

Membrane organization in G-protein mechanisms

RICHARD R. NEUBIG¹

Departments of Pharmacology and Internal Medicine (Hypertension), The University of Michigan, Ann Arbor, Michigan 48109-0626, USA

ABSTRACT A prevailing view of receptor and G-protein function in cells includes random collisions between the proteins with a great specificity at the sites of protein-protein interaction. Recent evidence suggests that receptors, G-proteins, and effectors may be less mobile and that these systems are more highly organized than previously appreciated. Several types of evidence suggest that receptors do not have free access to all G-protein with which they are capable of coupling. Also, the specificity of signaling in intact cells appears to be significantly greater than in reconstituted systems. The distribution and mobility of G-proteins in cells are restricted to a surprising degree. Thus, complex interactions of the receptors and G-proteins with their effectors and cell membrane machinery appear to play an important role in their function. A full understanding of G-protein-coupled receptors must include a better description of the organization of these systems in cell membranes. Possible roles for noncoated pits (caveolae) and a novel pleckstrin homology domain need to be examined.—Neubig, R. R. Membrane organization in G-protein mechanisms. *FASEB J.* 8: 939-946; 1994.

Key Words: adrenergic receptor • diffusion • fluorescence • cytoskeleton • membrane fluidity • pleckstrin homology

SCOPE OF REVIEW

Signal transduction mediated by guanine nucleotide-binding proteins (G-proteins)² has been the focus of much interest since the early 1980s. These proteins play a central role in signal transduction and cell biology far beyond just activation of adenylyl cyclase (1-4). A tremendous amount is known about the multitude of different proteins involved in G-protein-coupled signal transduction (5-9). There are more than 200 receptor types and subtypes, at least 21 G-protein α subunits, 4 β subunits, and 6 γ subunits (10). There are at least six different subtypes of mammalian adenylyl cyclase (11), many phospholipases A and C (12), and numerous potassium and calcium channels that are regulated by G-proteins (3). In olfactory signaling there may even be an immunoglobulin-like diversity of receptor structure (13). How these diverse components are organized in the cell is an important area for future exploration. This review describes several types of evidence for a more complex organization of G-protein-coupled signal transducing molecules in cells and membranes than has been appreciated previously.

Much current thinking about G-protein-coupled receptors is based on the idea of freely mobile receptors, G-proteins, and effectors in which the specificity of their interactions derives solely from the three-dimensional structure of the sites of protein-protein interaction. This concept arose in part from the elegant studies by Orly and Schramm (14) showing that signal transducing components could be exchanged be-

tween cells. Additional support came from Tolkovsky and Levitzki (15) with their "collisional coupling model" and the demonstration of catalytic activation of adenylyl cyclase by β -adrenergic receptors. These ideas were further supported by studies of purified and reconstituted proteins (16-20) and the rhodopsin-transducin system (21). Although these approaches have yielded many useful insights into the mechanism of receptor action, rod outer segment disks provide an unusual membrane environment with a unique lipid composition (22). The lateral mobility of rhodopsin (translational diffusion coefficient, D_T , $5 \times 10^{-9} \text{ cm}^2 \cdot \text{s}^{-1}$) is much higher than that of most mammalian membrane proteins (23). Receptors and G-proteins are also likely to be quite mobile in reconstituted lipid vesicles, making extrapolation to intact cells difficult.

A better understanding of the role of cytoskeleton in tyrosine kinase receptor signaling (24) raises the possibility of a specific organization of G-protein-coupled signal transducing molecules. The membranes of mammalian cells are far more complex than either reconstituted vesicles or rod outer segment membranes. Cells exhibit distinctive morphologies with functional proteins segregated into distinct regions (e.g., polarization of epithelial or neuronal cells), and even within relatively homogeneous regions there is an organized cytoskeletal network of proteins. Thus, it would not be surprising if receptor-G-protein-effector interactions in reconstituted systems differed in many ways from those in a complex plasma membrane or cell.

