Signal Transfer from GPCRs to G Proteins

ROLE OF THE Gα N-TERMINAL REGION IN RHODOPSIN-TRANSDUCIN COUPLING*

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Catalysis of nucleotide exchange in heterotrimeric G proteins (Gαβγ) is a key step in cellular signal transduction mediated by G protein-coupled receptors. The Gα N terminus with its helical stretch is thought to be crucial for G protein/activated receptor (R*) interaction. The N-terminal fatty acylation of Gα is important for membrane targeting of G proteins. By applying biophysical techniques to the rhodopsin/transducin model system, we studied the effect of N-terminal truncations in Gα. In Gαβγ, lack of the fatty acid and Gα truncations up to 33 amino acids had little effect on R* binding and R*-catalyzed nucleotide exchange, implying that this region is not mandatory for R*/Gαβγ interaction. However, when the other hydrophobic modification of Gαβγ, the Gγ C-terminal farnesyl moiety, is lacking, R* interaction requires the fatty acylated Gα N terminus. This suggests that the two hydrophobic extensions can replace each other in the interaction of Gα with R*. We propose that in native Gαβγ, these two terminal regions are functionally redundant and form a microdomain that serves both to anchor the G protein to the membrane and to establish an initial docking complex with R*. Accordingly, we find that the native fatty acylated Gα is competent to interact with R* even in the absence of Gβγ, whereas nonacylated Gα requires Gβγ for interaction. Experiments with N-terminally truncated Gα subunits suggest that in the second step of the catalytic process, the receptor binds to the αN/β1-loop region of Gα to reduce nucleotide affinity and to make the Gα C terminus available for subsequent interaction with R*.

Signal transduction by heterotrimeric G proteins (Gαβγ) and G protein-coupled receptors (GPCRs)4 is a fundamental process in the regulation of cellular function (1, 2). A prototypical GPCR and the eponym of the largest family of the GPCR superfamily is rhodopsin, the visual pigment of retinal rod photoreceptor cells. Photon capture by rhodopsin’s covalently bound chromophore 11-cis-retinal causes retinal cis/trans isomerization and thus activates rhodopsin. Within milliseconds, subsequent conversions in the protein moiety lead to the active rhodopsin conformation (R*), which is capable of catalyzing GDP/GTP exchange in the retinal G protein transducin (G) (3, 4). In the inactive state, Gt consists of Gαt-GDP and the Gβγ dimer (5) (see Fig. 1A).

A great deal of data regarding rhodopsin and Gt are available: high resolution structures of both rhodopsin (6–8) and Gt, (5, 9, 10); information on interaction sites on receptor and G protein (reviewed in Refs. 2, 4, and 11); experimental (e.g. see Refs. 2 and 12–16) and theoretical studies (17, 18); and various concepts of R*/Gt interaction (11, 15, 19–28). Despite the abundance of this information, however, it has not been sufficient to comprehensively describe how R* catalyzes nucleotide exchange in the G protein. The side of Gt facing R* (side oriented downward in Fig. 1A) (29) contains two key interaction sites, the C-terminal tail of Gαt (CTα) and the C-terminal tail of Gγ1 with its farnesyl modification (CTγ). CTα and farnesylated CTγ selectively recognize R* (30, 31) and adopt helical conformations upon binding (32–34). Involvement of the fatty acylated N-terminal region of Gαt (NTα; see Fig. 1A) in catalytic R*/Gt protein interaction was suggested by a study on a form of Gαβγ lacking a farnesyl moiety (15). There, fatty acylation of the Gα N terminus enabled interaction with R*.

This work, we set out to elucidate the role of NTα in G protein/receptor coupling by investigating N-terminal truncated Gα subunits. We confirm that lack of NTα has an effect on subunit interaction with Gβγ (39–41). We show that the G protein’s two terminal regions carrying hydrophobic modifications (i.e. myristoylated NTα and farnesylated CTγ) are functionally redundant parts of an amphiphilic microdomain that serves to anchor the G protein to the membrane and affords collisional coupling with R*. The actual trigger of nucleotide exchange requires a subsequent step that makes the Gα C terminus available for interaction with cytoplasmic binding sites of R* (15).

