Biochemistry

 Volume 35, Number 26 July 2, 1996

New Concepts in Biochemistry

Isoprenylation/Methylation of Proteins Enhances Membrane Association by a Hydrophobic Mechanism†

Craig A. Parish and Robert R. Rando*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 250 Longwood Avenue, Boston, Massachusetts 02115

Received March 11, 1996; Revised Manuscript Received May 1, 1996

Proteins of diverse types are posttranslationally modified at their carboxyl termini by isoprenylation/methylation (Sinensky & Lutz, 1992; Schafer & Rine, 1992; Omer & Gibbs, 1994; Clarke, 1992). These proteins include heterotrimeric G protein γ subunits (Yamane et al., 1990; Lai et al., 1990; Fukada et al., 1990), small G proteins (Takai et al., 1992), protein kinases (Inglese et al., 1992), nuclear lamins (Farnsworth et al., 1989), and viral proteins (Glenn et al., 1992). The modifications themselves are not of a single type and can be broadly divided into singly and doubly isoprenylated species (Figure 1). In the singly modified series, geranylgeranylation is by far the most prevalent modification. All heterotrimeric G proteins are geranylgeranylated with the exception of retinal transducin, which is farnesylated (Lai et al., 1990; Fukada et al., 1990). The growth-controlling small G protein *ras* is farnesylated/methylated, although some recent evidence indirectly suggests that the K-*ras* family member may possibly be geranylgeranylated/methylated (Lerner et al., 1995). Small G proteins which terminate in CC are bisgeranylgeranylated, while those terminating in CAC are both bisgeranylgeranylated and carboxymethylated (Farnsworth et al., 1991; Smeland et al., 1994; Yamane et al., 1991).

The modifications themselves are thought to allow otherwise soluble proteins to associate with membranes. The mechanism through which this enhanced membrane association occurs is of considerable interest. There are two major mechanisms through which this might occur, as indicated in Figure 2. The simplest mechanism involves lipid−lipid-based hydrophobic interactions, in which the isoprenylated/methylated cysteine moiety is held at the membrane's surface through hydrophobic interactions with membrane phospholipids. A second mechanism involves specific lipid−protein interactions in which the isoprenylated/methylated cysteine moiety is specifically recognized by a receptor protein (Marshall, 1993). It is the purpose of this article to review recent data concerned with the mechanism of membrane association of isoprenylated/methylated proteins.

A. ISOPRENYLATION/METHYLATION AS A HYDROPHOBIC MODIFICATION

One issue that must be addressed concerns the overall strength of the hydrophobic interactions of farnesylated and geranylgeranylated molecules and how methylation affects this hydrophobicity. While the simple addition of a methyl group per se would not be expected to have a dramatic effect on hydrophobicity, the fact that the methyl group neutralizes an otherwise negatively charged carboxylate would suggest a significant role for the methyl group here. Determinations of the *n*-octanol/water partition constants for isoprenylated cysteine derivatives show this to be the case. The data are as follows: *N*-acetyl-*S*-farnesyl-L-cysteine (AFC) (6.8 ± 0.2), AFC methyl ester (719.1 ± 13.4), *N*-acetyl-*S*-geranylgeranyl-L-cysteine (AGGC) (575.3 ± 99.3), and AGGC methyl ester (1436.8 ± 195.5) (D. Pérez-Sala and R. R. Rando, unpublished data). What is noteworthy here is that while all of the hydrophobic amino acids are more readily dissolved in *n*-octanol than in water (with the effect being considerably more pronounced with geranylgeranylated molecules), a very interesting effect of methylation is observed. In the farnesylated case (AFC), methylation is an extremely important

† The work described here from the authors' laboratory was funded by the U. S. Public Health Service National Institutes of Health Grant EY-03624.

* To whom correspondence should be addressed.

