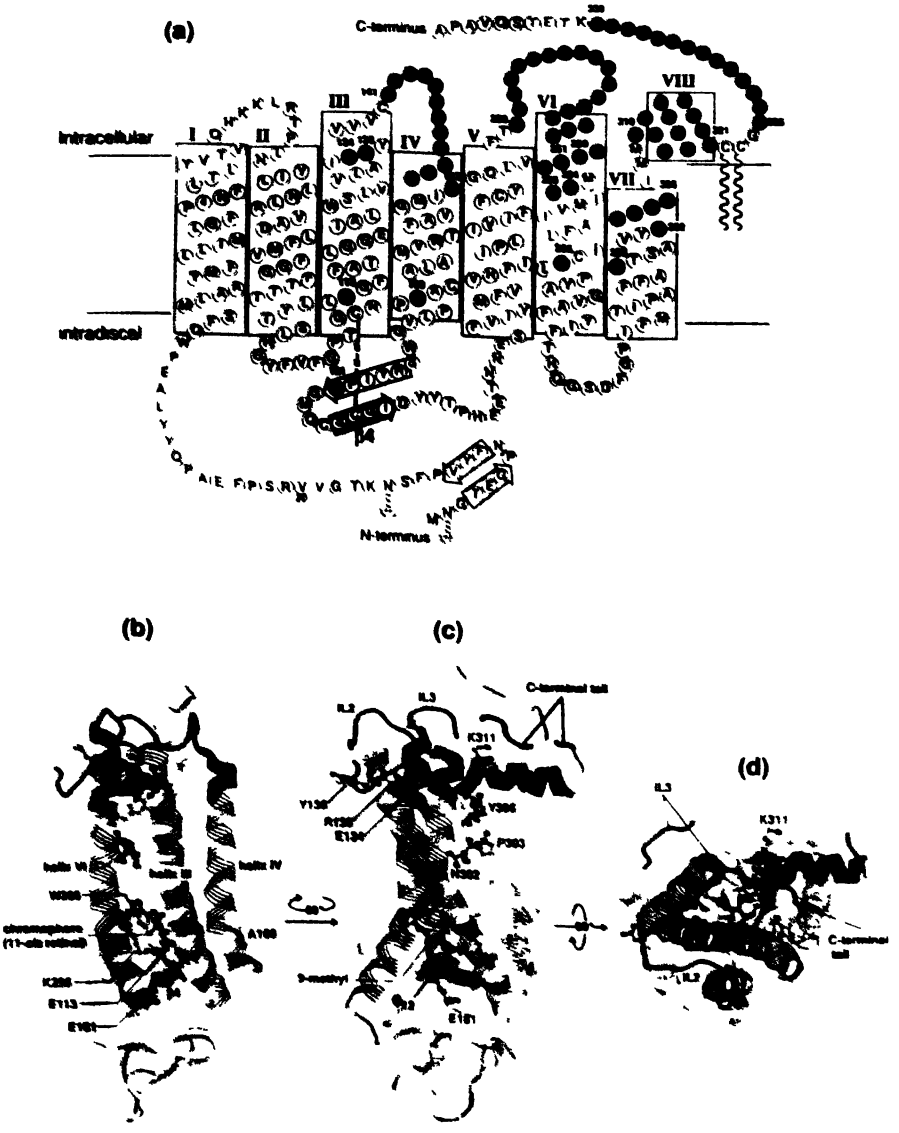

Fig. 1. Structure of bovine rhodopsin Secondary structure of rhodopsin is shown in (a) The residues constituting the chromophore binding site are expressed in white letters in black circles. The residues that form the interaction sites with transducin (Gt) are colored pink Important residues for coupling with transducin are shown in thick black letters in gray circles. The three-dimensional crystal structure of rhodopsin is shown in (b), (c) and (d). The coordinate was retrieved from the Protein Data Bank (Okada *et al*, 1F88) Interaction sites with transducin are colored pink

of the active state, the crystal structure in the resting state might offer some information on the molecular architecture of the active state. The important finding from the crystal structure of rhodopsin is that the putative IL4 forms an α-helix (termed helix VIII) whose *N*-terminal region is close to the *C*-terminal region of IL3 (Fig. 1b-d). Accumulated evidence has revealed that IL3 of rhodopsin is responsible for coupling with specific G protein subtypes[29] and that the coupling specificity of the G protein is determined by the *C*-terminus sequence of the α-subunit[30]. Furthermore, detailed experiments suggested that the *C*-terminus segment of Gtα interacts with the *C*-terminal segment of IL3 and the *N*-terminal of helix VIII[28,31,32].