EXPERIMENTAL PROCEDURES

Materials—Rod outer segments and disk membranes were prepared from frozen dark-adapted retinas as described (42). Gt	

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* The abbreviations used are: GPCR, G protein-coupled receptor; Gα, G protein of the rod cell, transducin; CTα, C-terminal tail of Gα; CTγ, (farnesylated) C-terminal tail of Gγ; NTα, (myristoylated) N-terminal region of Gα; MII, metarhodopsin II; R*, active state of rhodopsin; GTPγS, guanosine 5’-O-(thiotriphosphate).

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heterotrimers were purified from rod outer segment preparations essentially as described previously (42, 43). Subsequent separation into the Go subunit and the Gβγ dimer was performed by chromatography on a HiTrap Blue column (GE Healthcare Life Sciences) as described (42). The contamination of Go preparations by Gβγ was estimated to be less than 1%. This result was based on evaluation of the initial rates of R*-induced GTPγS uptake by Go, with decreasing Gβγ concentrations using the Gt fluorescence activation assay.

Peptide Synthesis—Peptide synthesis, myristoylation, and purification were as described previously (44). Lyophilized peptides were dissolved in water, and the pH was adjusted with 1 M NaOH to pH 6. Peptides were either unmodified or myristoylated at the N-terminal Gly.

UV-visible Spectroscopy—The amount of “extra MII” was recorded by time-resolved UV-visible spectroscopy as described (45). Samples contained 10 μM rhodopsin in disk membranes. Measurements were performed at pH 8.0 and 4 °C. Cuvette path length was 2 mm. 11.5% of rhodopsin was flash-activated by 500 ± 20-nm light.

Near Infrared Kinetic Light Scattering—Changes in intensities of scattered light were measured as described (43). All measurements were performed in 10-mm path cuvettes at pH 7.4 and 23 ± 1 °C. Reactions were triggered by flash photolysis of rhodopsin (3 μM) in disk membranes using flashes of green light (500 ± 20 nm; the flash activated 32% of rhodopsin).

Transducin Fluorescence Activation Assay—Gt activation was monitored by relative changes in intrinsic fluorescence emission at 340 nm after excitation at 300 nm as described before (45). Samples containing rhodopsin in disk membranes (50 nM), Go (0.6 μM), and Gβγ (0.5 μM) were illuminated with orange light (>495 nm, 10 s). Nucleotide uptake by the G protein was induced by adding GTPγS (5 μM final concentration) at 20 °C and constant stirring. The intrinsic GDP/GTP exchange of Go subunits (0.6 μM) was monitored in the absence of Gβγ and rhodopsin (see Ref. 45 and references therein). To complete activation of the entire Go pool, NaF and AlCl3 were added sequentially with 

RESULTS

Intrinsic Nucleotide Exchange and Heterotrimer Formation of Go Subunits with N-terminal Deletions—In order to study the role of NTα in catalytic R*/G protein interaction and subunit interaction with Gβγ, we expressed three sets of Go subunits in E. coli. Each set was composed of full-length Go and mutants with N-terminal truncations of various length (see Fig. 1B). The most extensive truncation included the end of the N-terminal helix and first residues of the β1 strand, which were reported to be involved in receptor contact (36, 37). Since light-activated rhodopsin can interact with both Goi/Gβ1γi and Goi/Gβ1γi mutants with N-terminal truncations of various length (see Fig. 1B). The other two sets were based on Go (Goi and Δ29-Go), and a Goi mutant containing the N346E substitution, which changes the C-terminal tail to the corresponding Goi sequence (Goi-t, Δ29-Go-t, Δ31-Go-t, Δ33-Go-t, and Δ35-Go-t). Gt-t was preferentially used in the study, because the protein’s termini are not modified by an affinity tag, and interaction with R* is similar to Go (11, 47, 48).