S0006-2960(96)00603-4 CCC: $12.00

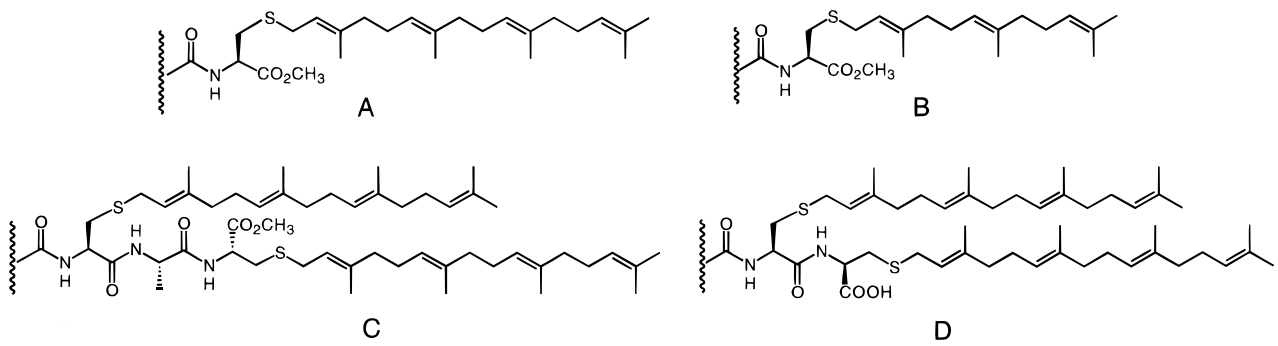

FIGURE 1: Biochemical scope of isoprenylation/methylation. (A) Singly geranylgeranylated/methylated protein; (B) singly farnesylated/methylated protein; (C) doubly geranylgeranylated/methylated CAC containing protein; (D) doubly geranylgeranylated CC containing protein.

determinant of the relative hydrophobicity, as indicated by the octanol/water partition coefficients, whereas in the case of geranylgeranylation, its effect is far less. This suggests that the role of methylation will be more important in farnesylated proteins than in the more hydrophobic geranylgeranylated proteins.

The results described above are also consistent with studies on the association of various isoprenylated/methylated peptides with synthetic liposomes (Silvius & l'Heureux, 1994; Shahinian & Silvius, 1995). It has been shown that, unlike their methylated counterparts, unmethylated farnesylated peptides associated only poorly with membranes. Moreover, methylation proved to be less important with respect to membrane binding when geranylgeranylated peptides were studied (Silvius & l'Heureux, 1994). The main conclusion here is that while isoprenylation by itself increases the ability of a peptide to associate with a membrane, methylation can also be important, especially in the case of farnesylation.

Patterns similar to those observed above were also seen in isoprenylated/methylated heterotrimeric G protein $\beta\gamma$ subunits. For example, farnesylated/methylated transducin binds to membranes, whereas its demethylated counterpart does not (Fukada et al., 1994; Bigay et al., 1994). On the other hand, geranylgeranylated $\beta_1\gamma_2$ binds tightly to membranes, irrespective of its state of methylation (Parish et al., 1996). Thus, if simple hydrophobic effects are central to isoprenylated/methylated protein interactions with membranes, then the state of methylation should be more important in the case of farnesylated proteins than in the case of geranylgeranylated proteins.

B. ROLE OF ISOPRENYLATION/METHYLATION IN HETEROTRIMERIC G PROTEIN FUNCTION

In attempting to understand the role of isoprenylation/methylation in protein function, it is important to study an isoprenylated/methylated system that can be described quantitatively. The retinal photoreceptor is the best understood of all the heterotrimeric G protein coupled receptor systems and often serves as a paradigm for the study of seven-transmembrane-helical receptor signal transduction (Stryer, 1991). While rhodopsin is an integral membrane protein, transducin (T) is farnesylated/methylated on its γ subunit (Lai et al., 1990; Fukada et al., 1990). Transducin's target, the phosphodiesterase, is farnesylated/methylated on one subunit and geranylgeranylated on the other (Anant et al., 1992), and rhodopsin kinase is farnesylated/methylated (Inglese et al., 1992). In this system, photoactivated rhodopsin (R*) interacts with T$\alpha\beta\gamma$ at the surface of the disk membrane and catalyzes the exchange of GDP for GTP. T$\beta\gamma$ is essential in this exchange reaction. Tα*GTP then interacts with the phosphodiesterase, relieving it of inhibition and allowing the latter enzyme to hydrolyze cGMP. As cGMP controls Na^+/Ca^{2+} conductance across the ROS plasma membranes, the hydrolysis of cGMP results in photoreceptor hyperpolarization and the initiation of the visual response (Stryer, 1991).