In addition to the above broad features of the interaction sites between Rh* and Gt, there are may experimental results that suggest the activation mechanism of Gt by Rh*. Several specific residues in rhodopsin have been reported to be critical for activation of Gt. The Asp(Glu)/Arg/Tyr triad located at the helical extension of the helix III is highly conserved among most GPCRs (Fig. 1). Point mutation of Arg135 of rhodopsin involved in the traid results in the loss of ability to activate Gt, although the ability to interact with the synthetic peptide derived from the *C*-terminus of Gtα is preserved[33]. In contrast, substitution of Glu134 with Gln, Ile, Ser, Leu or Phe enhances the light-dependent activity. Furthermore, Glu134Gln, Glu134Ile, Glu134Ser mutants exhibit a partial activity for Gt even in the dark (constitutive activation)[33]. Because Glu134 and Arg135 form a 'charge pair', as evidenced by the crystal structure of rhodopsin, its disruption causes a conformational change in the cytoplasmic border to be a G protein activating form. Recent FTIR studies in combination with site-directed mutagenesis report that the Glu134 becomes protonated when rhodopsin converts to the activated state[34]. On the other hand, chimerical and point mutational studies indicated that the seven amino acid region in the helical extension of the helix III of rhodopsin (from Glu134 to Cys140) is not exchangeable with those of other receptors, and three amino acids (Glu134, Val138 and Cys140) in the region are crucial for efficient G protein activation[29]. Therefore, the seven amino acid region of rhodopsin is not the binding site of G protein but the region essential for formation of the active form of rhodopsin. Because the region contains the Asp(Glu)/Arg/Tyr triad which is highly conserved among GPCRs, it is unlikely that only rhodopsin exhibits an unique activation mechanism quite different from those of the other GPCRs.

Several lines of evidence have indicated that IL3 has two distinct roles in the activation of Gt; the one is recognizing the pertinent G protein and another is catalyzing GDP release from Gtα. Substitution of IL3 with that of other Go-coupled receptors belonging to the rhodopsin family results in the significant Go activation, indicating that IL3 of rhodopsin as well as the other Go-coupled receptors possibly have a putative site(s) related to the in-

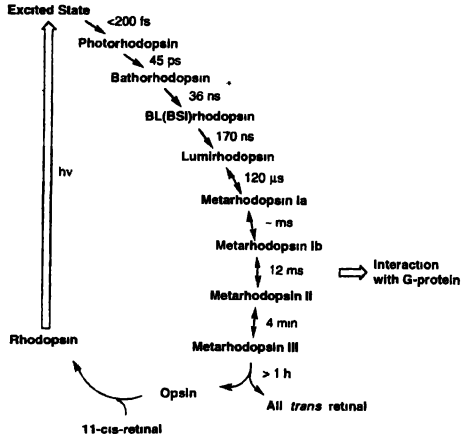

Fig. 2. Photobleaching process of rhodopsin Time constants of the transitions between intermediates observed at room temperature are shown on the right side of the arrows

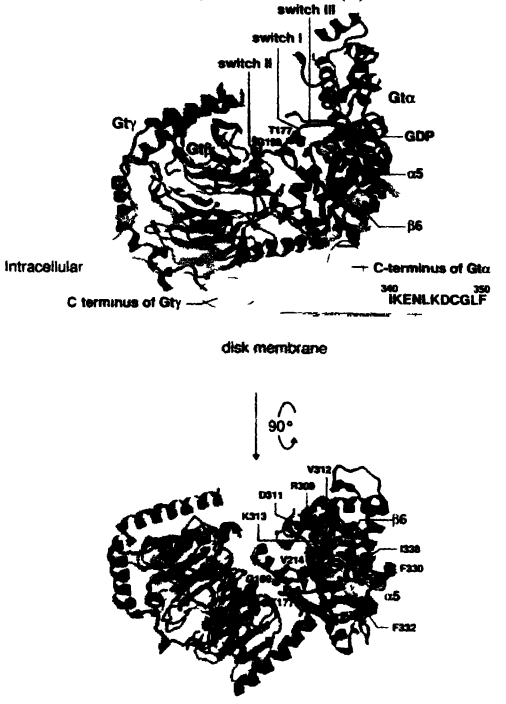

Fig. 3. Three-dimensional structure of transducin The coordinate was retrieved from the Protein Data Bank (Lambright *et al*, 1GOT) Both the *C*-terminus of α and γ subunits have not been determined The β6-α5 region in the α-subunit is colored green Switches I, II and III are colored blue, light blue and cyan, respectively

teraction of the G protein[29]. Four hydrophobic residues located in the same surface of helix VI of the muscarinic acetylcholine receptor (mAChR) participate in selective ineraction with the G protein[35]. Similar residues exist at the cytoplasmic boundary of the helix VI in rhodopsin (Val 250, Thr 251, Val 254 and Ile 255). Therefore, these are supposed to interact with the *C*-terminus region (Cys347-Phe350) of Gtα, which is responsible for selective interaction with Rh*. On the other hand, the rhodopsin mutant in which the middle part of the IL3 (Gln237-Glu249) is deleted binds to Gt normally but fails to enhance GDP release from Gtα[36], which shows that this region is responsible for promoting GDP release from Gtα.