We measured intrinsic GTPγS-induced fluorescence increase of isolated Go subunits to study the influence of the different N-terminal truncations on intrinsic nucleotide exchange. Almost the same rates of GTPγS uptake were found for Go-t and Δ29-Go-t, in agreement with published data (41) (data not shown). This was also observed for Gα1-t, Δ29-Gα1-t, and Δ31-Gα1-t, whereas Δ33-Gα1-t showed a slightly faster GTPγS uptake (Fig. 2A). After the addition of AlF4−, all of these subunits formed Go-GDP-AlF4− complexes, thus demonstrating functional folding of the Go subunits. However, Δ35-Gα1-t did not show any fluorescence increase, after the addition of either GTPγS or AlF4− (Fig. 2A, bottom trace), indicating that no GDP was bound in the nucleotide binding pocket.

We used size exclusion chromatography to study heterotrimer formation of truncated nonacylated Go subunits with Gβγ. Nonacylated Gα1-t, Δ29-Go1-t, Δ31-Go1-t, and Δ33-Go1-t as well as Gβγ eluted as single peaks from the size exclusion column, demonstrating the homogeneity of the subunit prepa-
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FIGURE 1. Model of heterotrimeric G, and Ga subunits used. A, Ga is composed of Ga3, Gβ, and Gγ subunits, shown in green, blue, and magenta, respectively (5) (Protein Data Bank code 1GOT). CTα and the farnesylated CTγ (both shown in orange) are known as the key binding sites for the active receptor and were modeled according to NMR data (32, 34). The crystal structure reveals a helical conformation of the N-terminal region of Ga (NTα, residues 2–28, red). In transducin, NTα is heterogeneously fatty acylated at the N-terminal Gly residue (myristoylation as the predominant modification of Ga3, modeled, shown in gray). Structure alignment shows NTα of bovine Ga3, red and rat Ga3 (subtype Ga3γ, blue) with the αN helix linked by a short loop (αN/β1-loop) to the β1 strand (green). The dashed lines represent residues not resolved in the structures of the heterotrimeric G proteins (GAGA3 for Ga3 and GCTL5 for Gβ3γ). Data Bank codes 1GOT and 1GP2, respectively. The Ga3 subunits investigated were native rod cell-specific bovine Ga3, a Ga3/Gα chimera termed rGa3 (containing an N-terminal His, tag and 16 residues from Ga3), and rGa3 (containing an N-terminal His tag and residues 1–35), Ga3, and mutants with N-terminal deletions (Δ25-, Δ29-, Δ31-, Δ33-, and Δ35-, respectively). Subunits termed Ga3-t were derived from Ga3 and contained the Ga3 C-terminal sequence due to an Asn → Glu mutation (IENNLKDCGLF).

Residues comprising the αN helix and β1 strand are underlined and shaded.

In the presence of Gβγ and excess GTP, light activation of rhodopsin did not give rise to binding signals of Ga3-t, Δ29-Ga3-t, Δ31-Ga3-t, or Δ33-Ga3-t (Fig. 3B). This result indicates that for all four Ga3 subunits, binding of Gβγ heterotrimers to R* is followed by rapid GTP uptake and Gβγ dissociation from both the receptor and the membrane. Furthermore, the lack of any light scattering change suggests that Gβγ heterotrimers were not bound to the disk membrane in the dark (see Refs. 15 and 42). The same results were obtained with rGa3, and Δ25-rGa3 as well as with Ga3 and Δ29-Ga3 (data not shown).

As another approach to address receptor-catalyzed G protein activation, GTPγS uptake by Ga3 subunits in the presence of Gβγ and catalytic amounts of R* containing disk membranes was monitored by fluorescence spectroscopy (Fig. 4). Consistent with the results from the light scattering assay, the lack of the N-terminal helix in rGa3 and Ga3-t had no severe effect on R*-catalyzed nucleotide exchange (Fig. 4). Similar observations were made for Ga3 andΔ29-Ga3 (data not shown). A gradual decrease in the kinetics was observed as the length of the deletion increased (compare Ga3-t, Δ29-Ga3-t, Δ31-Ga3-t, and Δ33-Ga3-t), which may arise from reduced Gβγ formation. No GTPγS uptake was observed for Δ35-Ga3-t/Gβγ, again demonstrating the complete functional loss of this Ga3 mutant.