The visual cycle provides an ideal system for addressing the importance of isoprenylation/methylation since transducin and rhodopsin can be obtained as highly purified preparations and quantitative assays are available to thoroughly describe the effect of protein modifications. In order to study the roles of the farnesyl and methyl moieties in transducin function, biochemical means are required to remove these two functionalities. This is possible because of the enzymatic activities, shown in Figure 3, which are capable of processing T$\beta\gamma$ to provide mechanistically informative fragments. The role of the farnesyl/methyl group can be studied directly in the case of transducin, because a specific photoreceptor protease cleaves off the carboxyl terminal Gly-*S*-farnesylcysteinyl moiety of T$\beta\gamma$ (Figure 3) (Cheng et al., 1995). Freshly prepared proteolyzed T$\beta\gamma$ (Cheng et al., 1995) and material isolated from retina (Ohguro et al., 1991) are inert with respect to activating Tα in the presence of R* in detergent and in disk membranes. In addition, proteolyzed T$\beta\gamma$ is incapable of supporting the pertussis-toxin-catalyzed ADP-ribosylation of Tα·GDP (Ohguro et al., 1990). This reaction is a measure of the interaction between Tα and T$\beta\gamma$, since Tα is ADP-ribosylated only in the heterotrimeric form (Neer et al., 1984). These experiments show that isoprenylation/methylation is essential for fruitful interactions between Tα and T$\beta\gamma$ at the membrane. However, removal of the carboxyl terminal Gly-*S*-farnesylcysteinyl moiety of T$\beta\gamma$ is a substantial molecular alteration, and the fact that the resulting protein is inactive may be a consequence of several influences, including the gross alteration of protein structure. Protein demethylation is a more subtle modification, and studies on demethylated T$\beta\gamma$ would be expected to yield more definitive information on the functional role of isoprenylation/methylation. If a protein receptor exists which recognizes the farnesylated/methylated cysteine moiety, then binding should be quite sensitive to the state of methylation.

Transducin can be demethylated using immobilized pig liver esterase (Figure 3) (Parish & Rando, 1994). Moreover, as isolated, transducin exists in a partially demethylated state (Fukada et al., 1990; Pérez-Sala et al., 1991). When tested for its ability to support GTP for GDP exchange catalyzed

by activated rhodopsin (R*), demethylated T$\beta\gamma$ proved to be approximately 50% as active as methylated T$\beta\gamma$ in photoreceptor disk membranes (Parish & Rando, 1994) and in reconstituted liposomes containing rhodopsin (Fukada et al., 1994). In detergent, no difference was observed between methylated and demethylated T$\beta\gamma$, suggesting no role at all for the methyl group in functional interactions between Tα, T$\beta\gamma$, and R* (Parish & Rando, 1994). The 2-fold activity difference observed in membranes (Parish & Rando, 1994) can be accounted for by the 2-fold lessened affinity of the demethylated T$\beta\gamma$, compared with its methylated counterpart, for membranes in the presence of R* and Tα (Fukada et al., 1994). While a 2-fold effect is not very large quantitatively, it still may play a role in fine-tuning the visual response *in vivo*. It is interesting to note that a substantially larger difference (>10-fold) in the relative binding of methylated versus demethylated T$\beta\gamma$ to membranes is observed in the absence of R* and Tα (Fukada et al., 1994). As expected from the model systems described above, the state of methylation would be expected to have a profound effect on the membrane associative properties of farnesylated proteins, but not geranylgeranylated ones. However, R* has a substantial affinity for T$\alpha\beta\gamma$, and the influence of R* and Tα greatly reduces any differences resulting from the presence or absence of a methyl group on T$\beta\gamma$.

The results from studies on demethylated T$\beta\gamma$ demonstrate that specific lipid−receptor interactions are unlikely to play an important role in the rhodopsin/transducin system and further show that the effect of methylation is probably due to the increased hydrophobicity of methylated T$\beta\gamma$ versus its unmethylated counterpart. These studies are, of course, relevant to heterotrimeric G proteins, and specifically to the interactions of receptor (R*) with Tα and T$\beta\gamma$. In addition, heterotrimeric G protein $\beta\gamma$ subunits can have effectors other than α subunits (Clapham & Neer, 1993; Sternweis, 1994). Heterotrimeric G protein $\beta\gamma$ subunits can activate a number of enzymes, including certain isoforms of PI-3 kinase (Stephens et al., 1994; Thomason et al., 1994) and PLC-β (Smrcka & Sternweis, 1993; Dietrich et al., 1994), adenylate cyclase (Tang & Gilman, 1991), and certain ion channels (Wickman et al., 1994; Reuveny et al., 1994). It would be of interest to determine whether $\beta\gamma$ methylation is important for the activation of some of these effectors.