A pentapeptide sequence (Asn/Pro/X/X/Tyr; a large hydrophobic residue, namely, Val, Leu, Ile or Phe, located at 'X') at the cytoplasmic border of the helix VII is highly conserved among GPCRs. This Asn/Pro/X/X/Tyr motif plays various important roles in GPCR-mediated signal transduction such as the high-affinity agonist binding of β2-adrenergic receptor (β2-AR)[37]. Cysteine substitution of Tyr306 or Ile307 next to Tyr306 in the motif of rhodopsin causes depression in the activation of Gt[38]. Substitution of Met257 in helix VI with other amino acids except for Asn and Leu brings about constitutive activity[39]. Met257 points toward the Asn/Pro/X/X/Tyr motif in the crystal structure and could form an interhelical domain responsible for Gt activation.

The *N*-terminal region of the cytoplasmic tail of Rh* (Lys325-Ser338) is a part of the binding site to Gt[27]. Another role of this region may be to cover other interaction sites in the resting state of rhodopsin.

The crystal structures of Gtα indicate that the regions of Ser173-Thr183, Phe195-Thr215 and Asp227-Arg238 in the GDP-bound form exhibit a conformation different from those in the GTPγS-bound form[3,6] (Fig. 3). These regions are termed as switches I, II and III, respectively. Thr177 in switch I and Gly199 in switch II are well conserved among the α-subunits of the heterotrimeric G proteins, Ras and EF-Tu[3]. The difference in the structure of switch I between the two forms is due to the formation of new hydrogen bonds between the guanine nucleotide of GTPγS and Thr177, while the difference in switch II is the formation of the hydrogen bond between the guanine nucleotide of GTPγS and the peptide amide of Gly199 (Refs. 3, 6). Switch III is unique to heterotrimeric G proteins, and the conformational change in this region seems to occur as a response secondary to that in switch II. All of the critical residues for the conformational changes in the switch regions are conserved among α-subunits of other heterotrimeric G proteins, suggesting the presence of a common activation mechanism through the switch regions.

Structural changes in the interaction between Rh and Gt* :

As already described, rhodopsin converts to a state that activates Gt through several intermediate states (Fig. 2). Thus to elucidate the detailed activation mechanism of Gt by rhodopsin, it is important to identify which intermediate(s) interacts with Gt. The first implication that metal II could be an active state of rhodopsin came from the biochemical evidence that phosphodiesterase, the effector enzyme of the phototransduction cascade in rod photoreceptor cells, was activated even at low temperature where meta II did not convert to the subsequent intermediate meta III[40,41]. Spectroscopic measurements then indicated that a large amount of meta II was accumulated in the presence of Gt and that the accumulation is abolished by GTP[42]. The exchange reaction results in the release of Gt from photoactivated rhodopsin[43]. Thus, the spectroscopic experiments clearly showed that metal II forms a complex with Gt and catalyzes the GDP-GTP exchange reaction on Gt. Recently, we showed that an intermediate state other than meta II also interacts with Gt[44,45]. This intermediate is a precursor of meta II and we termed it meta Ib because it exhibits an absorption maximum similar to but slightly blue-shifted from that of the previously identified meta I. The interesting observation is that metal Ib can bind to Gt but induces no GDP-GTP exchange reaction, whereas the exchange reaction occurs at the metal II state. That is, Gt can form a complex with meta Ib, and the subsequent change in conformation of the complex reaches the state (meta II-Gt complex) that induces the exchange reaction (Fig. 4).

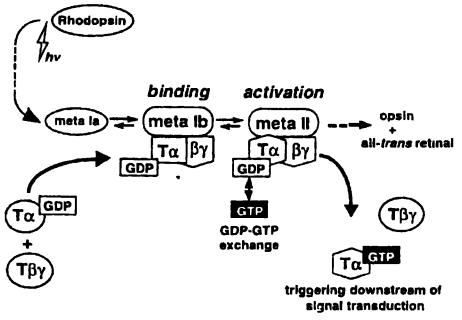

Fig. 4. Schematic drawing of the interaction process of photoactivated rhodopsin and transducin.

FTIR spectroscopy is one of the powerful tools to detect many structural features of proteins such as specific protein bands (amide I, II and III), the states of carboxylic acid residues and changes in hydrogen bonds. Thus we have tried to elucidate the detailed structure of the Rh*-Gt complex[46] by means of FTIR spectroscopy. The difference FTIR spectrum

of [Rh* *plus* Gt → Rh*-Gt complex] is shown in Fig. 5a, in which many bands in the 1800–1200 cm^{-1} region appear upon formation of the complex. The bands in the regions 1800–1730, 1720–1700 and 1700–1200 cm^{-1} reflect the changes in protonated carboxylic acids, asparagine and/or glutamine residues or protonated carboxylic acids, and peptide amides and carbonyls, respectively. These changes should arise from the interaction sites in both Rh* and Gt and/or the nucleotide binding site in Gtα. To elucidate more localized structural changes upon complex formation, we