Since the effect of N-terminal truncation of Ga3 on R* interaction was small, we tested two peptides derived from the N terminus of Ga3 and Ga3-t, Ga3-(2–28) and Ga3-t-(2–28), both prepared with and without a myristoyl moiety at the N-terminal Gly (see Fig. 1B). G3 holoprotein and peptides corresponding to the key binding sites of Ga3, CTα and farnesylated CTγ, are known to stabilize active metarhodopsin II (MII; “extra MII” assay) (30, 31). In contrast to these two peptides, the Ga3 N-terminal peptides did not stabilize MII (peptide concentrations up to 3 mM; data not shown). Nevertheless, the myristoylated Ga3 N-terminal peptides inhibited G3-induced MII stabilization. Similar inhibition was observed for the interaction between R* and CTγ (measured with MBP-CTαHA1 fusion protein as in Ref. 15) (data not shown). Importantly, the inhibition was only observed with myristoylated peptides. On the other hand, changes in the sequence of the Ga3 N-terminal peptides had only minor effects on the inhibition, suggesting that interaction
of the N-terminal peptide with the receptor is mainly driven by the myristoyl moiety. However, we cannot entirely exclude the possibility that inhibition is due to a more indirect effect induced by membrane partitioning of the peptides.

Gβγ-independent Interaction of Ga Subunits with R*—We performed kinetic light scattering to investigate the interaction of the nonacylated (Fig. 5A) and fatty acylated (Fig. 5B) Ga subunits with R* in the absence of Gβγ. Even at high concentrations (10 μM), the nonacylated subunits rGaα, Δ25-rGaα, Gaα-t, Δ29-Gaα-t (Fig. 5A, upper four traces), and Gaα, as well as Δ29-Gaα (not shown), failed to bind R*. Interestingly, and in contrast to all other nonacylated Gaα subunits investigated, the further truncated subunits Δ31-Gaα-t and Δ33-Gaα-t bound to R* even in the absence of Gβγ. Binding occurred with low affinity, as indicated by the small amplitude of the binding signal, despite the high concentration of the Gaα subunit (10 μM; Fig. 5A). The low but significant light-induced binding was abolished in the presence of GTP (Fig. 5A), indicating a Gβγ-independent activation of these Gaα subunits.

We then tested native Gaα, which is heterogeneously acylated at its N-terminal Gly (51, 52). Whereas no binding to R* was observed for nonacylated rGaα (Fig. 5A, upper trace), native Gaα bound to R* in a dose-dependent manner even in the absence of Gβγ (Fig. 5B, three upper traces). From the amplitude of the binding signal (ΔI/I ~ 0.01), we can calculate that with 0.5 μM Gaα in the sample, about 40% of the subunit was bound to R* (ΔI/I = 0.01 corresponds to ~0.1 μM Gaα or ~0.2 μM Gaα bound to the membrane and/or receptor (42)). Hence, the binding could not be attributed to Gaα formed from trace amounts of Gβγ contamination (<1%) present in the Gaα and rod outer segment membrane preparation (see “Experimental Procedures”). Furthermore, Gβγ-independent

**FIGURE 2.** Intrinsic nucleotide exchange and heterotrimer formation of N-terminal truncated Gaα subunits. A, intrinsic nucleotide exchange of the Gaα subunits (0.6 μM) Gaα-t (black), Δ29-Gaα-t (blue), Δ31-Gaα-t (green), Δ33-Gaα-t (red), and Δ35-Gaα-t (gray) was monitored by measuring the increase in fluorescence emission after the addition of GTPγS (5 μM black arrow). The remaining GaαGDP was activated by the addition (gray arrows) of NaF and AlCl3 to form GaαGDP-AlF4. Traces were normalized to the final fluorescence intensity. Nonnormalized final fluorescence intensities of different Gaα subunits varied within 15%. B, heterotrimer formation of Gaα-t, Δ29-Gaα-t, Δ31-Gaα-t, and Δ33-Gaα-t with Gβγ investigated by size exclusion chromatography. Elution profiles are shown for Gaα alone (black), Gβγ alone (red), and the mixture of Gaα/Gβγ (green). Calculated superpositions of the single Gaα and Gβγ elution profiles are shown as dotted lines. Lower panel, SDS-PAGE analysis of the elution fractions. Note that in the right panel the Gaα and Gβγ bands are close to each other due to the weaker separation performance of the SDS gels used.