Isoforms of PI-3 kinase and PLC-β are normally activated by geranylgeranylated $\beta\gamma$ (Stephens et al., 1994; Ueda et al., 1994). The role of geranylgeranylated $\beta\gamma$ is presumably to direct the enzymes to membrane sites, where they act on their membrane substrates. The geranylgeranylated $\beta_1\gamma_2$ isoform can be readily demethylated by PLE, so the role of methylation can easily be determined (Parish et al., 1996). Interestingly, methylation proved to have only a small effect on activation. An approximately 25% diminution in efficacy, but not potency, was observed for the demethylated geranylgeranylated $\beta_1\gamma_2$ versus its methylated counterpart, for both enzymes. This again shows that specific lipid−protein interactions are unimportant. The effect of methylation on membrane binding would be expected to be small, given that $\beta_1\gamma_2$ is geranylgeranylated. It is of interest to compare these results with those found with methylated and unmethylated T$\beta\gamma$ as potential activators of the two enzymes (Parish et al., 1995). In this instance there was a very large effect noted, with methylated T$\beta\gamma$ being at least 10-fold more potent than its unmethylated counterpart with respect to activating either enzyme. This result is readily understandable in light of the role of methylation in selectively enhancing hydrophobicity of farnesylated proteins as opposed to geranylgeranylated proteins.

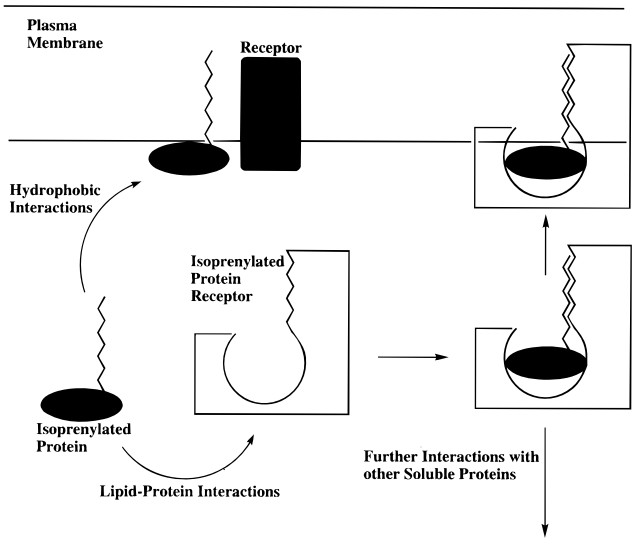

FIGURE 2: Modes of interaction of isoprenylated/methylated proteins with membranes and receptors.

The possibility that specific lipid−protein interactions are involved in the interaction of T$\beta\gamma$ with other proteins would suggest that isoprenylated/methylated peptides would be competitive inhibitors of enzymatic assays involving T$\beta\gamma$. While these molecules can inhibit the exchange of GTPγS for GDP on Tα or the pertussis toxin mediated ADP-ribosylation of Tα at high concentrations ($\sim$100 μM) of isoprenylated peptides (Scheer & Gierschik, 1995; Matsuda et al., 1994), it is unclear if this inhibition is specific in nature. Isoprenylated peptides have been shown to stabilize metarhodopsin II (Kisselev et al., 1994, 1995), but the specificity of this result is in question due to the high concentration of peptides required, the inability of T$\beta\gamma$ alone to stabilize activated rhodopsin, and previous reports that meta II formation is sensitive to lipid and detergent composition (Baldwin & Hubbell, 1985; König et al., 1989). At high concentrations of farnesyl cysteine derivatives, rod outer segment membranes actually begin to dissolve (C. A. Parish, and R. R. Rando, unpublished data). Moreover, the inhibitory effect of the isoprenylated cysteine derivatives is not stereospecific and can be mimicked by nonspecific reagents such as farnesoic acid and palmitic acid (C. A. Parish, and R. R. Rando, unpublished data). Therefore, in our opinion, it appears that currently there is no strong evidence to support the view that specific lipid−protein interactions are involved in the transducin−rhodopsin system.