Recent evidence for more organized interactions of receptors, G-proteins, and effectors derives from several sources. First, several G_i -coupled receptors in neuronal cells appear to interact functionally with distinct "pools" of G-protein. Also, the existence of an uncoupled form of many G_i -coupled receptors is not compatible with free accessibility of homogeneous pools of receptors with excess G-protein. Second, the specificity of signaling by G-proteins and receptors in intact cells (25-27) appears to be greater than that seen in reconstitution systems (28, 29). Finally and most compellingly, direct demonstrations of G-protein-cytoskeleton interactions and limited mobility of receptors and G-proteins are becoming common. Indeed, there is recent recognition that

¹Address correspondence and requests for reprints to Dr. Neubig at: Department of Pharmacology, University of Michigan M6322 Medical Science Bldg. 1, Ann Arbor, MI 48109-0626, USA.

²Abbreviations: β -ARK, β -adrenergic receptor kinase; D_T , translational diffusion coefficient; FPR, fluorescence photobleaching recovery; G-protein, guanine nucleotide binding protein; G_i , inhibitory G-protein; G_s , stimulatory G-protein; SH2, src homology 2; PH, pleckstrin homology domain; RG, receptor and G-protein complex; TMR, tetramethylrhodamine; TMR- $\beta\gamma$, tetramethylrhodamine-labeled $\beta\gamma$ subunit of G-protein; I_m , voltage-sensitive potassium current; LHRH, luteinizing hormone releasing hormone.

signal transduction events in intact cells are more complex and interesting than in reconstituted systems (9).

The multiplicity of receptors, G-proteins, and effectors (8, 11, 12, 30), structural features of the components (31, 32), mechanisms in reconstituted systems (33), and regulation (32) have been well reviewed recently. Thus, in this review I will focus on information regarding regulation of G-protein-receptor interactions in intact cells and membranes. I will describe accumulating data that support the idea of a higher-order organization of G-proteins and receptors in intact cells and membranes. Finally, I will speculate about possible cellular proteins that may play a role in this organization.

CAN MULTIPLE RECEPTORS ACCESS THE SAME POOL OF G-PROTEINS IN CELLS OR CELL MEMBRANES?

Evidence for shared pools of G-proteins

The nonadditivity of glucagon- and ACTH-stimulated adenylyl cyclase activity in adipocyte membranes showed that these receptors were both able to activate a common pool of effector enzyme (34). Additional evidence that multiple receptor types can access the same pool of G-protein comes from nonadditivity of receptor-stimulated GTPase activity. By this criterion, vasopressin and platelet-activating factor appear to share the same pool of G-protein in human platelet membranes (35). With agonist binding methods, Dasso and Taylor (36) showed that, in rat hepatocyte membranes, vasopressin was able to markedly reduce high-affinity binding of epinephrine to α_1 -adrenergic receptors apparently by tying up the G-protein required for the high-affinity conformation of the α_1 receptor.

Evidence for compartmentation of receptors and/or G-proteins

In other systems, there is evidence for compartmentation of receptors and/or G-proteins into distinct pools. This conclusion derives from functional data, ligand binding studies, and more direct physical evidence such as fluorescence photobleaching recovery measurements with G-protein subunits and coprecipitation with cytoskeletal proteins.

Lack of cross-talk in agonist binding to G_i -coupled receptors

In contrast to the results with vasopressin and α_1 -adrenergic receptors, α_2 -adrenergic and δ -opioid receptors, both of which couple to the G_{i2} , showed no evidence for shared pools of G-protein in NG-108-15 neuroblastoma-glioma cells. Despite predictions of a two-receptor ternary complex model (37) there was no decrease in α_2 -adrenergic or δ -opiate agonist binding by the heterologous agonist. This was true even when G-protein was made limiting by partial pertussis toxin treatment. This lack of cross-talk does not result from G-protein α subunit heterogeneity, as both the δ opiate and α_2 receptor in NG 108-15 cells have been shown to efficiently couple to the G_{i2} subtype of G_i ; however, it could result from $\beta\gamma$ subunit heterogeneity (see below) or physical compartmentation of the G-protein.