**FIGURE 3.** Influence of N-terminal deletions of Gaα on the interaction between heterotrimeric G proteins and R* monitored by kinetic light scattering. A, light-induced binding of non-fatty acylated Gaα subunits (1 μM) in the presence of Gβγ (1 μM) and in the absence of exogenous nucleotides was monitored by kinetic light scattering. Binding is reflected by the increase in the relative intensity of scattered light (ΔI/I) observed after activation of rhodopsin (flash symbol; 3 μM rhodopsin in disk membranes; 32% rhodopsin was activated by a green flash, pH 7.4, 23 °C). The Gaα subunits investigated are indicated (abbreviated as in the legend to Fig. 1B). B, measurements as in A but in the presence of 50 μM GTP.
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binding to R* was also seen with recombinant myristoylated Gα, which is essentially Gβγ-free (data not shown). Light activation of rhodopsin did not evoke binding signals of acylated Gαi in the presence of GTP (Fig. 5B, bottom). These results indicate that fatty acylation at the N-terminal Gly residue of Gαi or Gαε enables Gβγ-independent binding to R*. Furthermore, binding is followed by GDP/GTP exchange, leading to Gα activation and subsequent fast dissociation of the R*-Gα complex.

DISCUSSION

In the crystal structures of Gαβγ, NTα forms an α-helical stretch that protrudes from Gα and links it to the Gβγ dimer (Fig. 1). This prominent structural feature was found in heterotrimers of Gαi and Gαγ, and is probably shared by all G proteins (5, 53). NTα is also part of the R*/G protein interface (35, 36), and its characteristic fatty acylation at the N-terminal Gly is mandatory for membrane association of the G ε heterotrimer (15, 54). N-terminal truncations of Gα were therefore found to have effects on subunit association and R* interaction (35, 39, 40, 49). Here we resolve the function of NTα into membrane binding, receptor binding, and nucleotide exchange catalysis.

Membrane Attachment and R* Docking—The available Gαβγ crystal structures suggest that the N terminus of Gα and the C terminus of Gγ are close to one another in space. Accordingly, NTα appears to serve as a spacer that enables the two hydrophobic modifications of Gα and Gγ (both not resolved in the crystal structures) to form a common site for membrane anchoring (Fig. 1). This notion is supported by binding studies of Gt to model membranes (55) and electron crystallography of Gt in the membrane-bound state (29). The latter investigation further revealed that only about 2% of the G protein surface is in contact with the membrane. This conclusion coincides with the weak lipid/protein interactions found for Gt, which engages a small amount of phosphatidylserine (56). Taking these facts into account, it is easy to understand that the lack of one of the two hydrophobic modifications (15, 54) or N-terminal truncation of Gα renders the G protein heterotrimer soluble.

The Gα N terminus and the Gγ C terminus with their hydrophobic modifications are also involved in receptor binding (15, 30, 35, 36) and may be viewed as an amphiphilic microdomain that is engaged in R* interaction. However, removal of the N-terminal myristoyl moiety or even truncation of whole NTα did not abolish R*/G protein coupling (Figs. 4 and 5). The same was observed when the farnesyl moiety or the C-terminal Gly-Cys-farnesyl fragment was removed from Gα (15). This indicates that fatty acylated NTα and farnesylated CTγ are functionally redundant and that one site is sufficient for interaction with R*. The initial docking to R* appears to be of predominantly hydrophobic nature with rather low specificity, since the sequences of CTγ and NTα are different and vary between subtypes (e.g. see Fig. 1B). The NTα-derived peptides fail to selectively stabilize MII. Furthermore, farnesylated CTγ-derived peptides exhibit a lower MII-stabilizing efficiency compared with CTα-derived peptides (30). These observations suggest that the Gα N terminus and Gγ C terminus, particularly

FIGURE 4. R*-catalyzed nucleotide exchange of Gα subunits monitored by the fluorescence activation assay. GTPγS uptake by non-fatty acylated Gα subunits (0.6 μM) in combination with Gβγ (0.5 μM) and in the presence of R* (50 nM) was initiated by the addition of GTPγS (5 μM final concentration, arrow).