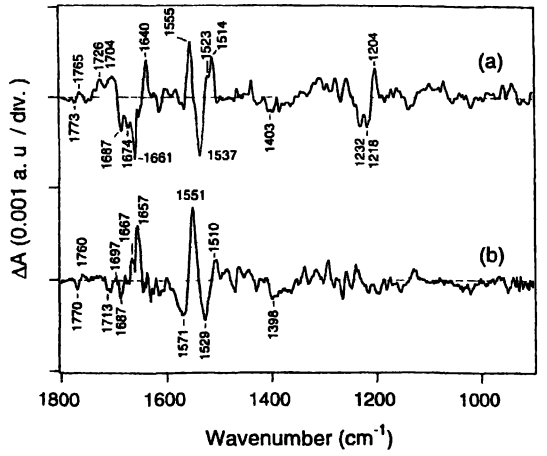

Fig. 5. Difference FTIR spectra for the conversion from Rh* to the complex The [Rh* plus Gt → Rh*-Gt complex] spectrum and the [Rh* plus the peptide → Rh*-peptide complex] spectrum are shown in (a) and (b), respectively, which are reproduced from Refs 46 and 47

have investigated structural changes upon complex formation between Rh* and a synthetic peptide derived from the *C*-terminus of Gtα (Ile340-Phe350)[47] (Fig. 5b). Comparison of these two spectra clearly shows that the change in the bands at 1770(–)/1760(+) cm^{-1} observed in the Rh*-Gt complex originates from the perturbation of the protonated carboxylic acid present in the interaction surface of Rh* and *C*-terminus of Gtα. The candidate is either Glu342 or Asp346 in the *C*-terminus of Gtα, or Glu249 in the *C*-terminal region of IL3 of Rh* (Figs. 1 and 3). Positive bands at 1726 and 1704 cm^{-1} in the Rh*-Gt complex are probably due to the protonation of the carboxylate, because the band at 1403(–) cm^{-1} due to the carboxylate is seen in the negative side of the spectrum. These bands are not seen in the Rh*-peptide complex, so that they originate from the carboxylate present in the region other than the *C*-terminus of the Gtα. Among the frequency shifts of amide I bands, only the 1687(–) cm^{-1} band remains in the spectrum for Rh*-peptide complex. Bands at 1232, 1218 and 1204 cm^{-1} (Fig. 5a) due to amide III are not observed in the

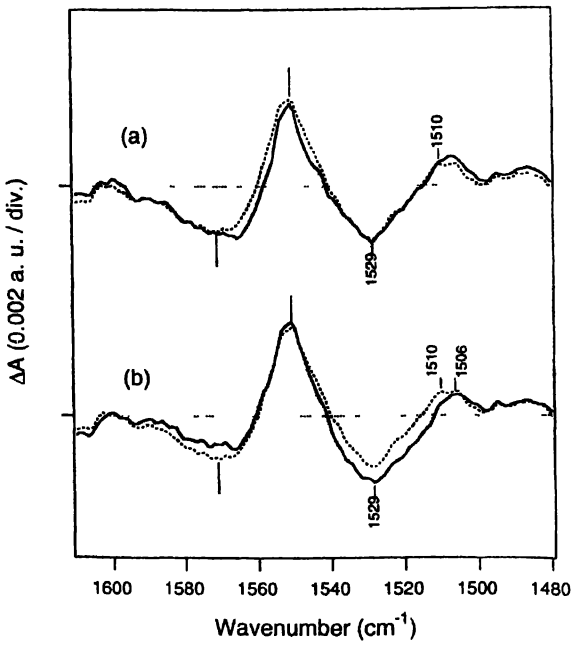

Fig. 6. Difference FTIR spectra for the formation of the complex between Rh* and the L344-^{15}N labeled (solid line in a) and that between Rh* and the L349-^{15}N labeled (solid line in b) peptides The [Rh* plus the peptide → Rh*-peptide complex] spectrum for the parent peptide (dotted lines) is overlaid which is reproduced from Ref 47

spectrum for Rh*-peptide complex (Fig. 5b).

Furthermore, amide II bands at 1555(+), 1537(–),

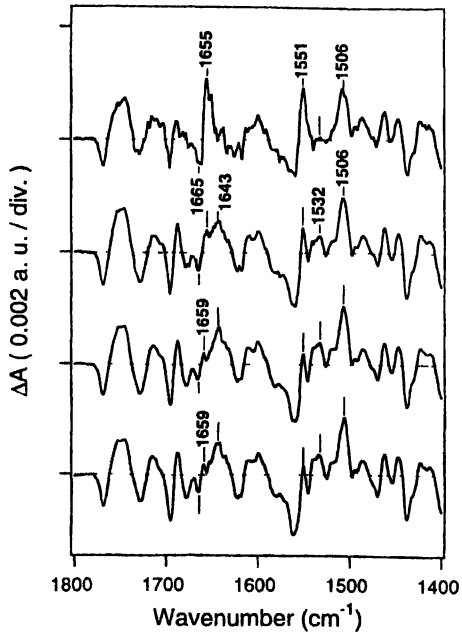

Fig. 7. The [rhodopsin plus peptide → complex] spectra just after the illumination (a), at 120 s (b), 320 s (c) and 1360 s (d) after the illumination, which are reproduced from Ref 47