Agonist-specific GDP β S inhibition

Another piece of data to indicate that multiple G_i -coupled receptors use distinct pools of G_i comes from patch-clamp experiments with bullfrog sympathetic neurons (38). In this system, muscarinic agonists, substance P, and luteinizing

hormone releasing hormone (LHRH) all inhibit a voltage-sensitive potassium current (I_m) through a pertussis toxin-sensitive G_i -like protein. Each agonist is capable of fully inhibiting the I_m current in the cell and there is no additivity of responses. Inclusion of GDP β S in the pipette results in a time-dependent inhibition of responses that is accelerated by agonist. It is interesting that the effect is selective for the agonist applied with GDP β S. Treatment of cells with GDP β S, followed by several pulses of LHRH, causes a selective loss of the LHRH response whereas the muscarinic and substance P responses are spared. This is not just a receptor-desensitization phenomenon as it does not occur when GTP is in the pipette rather than GDP β S. The simplest explanation of these results is that agonist enhances the rate of GDP β S binding to the pool of G-protein that is coupled to that receptor. The fact that only the response to one receptor is blocked implies that the receptors do not share a common pool of G-protein; rather, each receptor seems to prefer its own pool of G-protein. This idea is a bit surprising because each receptor can fully activate the response (i.e., inhibition of I_m), but this can be rationalized by allowing an excess of G protein over effector and permitting the α subunits (which presumably mediate this effect (3)) to be mobile (Fig. 1).

G-protein heterogeneity

As the importance of β and γ subtypes becomes better understood, G-protein heterogeneity seems to play a much bigger role in receptor specificity than previously appreciated. Many different α and $\beta\gamma$ subunits have been identified (39, 40). Recent evidence of a strong selectivity of β (26) and γ (27) subunits for particular receptors may explain the limited G-protein pool available to receptors coupled to G_i -like G-proteins. For example, the selective interaction of somatostatin receptors with $\alpha_{o2}\beta_1\gamma_3$ and muscarinic receptors with $\alpha_{o1}\beta_3\gamma_4$, as shown by the data of Kleuss et al. (27), could account for the existence of G-protein "pools." This degree of selectivity, however, is not seen in reconstitution studies. This raises the interesting possibility that the $\beta\gamma$ subunits may impart their selectivity by localizing receptor, G-protein, and effector complexes in cell membranes rather than simply by imparting specificity at the receptor- $\beta\gamma$ interface.

Restricted collision-coupling model

Limited access of receptors to G-proteins is also supported by data on activation of adenylyl cyclase by adenosine A_2 receptors both in membranes and intact cells. Indeed, the original analysis by Braun and Levitzki (41) of adenosine receptor activation of adenylyl cyclase indicated that the adenosine receptor was more "tightly coupled" to the effector than was the β -adrenergic receptor. Gross and Lohse (42) describe a "restricted collision-coupling model" in which receptors diffuse freely but are only able to contact a small number of effectors. They do not, however, report how low the diffusion coefficient must be to account for their observations.

Evidence for restricted mobility of receptors and/or G-proteins derived from ligand binding studies

A series of binding studies with the α_2 -adrenergic receptor led us to conclude that free diffusion was not the only factor regulating receptor-G-protein interactions in cell membranes (43-45). The first evidence for this conclusion was the existence of an uncoupled state of the α_2 -adrenergic receptor