FIGURE 5. Interaction between Gα subunits and R* in the absence of Gβγ investigated by kinetic light scattering. The flash symbol indicates time of rhodopsin activation, measuring conditions as in Fig. 3A. Interaction of non-fatty acylated Gα subunits (10 μM, subunits as indicated) with R* (1 μM) in the presence of GTPγS (0.5 μM) and in the presence of GTP (50 μM).

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their hydrophobic modifications, may recognize binding sites that are already present in precursors of MII or even in the inactive ground state. The apparently weak contribution of the NTα sequence in R* interaction suggests that the main role of the N-terminal helix is to position the myristoyl moiety in the vicinity of the farnesyl moiety.

Role of NTα in Nucleotide Exchange Catalysis—We concluded previously that upon formation of the docking complex between R* and Gaβγ, via the amphiphilic microdomain defined above, the second key binding site of the G protein, namely CTα, becomes available for R* interaction (15). The experiments described in this study on truncated versions of isolated Gaα subunits (i.e. without Gaβγ) suggest a role of NTα in this process (Fig. 5). We found a weak but significant interaction between R* and native fatty acylated Gaα (i.e. in the absence of Gaβγ). This interaction did not occur when fatty acylation was missing. However, truncation of NTα, including the loop linking the αN helix and the β1 strand (αN/β1-loop; see Fig. 1), rendered isolated Gaα competent to couple to R*, even without the initial hydrophobic interaction described above. This result suggests that in such truncated forms of Gaα, the CTα binding site is already unleashed for interaction with R*. What then makes the CTα binding site available in the native holoprotein? Based on our results, we propose that structural alterations in the αN/β1-loop region contribute to the unleashing of CTα in Gaβγ. It is conceivable that cytoplasmic receptor loops directly target the αN/β1-loop region upon formation of the initial docking complex between R* and Gaβγ. Experimental evidence was provided by cross-linking experiments on rhodopsin/Gi interaction. An interaction between the third cytoplasmic receptor loop and the Gaα fragment Leu19–Arg28 covering the αN/β1-loop was mapped (36). Furthermore, it was reported that the αN/β1-loop allows GPCRs to distinguish between Gaα15 and Gaα16 subtypes (37). The αN/β1-loop region also appears to be an element in controlling the affinity of GDP, because truncation of this region in Gaα increases intrinsic GDP/GTP exchange (Fig. 2). Interestingly, a similar effect was found previously for a point mutation close to this loop in the β1 strand (57).

EPR studies on Gaα15 showed that NTα adopts an ordered structure in the myristoylated Gaα subunit (58, 59), suggesting that even in isolated Gaα a structure corresponding to the αN helix in Gaβγ can be formed. NTα may act as spacer between the lipid anchor site and the αN/β1-loop region, thus establishing a geometric constraint between these two regions. Such a constraint, enhanced by Gaα/Gaβγ association in the heterotrimer, would be employed to bring the αN/β1-loop region and respective sites on R* in proximity for subsequent interaction to unleash CTα. NMR studies and fluorescent labeling of Cys347 in Gaα showed that CTα, which is not resolved in the crystal structures, adopts different conformations in solution (16, 60–62). The conformational changes were dependent on the type of nucleotide bound by Gaα, association of Gaα and Gaβγ, and interaction of Gaβγ with R*. Further studies will be required to identify the specific structural changes needed to make CTα available for R*. Interestingly, a point mutation close to the nucleotide-bind-

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Freissmuth and co-workers (41, 64) showed Gβγ-independent activation of Gaα15 and Gaα subunits by a receptor-mimetic peptide derived from the N-terminal portion of the third cytoplasmic loop of the D2-dopamine receptor. Interestingly, Gaα activation by the peptide was not affected by the lack of myristoylation or a 30-amino acid truncation of the Gaα1 N terminus (41). Furthermore, the presence of Gβγ reduced Gaα activation, which could be overcome by increasing the receptor-mimetic peptide concentration. This shows that (i) the peptide can directly target its binding site on Gaα, which was mapped to be CTα in close proximity to the αN/β1-loop, and (ii) Gβγ contributes to shielding of CTα. In the present study, Gaα and heterotrimeric G protein lacking hydrophobic modifications failed to interact with light-activated rhodopsin. We thus conclude that, in contrast to the receptor-mimetic peptide, the corresponding endogenous cytoplasmic third loop fragment of rhodopsin cannot directly target CTα. Accordingly, this interaction would require the above described initial docking via the G protein’s amphiphilic microdomain and subsequent conformational changes within the receptor.