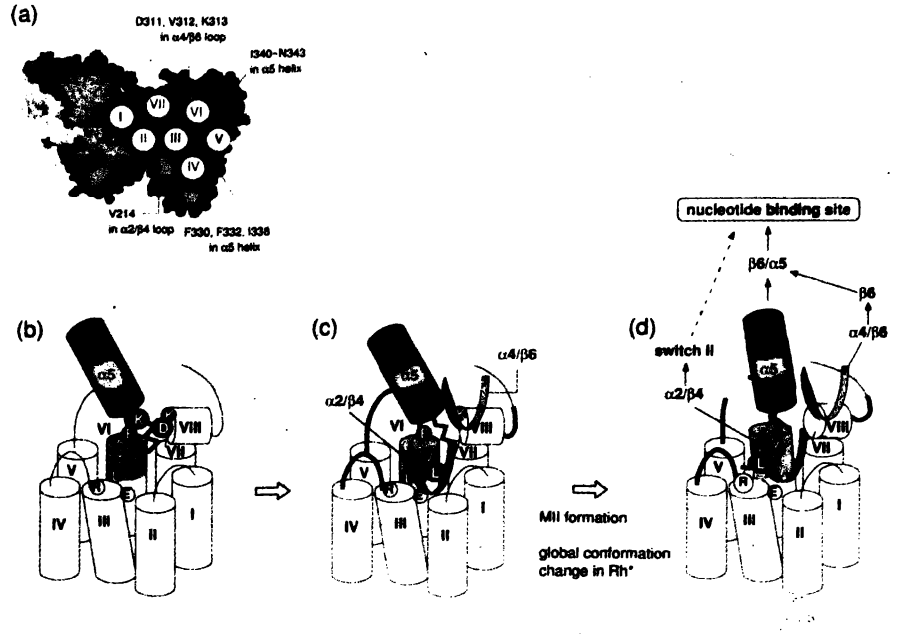

Fig. 8. Proposed mechanism of the interaction between Rh* and Gt. A three-dimensional structure of Gt viewed from the ROS membrane in which α-helical arrangements of rhodopsin are projected (a). The Gt α, β and γ subunits are colored blue, green and yellow, respectively. The *C*-terminus of Gtα and the preceding α5 helix are colored red and cyan, respectively. The β6 sheet in Gtα is orange. (b)-(d) illustrate the first, the second and the third step in the process of Rh*-Gt interaction.

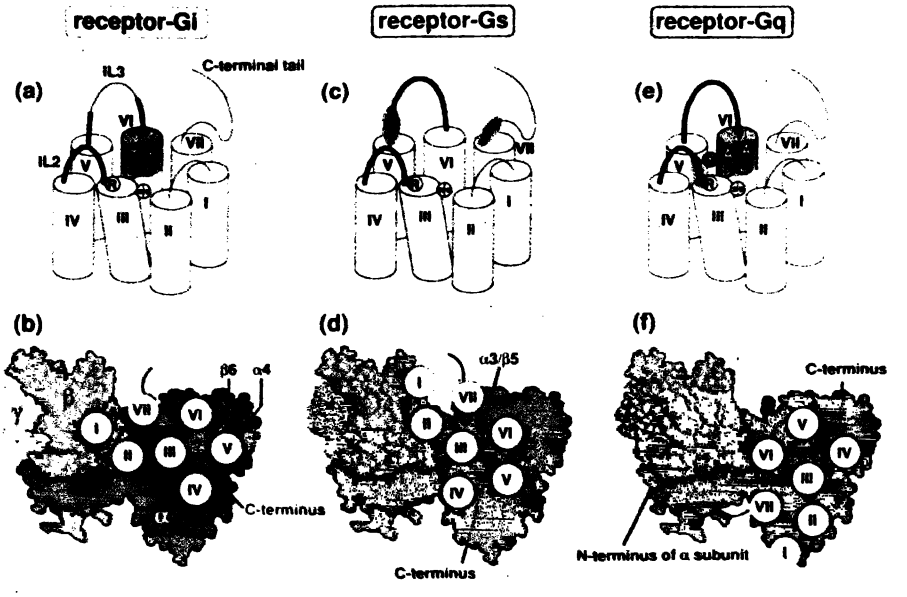

Fig. 9. Models of the interaction surface between Gi/o, Gs, Gq/11 and their cognate GPCRs. In (a), (c) and (e), the binding sites of the G proteins in GPCRs are illustrated. The intracellular loops and residues essential for the interaction with the G protein are shown by thick black lines and white letters, respectively. Regions that determine the receptor-G protein selectivity are shadowed. Residues shown in black letters are in the highly conserved Asp(Glu)/Arg/Tyr motif. Proposed models of the receptor-Gi, Gs and Gq complexes are shown in (b), (d) and (f), respectively. In these models, we take the structure of Gi1α(G203A)β1γ2 with bound GDP as a representative structure of the G protein $\alpha\beta\gamma$ complex, because all the G proteins share a common motif. The α, β and γ subunits are shown in blue. green and yellow, respectively. The *C*-terminus of the α-subunit is colored red. Other regions critical for receptor-mediated activation are colored orange and magenta.