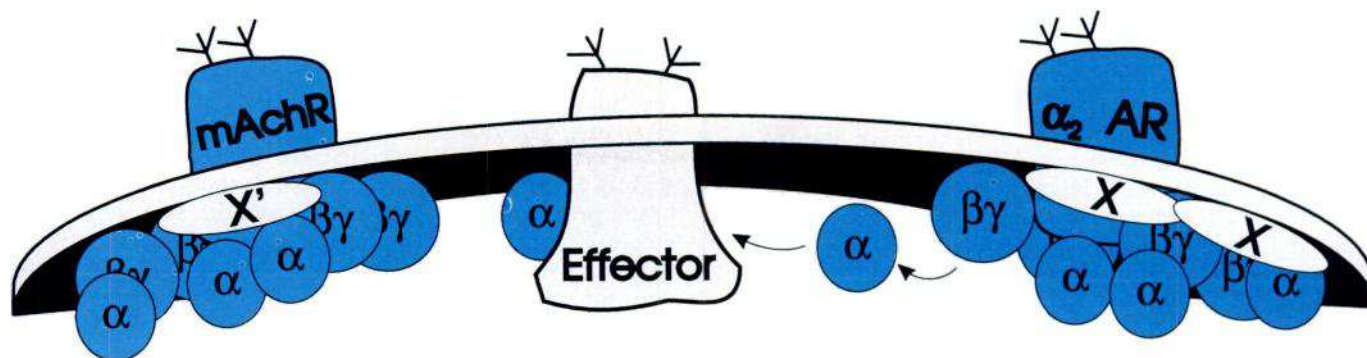


Figure 1. Possible membrane organization of receptors and G-proteins. The interactions of receptors and G-proteins do not appear to be governed entirely by a random collision of proteins in the plasma membrane. Rather, sorting of the distinct components of the G-protein-mediated signal transduction system into organized regions or supramolecular complexes may be important for the specificity of receptor-G-protein interactions in cells. Different receptors (e.g., α_2 adrenergic and m4 muscarinic) that are capable of coupling to the same G-protein *in vitro* do not seem to share the same pool of G-protein in intact cells. It will be important to identify any additional cellular machinery (X or X') that may be responsible for the organization of these systems in cells. The interaction of one receptor with multiple $\beta\gamma$ subunits suggested in this model is consistent with the significant excess of G-proteins over receptors in most tissues. It is also possible that some effector molecules could be incorporated into the complexes of receptors and $\beta\gamma$ subunits as there is increasing evidence for signal transmission by $\beta\gamma$ subunits as well as α subunits of G-proteins.

in equilibrium binding studies despite a 20- to 50-fold excess of G-protein (43). Second, there was a slow agonist-stimulated association of the α_2 -adrenergic receptor and G-protein in kinetic studies that was more than an order of magnitude slower than that predicted for simple protein diffusion in lipid membranes (44, 45).

To appreciate the significance of low-affinity agonist binding in the presence of excess G-protein we must closely examine the predictions of the ternary complex model of ligand binding (46). Many investigators have pointed out that the ternary complex model only accounts for the biphasic agonist binding if the G-protein is present in amounts less than or equal to that of receptor (43, 46-48). We showed that in human platelet membranes there is a 20- to 50-fold excess of G_i over α_2 -adrenergic receptor (43). This discrepancy made it clear that the simple ternary complex model was not sufficient to explain the existence of low-affinity binding. Ransnäs and Insel (50) quantitated G_s in S49 lymphoma cells and also found a great excess of G_s (19 pmol/mg) over β -adrenergic receptors (170 fmol/mg) in that system as well. There is also a significant excess of G-protein over receptor for formylpeptide (51), muscarinic (52), and opiate (52) receptors, which all show biphasic agonist binding.

Thus, for many G_i -coupled receptors and at least one G_s -coupled receptor, an explanation for the low-affinity binding was needed beyond the simple ternary complex model. There are two general types of explanations: 1) biochemical heterogeneity of receptor or G-protein, and 2) compartmentation of receptor and/or G-protein in cells to prevent access. It is possible that phosphorylation of receptors (53-55) could result in an uncoupled receptor with low affinity for agonist, but other sources of receptor heterogeneity including receptor subtypes, alternative splicing of mRNA, glycosylation, and palmitoylation do not account for the many systems in which low-affinity binding is seen. The α subunits do not exhibit enough heterogeneity to result in a limiting amount of G-protein for coupling to α_2 - and β -adrenergic or neutrophil formylpeptide receptors.³ A further level of heterogeneity may also be present in the interactions of receptors with coated pits and noncoated membrane invaginations (caveolae). This is discussed later in this review under the rubric of compartmentation.

Detailed agonist binding time course studies have been described for the α_2 -adrenergic receptor (44, 45, 57) and the neutrophil formylpeptide receptor (58-60). In both systems, there appear to be three states of the receptor: 1) the low-affinity resting state of the receptor (R), which couples to G-protein after agonist is added, 2) a precoupled complex of receptor and G-protein (RG) with high affinity for agonist, and 3) an "uncouplable" state of the receptor (R') that is never able to couple to the G protein and has low affinity for agonist.

Approximately one-third to one-half of the receptors appear to be in the precoupled state for both receptors. Upon addition of agonist, slow coupling of an additional 30-50% of the receptor occurs with a half-time of 300 to 700 s while the predicted value for simple diffusion with a D_T of $5 \times 10^{-10} \text{ cm}^2 \cdot \text{s}^{-1}$ is less than a second (44).

"Structural" studies

More direct methods also indicate that the simple concept of receptors and G-proteins moving freely in cell membranes needs to be modified. Indeed, the existence of cytoplasmic barriers to free motion of proteins is well supported (61).