Model of R*G Protein Coupling—Our data are consistent with a coupling mechanism in which an initial contact is established between R* and a microdomain on the G protein composed of the hydrophobic modifications of Gaα and Gγ and adjacent N-terminal residues (Fig. 6). This microdomain therefore not only constitutes the membrane anchoring site of the G protein but also serves to establish a docking complex with R*. This complex in turn allows the G protein to sample different orientations until receptor loops can engage with the αN/β1-loop region to lower nucleotide affinity and to unleash the CTα key binding site. Eventually, the interaction of CTα with R* triggers GDP release and weakens the contact between the initial R*/G protein docking sites (15). A gain in fidelity of signal transduction might be the reason for the multiple steps involved in G protein activation. Future work will aim at testing this working model with G proteins containing all native hydrophobic modifications.

An outcome of the present study as well as a study by others (41) is that Gβγ is not unconditionally required for nucleotide exchange catalysis. A Gβγ-independent Gaα activation could allow the cell to maintain intracellular signaling even in the case where Gβγ is involved in effector interaction. Notably, the mechanism proposed above can also be used to describe Gβγ-independent activation of Gaα: initial contact between R* and the hydrophobically modified N-terminal region of Gaα enables unleashing of CTα and subsequent CTα interaction with R*. However, lack of Gβγ in the amphiphilic microdomain employed in the docking step decreases efficiency of R*G protein coupling. The question remains whether in the course of nucleotide exchange catalysis Gβγ also directly affects nucleotide affinity. Models
were proposed in which, upon receptor/G protein interaction, (i) Gα and H9252/H9253 undergoes a conformational switch that creates an escape route for GDP (19) and (ii) Gα and induces conformational changes in switch regions of Gα5 helix to destabilize nucleotide binding (12, 22, 25). Such contributions by Gα could improve nucleotide exchange catalysis, although CTα and the preceding α5 helix are considered to be the key elements needed to prompt nucleotide release from the "back side" of the nucleotide binding pocket (13, 14, 28, 41, 65). Our sequential fit mechanism of G protein activation includes a transitory state with multisite interaction between R* and the G protein (Fig. 6) (15, 30). This state enables GDP release and accounts for contributions by Gβγ to enhance GDP release.

It should be further noted that the functional unit of active rhodopsin is not yet clear (e.g. see Refs 17 and 26). However, our docking concept is compatible with various scenarios (Fig. 6B). Among them are docking between (i) Gα and an activated rhodopsin monomer, (ii) Gα and a preformed rhodopsin dimer or higher oligomer with at least one activated rhodopsin molecule, and (iii) a preformed Gα-rhodopsin complex with an activated rhodopsin monomer. It is further conceivable that docking between Gα and an activated rhodopsin monomer induces receptor dimerization or oligomerization.

As to the docking site on rhodopsin, previous work suggests a potential hydrophobic site comprised of the NPXYY(F) motif in transmembrane helix VII and cytoplasmic helix VIII of rhodopsin that includes the palmitoylated Cys322 and Cys323 (44, 66–68). The crystal structure of the rhodopsin ground state revealed two lipid molecules bound close to this region (8). Furthermore, it was shown that rhodopsin/lipid interactions change upon rhodopsin illumination (69, 70). It is conceivable that upon this change, a docking site for the G protein’s amphiphilic microdomain is formed. A high resolution structure of the R*G protein complex should provide information regarding specific interactions between GPCR and G protein as well as structural alterations enabling nucleotide exchange.

However, as this work shows, interaction between the two proteins requires several steps to be productive. Inherent problems with crystallizing such a complex may be attributable to the dynamic nature of signal transfer.

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