1523(+) and 1514(+) cm^{-1} in the spectrum for the Rh*-Gt complex are replaced by 1551(+), 1529(-) and 1510(+) cm^{-1} bands with slight downshifts in the spectrum of the Rh*-peptide complex. All these results indicate the dynamic changes in the peptide backbone of Rh* and Gt (peptide) upon formation of the complex. To visualize the structural changes, further investigations using site-directed mutagenesis and/or isotope-labelling of the specific residues are necessary. In this context, our recent investigations clearly show that the amide II band at 1510 cm^{-1} originates from the peptide amide of Leu349 (Fig. 6). That is, incorporation of the ^{15}N label in the peptide amide of Leu349 causes a clear isotope-shift (about 4 cm^{-1}) of the 1510 cm^{-1} band, while the lower frequency side of the negative 1529 cm^{-1} band loses some negative absorbance. Thus the amide II vibration of the Leu349 undergoes a frequency change from the 1530–1515 cm^{-1} region to 1510 cm^{-1} upon complex formation. Because the identified frequencies are extremely low compared with those of the normal amide II, Leu349 should be in an extremely apolar and restricted environment of the peptide binding site. Theoretical considerations by Bandekar and Krimm[48] predicted that the third peptide amide among four in a type II' β-turn exhibits an amide II band around 1520 cm^{-1}. Distortion around Leu349 upon complex formation may result in more pronounced perturbation of its peptide bond, implying interaction in an apolar environment. This also holds for the structure in the complex deduced by NMR spectroscopy, in which the N-H of Leu349 points toward the region surrounded by the hydrophobic side-chains[49].

During the investigations of the complex formation with the peptide, we found that the time constant of formation of meta II ($\tau_{1/e}$ ~330 s) monitored by visible absorption spectroscopy was considerably larger than that of the complex formation ($\tau_{1/e}$ ~100 s) monitored by FTIR spectroscopy[47]. These results suggested that the complex forms prior to the deprotonation of the Schiff base chromophore and changes conformation with elapsed time. The difference FTIR spectrum obtained at the early stage of complex formation shows the intense amide I and II band at 1655 and 1551 cm^{-1} that probably originate from a normal α-helical structure (Fig. 7a), while the later spectra exhibit 1659, 1643 and 1532 cm^{-1} bands due to the distorted α-helical structure (Fig. 6c, d). The absorbances of these bands suggest that 3~9 peptide amides and carbonyls are involved in forming the α-helix at the early stage, while 1~3 peptide amides and carbonyls change their conformation to form the distorted α-helical structure at the later stage. These results are consistent with those obtained by low temperature time-resolved visible spectroscopy[44] and further elucidate the detailed mechanism of complex formation.

Predicted model of Rh-Gt complex* :

Here we propose a possible mechanism of activation of Gt by Rh* speculated from the results obtained by both FTIR an low-temperature time-resolved spectroscopy. Fig. 8a shows a three-dimensional structure of Gt viewed from the ROS membrane in which α-helical arrangements of rhodopsin are projected.

The first step in the signal transduction from Rh* to Gt is the interaction between each other and the formation of the transient complex (Fig. 8b). The FTIR experiments strongly suggest that during the complex formation, a part of the *C*-terminus of Gtα forms an α-helical structure that participates in the interaction with Rh*. This region is likely to be Ile340-Asp346 and to contact with the binding pocket of Rh*, the region between the *C*-terminal of IL3 and the *N*-terminal of helix VIII. Lys311 in the *N*-terminal of helix VIII points toward the cytoplasm (Fig. 1 and 8b, 8c) and therefore, the positive charge on Lys311 could contact with a negatively charged amino acid residue, presumably Asp346, in the *C*-terminal of Gtα. The charged residues in the *C*-terminal region of IL3 of Rh* will interact with the hydrophilic region of Ile340-Asp346 in Gtα. Among these sites, Glu249 in IL3 of Rh*, completely conserved among vertebrate opsins, seems to play an important role. This negative charge may interact with Lys345 in Gtα. These hydrophilic interactions could stabilize the α-helical structure of the *C*-terminal Ile340-Asp346 region of Gtα and may induce a hydrophobic interaction between the four residues in the *C*-terminal of Gtα (Cys347-Phe350) and the cytoplasmic border of the helix VI of Rh* (Val250, Thr251, Val254, Ile255). The first contact as described above would occur before formation of meta II.