Target size analysis

Early work on the glucagon receptor- G_s -adenylyl cyclase system from Rodbell and co-workers (62) found that target sizes determined from radiation inactivation studies did not fit with a simple idea of receptor and G-protein monomers

³In the case of the α_2 -adrenergic receptor, there is a 50-fold excess of G_i over receptor in human platelet membranes (43) and the α_{12} subunit makes up approximately two-thirds of the total G_i pool (39), which still leaves a 30-fold excess of $G_{\alpha_{12}}$ protein that should be able to couple to α_2 receptors. An estimate of the number of G_i and receptor molecules in each platelet membrane fragment is 120 and 2.4, respectively (44), which if evenly distributed would result in an infinitesimal fraction of the membrane fragments with no G-protein. Thus, only a highly compartmentalized distribution of G-protein could result in membrane vesicles that contain receptor but no G-protein.

floating freely in cell membranes. Large complexes with predicted molecular weights in excess of 1 million were found. In addition, Nakamura and Rodbell (63) found that extraction of hepatic cell membranes with the detergent octylglucoside resulted in large complexes containing the G_i and G_o subunits. The size of these complexes was reduced by previous treatment of the cells with glucagon (64). The implications of these data have been reviewed recently and a model involving large macromolecular assemblies of G-protein was proposed (9).

Cytoskeletal associations

A number of laboratories have obtained data showing association of G-protein α or $\beta\gamma$ subunits with tubulin or other cytoskeletal proteins. An interaction between tubulin and adenylyl cyclase was suggested from effects of microtubule inhibitors on adenylyl cyclase activity (see review, ref 65). More direct evidence for interactions of G-proteins with microtubules came from transfer of guanine nucleotides from G-proteins to tubulin (66) and direct binding of ^{125}I -labeled tubulin to G-protein α subunits, particularly G_{i1} and G_i (67, 68). G_i -like proteins in a murine T lymphoma cell line colocalize in cap structures that are rich in actin, myosin, and fodrin (69). Also, in S49 lymphoma cells the $\beta\gamma$ subunit of G-proteins appears in a Triton X-100 insoluble pool that is enriched in actin (70). Finally, the formylpeptide receptor in neutrophils appears to be intimately involved in cytoskeletal interactions (71, 72). The possible role of spectrin or dynamin in binding $\beta\gamma$ subunits is discussed below with respect to pleckstrin homology domains.

Immunocytochemical localization of receptors and G-proteins

In epithelial tissues and in neurons, endogenous G-proteins (Table 1) and epitope-tagged adrenergic receptors (73, 74) both show markedly specific distributions within cells. By immunocytochemical methods, G_i and G_o both exhibit a clustered distribution in the plasma membrane and may be associated with microspikes enriched in actin (75, 76) (Table 1). G_o is also localized in growth cones of neurons (28). The cellular distribution of the β -adrenergic receptor in A431 cells exhibits a striking speckled pattern indicating a nonrandom distribution of the receptors in the plasma membrane (77). Immunofluorescence localization of G-protein β subunits in these cells (77) and in transfected COS cells (78) also showed a speckled pattern. These observations all suggest that a specific mechanism is responsible for the organization of components of the signal transduction cascade in cell membranes.

Fluorescence photobleaching recovery measurements with G-protein subunits

Despite the important role of the collisional-coupling model (15, 79) in the theory of G-protein-coupled receptors, there has been virtually no direct data on the mobility of G-proteins in membranes or intact cells (80). There are only two systems for which mobility has been studied for G-protein-coupled receptors (81, 82). We found that fluorescently labeled G-protein α and $\beta\gamma$ subunits (83) introduced into NG 108-15 neuroblastoma-glioma cells (83) by cell-vesicle fusion showed a strong degree of clustering, similar to that reported for immunofluorescence studies of endogenous G-proteins (see above). Although the labeled $\beta\gamma$ subunit was largely located in the plasma membrane, the α subunit showed both membrane and cytoplasmic distributions (83).

Most strikingly, the mobility of the tetramethylrhodamine-labeled $\beta\gamma$ (TMR- $\beta\gamma$) subunit of G_o in NG cells as determined by fluorescence photobleaching recovery (FPR) was very low. Only 16% of the fluorescence of TMR- $\beta\gamma$ recovered after photobleaching (83). The small fraction that did recover had a diffusion coefficient of $2 \times 10^{-9} \text{ cm}^2 \cdot \text{s}^{-1}$, slightly higher than that of typical mammalian cell membrane proteins. TMR-labeled α_o subunits also showed less recovery of fluorescence than expected (32% vs. 50–60% for typical membrane proteins) and had a slightly higher diffusion coefficient than $\beta\gamma$ ($4 \times 10^{-9} \text{ cm}^2 \cdot \text{s}^{-1}$). Thus, the restricted mobility of components of the G-protein-coupled signal transduction cascade does not appear to be due to a uniformly low diffusion coefficient; rather, a large fraction of $\beta\gamma$ subunit is virtually immobilized.

No effect on either the mobile fraction or diffusion coefficients of the α or $\beta\gamma$ subunits was seen upon addition of agonist or modification of cytoskeletal proteins with cytochalasin or nocodazole (83). The difference between the mobility of the α and $\beta\gamma$ is intriguing. It has been proposed that the $\beta\gamma$ subunits are a membrane anchor for the α subunit (84, 85) and the β subunit has been shown to associate with cytoskeleton (70). Consistent with this concept, fluorescence energy transfer studies show that fluorescent labels in β and γ subunits are closer to membrane lipids than the label in α subunit (86). Future work should focus on the specificity of $\beta\gamma$ subunit anchoring rather than just considering it a property of the "hydrophobic" nature of the $\beta\gamma$ subunit.

Molecular candidates for organizing factors

Two recent observations provide interesting speculations about possible cellular or molecular candidates for regulating movement of G-proteins in cell membranes. First, a striking enrichment of several types of G-proteins was found in Triton X-100 insoluble membranes with many characteristics of noncoated pits or caveolae (87). These membranes were also enriched in caveolin, the protein component of noncoated pits that binds glycosylphosphatidylinositol-linked proteins and plays a major role in protein sorting in epithelial cells. β -Adrenergic receptors were also shown to accumulate in noncoated pits (88) and to copurify with caveolin (89) when cross-linked with antibodies. It is interesting that both calcium ATPase and calcium release channels (inositol trisphosphate receptor) are also enriched in caveolae (90, 91). Thus, caveolae may represent sites of assembly of a signal transducing complex that could include receptors, G-proteins, effectors, and even the intracellular targets of the second messengers generated.

Second, at a molecular level the novel protein interaction motif called the pleckstrin homology (PH) domain (92, 93) may be important for $\beta\gamma$ subunit interactions with the cytoskeleton and other cellular proteins. The PH domain was named for pleckstrin, the major protein kinase C substrate in human platelets, and is similar to src homology 2 (SH2) domains. PH domains have been found in signal transducing molecules such as ras-GTPase activating protein and ras-guanine nucleotide releasing factor (93). They are also found in the cytoskeletal protein spectrin and the microtubule motor protein dynamin (92). It was recently observed that the carboxyl-terminal portion of β -ARK, which is the site of interaction with $\beta\gamma$ subunits (94), also contains a PH domain (95, 96). This region is missing from rhodopsin kinase, which is not activated by $\beta\gamma$ subunits. This provides an intriguing clue that PH domains are important for $\beta\gamma$ subunit interactions with other proteins. It leads to the