The next step is the formation of the stable contact between the cytoplasmic border of the helix VI of Rh* and the Cys347-Phe350 region of Gtα, followed by defolding of the α-helical structure in the Ile340-Asp346 region of Gtα (Fig. 8c, 8d). Interaction with the cytoplasmic border of the helix VI of Rh* causes a conformational change in the Cys347-Phe350 region of Gtα, resulting in a distortion of the peptide bond between Gly348 and Leu349. This causes the distortion of the α-helical structure of the Ile340-Asp346 region of Gtα, although the interaction with Rh* remains. Distortion of the peptide amides of Ile340 and Lys341, which form hydrogen bonds with the peptide carbonyls of the α5 helix of Gtα, should induce a conformational change in the α5 helix, thereby resulting in the cooperative interaction of other regions of Gt with Rh*

Fig. 8d shows the proposed signalling pathways from Rh* to the nucleotide binding site in Gtα. The binding of Gt with Rh* accompanies the conformational changes in the β6 sheet and the α2-β4 loop in Gtα. Structural change in the β6 sheet will affect the structure around GDP through the β6-α5 loop, while that in the α2-β4 loop will be propagated to the GDP binding site through α2 in switch II. The two-step structural changes in the *C*-terminus of Gtα could change the conformation of the guanine nucleotide binding site through the α5 helix and the β6-α5 loop. GDP release from Gtα will result in these structural changes.

Interactions between other types of G proteins and GPCRs

How do other GPCRs interact with the cognate G protein? Does a common mechanism exist in seven-transmembrane receptor mediated G protein activation? In order to gain a clue to answer these intriguing questions, we first discuss the features of the GPCR-G protein interaction surface on three types of G protein (Gi/o, Gs and Gq/11).

Gi and its cognate receptors :

While bovine rhodopsin couples with Gt in the native photoreceptor cells, it exhibits the ability to activate Gi and Go subtypes, but not Gq subtype, as evidenced by the *in vitro* studies[29,50]. Thus the interaction mechanism of Gi with its cognate receptors could be speculated to be similar to that of Gt with rhodopsin. In fact, several lines of evidence indicated that the regions responsible for the rhodopsin-Gt coupling are also important for the coupling of Gi with cognate receptors[29]. The region unique for the Gi coupling is the BBXX or BBXXB (B stands for a basic and X for a nonbasic residue) motif located near the helix VI. The motif is highly conserved among various Gi/Go-coupled receptors but not in rhodopsin, indicating that this motif is not the recognition site of Gi. It is, however, responsible for promoting nucleotide exchange in Giα[51]. Four hydrophobic residues located in the same surface of the helix VI probably participate in selective interaction with Gi[35].

Serotonin receptor couples with Gi/Go, but not with Gt. The *C*-terminal regions of α-subunits of these G proteins are highly homologous[30], suggesting that sites other than the *C*-terminus of α-subunit are involved in receptor-Gi selectivity. There are essential amino acid residues that determine the structure of Giα different from Gtα. Replacement of two residues (Gln304 and Glu308) in the α4 helix of Giα with the residues at the corresponding positions of Gtα impairs the receptor-mediated activation of Gi[52]. These residues are proposed to contact with the threonine residue at position 321 in the adjacent β6 sheet in the crystal structure, while no contact exists between α4 and β6 in Gtα. Thus, the interaction between α4 and β6 of Giα could be essential for forming the surface for interaction with the cognate receptors. These structural differences might account for the specific coupling of most Gi/Go-coupled receptors with Gi but not with Gt.

On the basis of the characteristics described above, we speculate a model for the complex between Gi/Go and the cognate receptors (Fig. 9a). In contrast to the rhodopsin-Gt complex (Fig. 8a), the *C*-terminal of IL3 of the receptor is supposed to locate between the α4 helix (shown in orange) and the β6 sheet (shown in magenta) of Giα.

Gs and its cognate receptors :

Like rhodopsin and Gi/Go-coupled receptors, Gs-coupled receptors interact with and activate Gs through IL2 and IL3. The uniqueness of the Gs-coupled receptors is the regions that determine the selective coupling with Gs. That is, the critical determinants are situated within the *N*-terminal region of IL3 and the *N*-terminal region of the cytoplasmic tail (now speculated to be helix VIII). The latter site is similar in position to those in rhodopsin and Gi/Go-coupled receptors, where the *C*-terminus of Gtα and Giα interact, respectively. Therefore, the presence of the specific site at the *N*-terminal region of IL3 is unique in Gsα and different from that of Giα and Gtα. Furthermore, chimerical and site-directed mutagenesis studies on the β2-adrenergic receptor reveal that the α3/β5 loop of Gsα is one of the critical sites that activate Gs[53], suggesting that the site contributes the receptor-Gs selectivity.

A proposed model for the receptor-Gs complex is shown in Fig. 9d. The *N*-terminal of IL3, the major site for the determination of the receptor-Gs coupling selectivity, is likely to contact the *C*-terminus of the α-subunit (shown in red), which is generally important in receptor-Gα interaction. The *N*-terminal of the cytoplasmic tail of the receptors is another determinant of the selectivity. It might interact with the α3/β5 loop of Gsα (shown in orange), which may be a receptor-binding site characteristic of Gsα. IL2 is supposed to play a role in interacting with Gsα and/or in promoting dissociation of the α-subunit from the $\beta\gamma$-subunits.

Gq and its cognate receptors :

Chimerical studies between Gq-coupled receptors with other types of receptors indicate that Gq-coupled receptors also interact with the cognate G protein by IL2 and IL3, but the interaction mechanisms are notably different from those of the other types of receptors. Chimerical Gi/Go and Gs-coupled receptors whose sequences of IL2s were replaced with Gq-coupled receptors gained the ability to activate Gq[54–56], while the replacement caused little effect on the

Gi/Go coupling in rhodopsin[29]. On the other hand, the VIa vassopressin receptor loses its ability to couple to Gq when IL2 is replaced with the cognate sequence of a Gs-coupled receptor[54]. These results suggest that IL2 of the Gq-coupled receptor is a specific binding site with Gq, while that of the Gi/Go-coupled receptor is not the major binding site to recognize the G-proteins.

The Ala/Ala/X/X/Leu/Ser motif in helix VI of the Gq-coupled receptor corresponds to the rhodopsin Val250/Thr251/Val254/Ile255 motif, and it is supposed to interact with the *C*-terminus sequence of Gqα. However, these residues form a hydrophobic cluster with the residues located in the cytoplasmic surface of helix VI[57–59]. These are consistent with the fact that the *C*-terminus segment of Gqα has a more hydrophobic character than those of other types of α-subunits.

Another interesting observation is that the *N*-terminus of Gqα is also the binding site of the Gq-coupled receptor[30,61]. Furthermore, the β-subunit also interacts with the middle part of I13 of the Gq-coupled receptor[62]. Accordingly, an interaction surface between Gq/11 and its cognate receptor could be speculated as shown in Fig. 9f. I12 of the receptor, which is major site determining the selectivity between Gq/11, could interact with the *C*-terminus of Gqα. Another site in Gq determining the selectivity is the *N*-terminus of the Gqα (shown in orange), which is apart from the *C*-terminus of Ggα. Thus the cytoplasmic tail of the receptor might interact with this region. I13 of the receptor is likely to be located near Gqβ.

Common mechanisms in the interaction between G proteins and seven-transmembrane GPCRs

As already described, the interaction surfaces between GPCR and the G protein are diversified depending on the different types of G protein. These are the molecular bases of the mechanism that triggers the specific signal cascade in the cells. On the other hand, all the receptors interact with cognate G proteins at the cytoplasmic surfaces of helices III to VII and IL2 and IL3, indicating that the global conformational changes in the transmembrane regions are essential for constructing the interaction surface with the G protein. The rhodopsin studies clearly show that the outward movements of the cytoplasmic ends of helix VI and III, which are accompanied by clockwise rotation of helix VI as viewed from the cell interior, are induced by *cis-trans* isomerization of the chromophore[22,23]. Similar movements were later observed in several receptors after agonist binding[63–65], indicating that the helix movements are common in all the receptors to create an activating state. Furthermore, the crystal structure of rhodopsin clearly shows that the arginine residue in the Asp(Glu)/Arg/Tyr triad of the cytoplasmic boarder of helix III serves as a hinge to contact helix VI and maintains the receptor conformation in an inactive state[8]. Because the arginine residue is completely conserved, this mechanism seems to be general among seven-transmembrane GPCRs.

While about 35 kcal of chemical free energy are utilized for the helical movements in rhodopsin, only several kcal of energy are needed for the movements in the ligand-binding receptors. This is because rhodopsin has the inverse agonist, 11-*cis*-retinal, in its protein moiety and the inverse agonist maintains the rhodopsin in a completely inactive state. Most receptors have a partial activity for G protein even in the absence of the agonist, and these phenomena account for the presence of an equilibrium between the inactive and active states[66]. Thus the role of the agonist is shifting the equilibrium toward the active state. In addition, many GPCRs in the resting state are proposed to bind to their cognate G proteins. This state is possibly analogous to the meta Ib-Gt complex we discovered. Thus more detailed analysis of the binding states of rhodopsin would shed light on the general activation processes of GPCRs.

There is ample evidence that the interaction surfaces of the G protein with GPCR are apart from the guanine nucleotide binding site in α-subunits, which means that there are some intramolecular signalling pathways in the G protein. Such signalling pathways are relatively well investigated in Gs, showing that there are two pathways; the one is [receptor $\rightarrow$ switch regions in Gsα $\rightarrow$ nucleotide binding site] and the another is [receptor $\rightarrow$ β6 and α5 in Gsα $\rightarrow$ β6/α5 loop $\rightarrow$ nucleotide binding site][67–72]. Because the relative positions of these sites in various α-subunits are almost identical[3,7,67], the same signalling mechanism is probably shared by α-subunits.

Concluding remarks :

The rhodopsin-transducin system has specific mechanisms for suppressing spontaneous (light-independent) activation strictly, which is required for normal visual sensation. The interaction process between them, however, has come to be known to contain common mechanisms that underlie other GPCR-G proteins. Future studies on rhodopsin-transducin interaction on the basis of the three-dimensional structures will bring us more detailed information about the general mechanisms of GPCR-G protein interactions.

Abbreviations

GPCR, G protein-coupled receptor, Rh, rhodopsin, meta II, metarhodopsin II, Gt, transducin, Gtα, transducin α-subunit, FTIR, Fourier transform infrared, IL1, the first intracellular loop, IL2, the second

intracellular loop IL3, the third intracellular loop, IL4, the fourth intracellular loop

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