TABLE 1. Localization and subcellular distribution of G-protein subunits*

Component	Method	Cell type	Result	Reference
Cells				
α_s, α_i	SCF	Adipocytes	1) α subunits eightfold enriched in PM compared with low-density membranes (Golgi) 2) $\beta\gamma$ not detected in low-density membranes (Golgi)	(97)
$\alpha_s, \alpha_i, \alpha_o$	IEM	Cardiomyocytes	All labeling in PM, no staining of sarcoplasmic reticulum	(98)
α_i	IC IEM	3T3 cells, NRKF, C6 glioma, HUVE HEK293	1) Cytoplasmic tubular structures were stained (mitochondria or microtubules) 2) Diffuse particulate fluorescence in PM 3) Concentrated staining of PM in microspikes	(76)
α_{i3}	IC	Transfected NIH-3T3 cells	1) Low α subunit densities—Golgi 2) High expression densities—Golgi and PM	(99)
α_{i3}	IC and SCF	LLPK ₁ , NRK, and rat liver and transfected LLPK ₁	α_{i3} present in Golgi	(100)
$\beta\gamma$	IC and SCF	Transfected COS cells	1) $\beta\gamma$ shows punctate distribution in PM but is also present in intracellular vesicles 2) Inhibition of γ subunit prenylation results in substantial cytoplasmic pool of $\beta\gamma$	(78)
Tissues				
α_{i3}	IC	A6 renal epithelial cells	1) α_{i3} and sodium channels in "distinct but adjacent domains of the apical cell surface" 2) Only α_{i3} in Golgi, no sodium channels	(101)
$\alpha_s, \alpha_i, \beta\gamma$	SCF	Kidney	1) Cortex— α_s and α_i greater in brush border vs. basolateral membrane 2) Medulla— α_s in both brush border and basolateral membrane but α_{i2} only in brush border	(102)
$\alpha_s, \alpha_{i1.2.3}$	IC	Rat kidney	1) α_{i1} apical pole 2) α_{i2} basolateral PM and cytoplasm of collecting duct cells 3) α_{i3} apical proximal tubule "sub-brush border invaginations" and perinuclear Golgi 4) α_s basolateral PM of thick ascending limb cells	(103)
α and $\beta\gamma$	IC and SCF	<i>Aplysia</i> nervous tissue	α and $\beta\gamma$ both in PM fraction of synaptosomes, not granules	(104)
α_i and α_o $\beta\gamma$	IC	Olfactory tissue	1) Epithelium— α_o, α_{i2} , and $\beta\gamma$ all present in basolateral surface but apical surface has $\beta\gamma$ only 2) Molecular and internal granular layer of olfactory bulb has α_o and $\alpha_{i1/3}$ present but no α_{i2}	(105)
α_{i2}	IC, SCF, and IEM	Brain	1) α_{i2} in axon terminals 2) α_{i2} in PM, ER, synaptic vesicles, and microtubules	(106)

*Studies from 1990–1993 of the subcellular distribution and immunocytochemical localization of G-protein subunits in cells and tissues reveal restricted localizations of different subunits within and between cells. IC, immunocytochemistry; SCF, subcellular fractionation; IEM, immunoelectron microscopy; PM, plasma membrane.

speculation that the PH domains in cytoskeletal proteins such as spectrin or dynamin may bind $\beta\gamma$ subunits and contribute to their immobility and organization in cell membranes.

SUMMARY

A wide variety of approaches have yielded data that are not consistent with the simple concept that G-proteins and their coupled receptors move freely in cells. This raises the possibility that the interaction of G-proteins and receptors with one another may not be based solely on the sequence of the interaction sites on the proteins themselves but depends on their organization in the cell. Rodbell (9) has proposed the idea that G-protein activation involves multimers of G-

proteins that exhibit a dynamic instability like actin. The concept proposed here incorporates his observations of large molecular complexes containing G-proteins and our data on the highly restricted mobility of the G-protein $\beta\gamma$ subunits in cells. Thus, there appears to be a more explicit organization of receptors, G-proteins, and probably effectors into supramolecular complexes that enhance the specificity of the protein-protein interactions (Fig. 1). Are there other components of the cell membrane and/or cytoskeleton (i.e., X or X' in Fig. 1) that limit the mobility of α and $\beta\gamma$ subunits of G-proteins? If so, are they spectrin- or dynamin-like proteins? Do they also organize the receptors and G-proteins in clusters? Is this organization specific for the type of receptor and G-protein? Is the role of the $\beta\gamma$ subunit subtype in specificity of receptor coupling (26, 27) due to a direct inter-

action of $\beta\gamma$ subunit with the receptor or is it due to a targeting of that $\beta\gamma$ subunit to the vicinity of the receptor? It is clear that the simple model of receptors and G-proteins floating in a Singer-Nicolson (107) sea of lipid needs to be reevaluated and more specific questions asked regarding the formation and maintenance of a sophisticated level of cellular organization of these important signal transducing components. [F]

The author thanks Drs. Ann Remmers and Bill Pratt for a critical review of the manuscript, Dr. Staffan Uhlén for helpful discussions, and Dr. Henry Bourne for many helpful comments. Support for the work described in this review was from the National Institutes of Health (GM 39561) and the author is an American Heart Association/Genentech Inc. Established Investigator.

Note added in proof: Two recent papers (Lisanti et al., *Trends Cell Biol.*, vol. 4, pp. 231-235, 1994, and Touhova et al., *J. Biol. Chem.*, vol. 269, pp. 10217-10220, 1994) discuss the role of caveolae and PH domains, respectively, in G-protein function.

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