The results described above show that isoprenylation/methylation of heterotrimeric G protein $\beta\gamma$ subunits is essentially a modification which operates by enhancing the hydrophobicity of the modified proteins. It has been suggested that the activity of isoprenylated/methylated proteins may be regulated by the protein's state of methylation (Philips et al., 1993; Marshall, 1993). This suggestion makes an analogy between the known role of protein methylation in bacterial chemotaxis (Kort et al., 1975; Shapiro et al., 1995) and a conceivably similar role in mammalian signal transduction mechanisms. The studies summarized here speak to the question of whether this

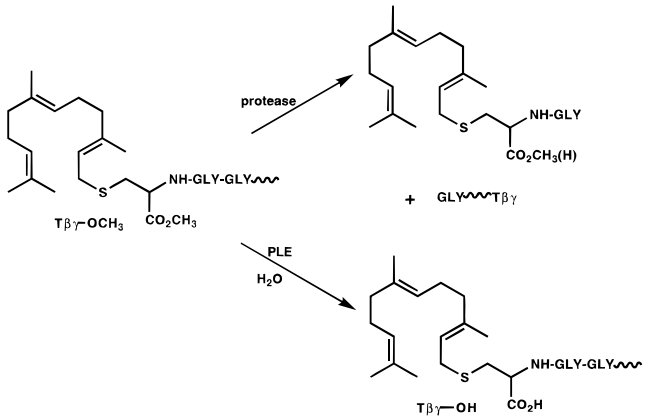

FIGURE 3: Enzymatic processing of retinal transducin. Pig liver esterase (PLE) can specifically hydrolyze the methyl ester, and a rod outer segment protease cleaves off the terminal dipeptide.

analogy is valid. At this time, no good evidence exists for a regulatory role for methylation in mammalian signal transduction, and there are many examples in the literature, in addition to the studies described above on demethylated transducin, which indicate that methylation has either no functional role or only a very small role. For example, the *rab* 3A small G protein contains a CAC motif and is normally bisgeranylgeranylated and methylated (Yamane et al., 1991). Expressed protein which is still bisgeranylgeranylated, but not methylated, is approximately as active as its methylated counterpart (Musha et al., 1992). Furthermore, the idea that methylation does not have a profound influence on isoprenylated protein function is strengthened by the observation that separate isoprenylated protein methyltransferases do not appear to exist for geranylgeranylated and farnesylated substrates, as would be anticipated if methylation were a regulatory event (Smeland et al., 1994; Pérez-Sala et al., 1992). Moreover, a yeast mutant which lacks isoprenylated protein methyltransferase activity differs from wild type only in growing more slowly and in being unable to mate (Hrycyna et al., 1991). The latter observation is explained by the fact that the isoprenylated undecapeptide yeast mating factor requires methylation for activity (Anderegg et al., 1988).

C. ADDITIONAL STUDIES ON THE FUNCTION ISOPRENYLATION/METHYLATION

Although the results described above are most directly relevant to heterotrimeric G protein function, it is possible that the role of isoprenylation/methylation in other systems, especially the small G proteins, involves specific recognition by a receptor. One instance where this appears to be the case is the interaction between *rab* and its GDP dissociation inhibitor (*rab*-GDI). These proteins form a 1:1 complex, translocating *rab* from the membrane to the cytosol (Pfeffer et al., 1995). However, the idea that isoprenylation/methylation is primarily a hydrophobic modification is supported by a number of other studies involving a diverse group of proteins in addition to the heterotrimeric G proteins. For example, when the normally farnesylated rhodopsin kinase is geranylgeranylated, the protein remains quite active, although it no longer shuttles between soluble and membrane-bound states (Inglese et al., 1992). In fact, rhodopsin kinase still shows some activity even when the isoprenoid is missing (Inglese et al., 1992). The small G protooncogene *ras* is rendered inactive when a serine residue, which is not farnesylated, is engineered in to replace the normally farnesylated cysteine residue (Hancock et al., 1989). Interestingly, some activity can be regained when a myristoyl site is added to the N-terminus of this protein (Buss et al., 1989). A mutant *rab*6 small G protein in which the two geranylgeranyl groups have been replaced with palmitoyl and farnesyl groups behaves like wild-type protein in membrane localization and functional studies (Beranger et al., 1994). Moreover, in no case has there yet been a demonstration of the importance of methylation in small G protein function. While there is always the possibility that putative receptors exist which recognize isoprenylated/methylated cysteine moieties, these receptors would appear not to be sensitive to the nature of the hydrophobic modification or to the state of methylation. It should also be noted that various isoprenylated proteins are targeted to particular membrane targets, such as the Golgi apparatus. While isoprenylation is important here to allow for secure membrane association, the actual targeting and specificity of the process appears likely to be mediated by protein−protein interactions.

While it is true that specific lipid−protein interactions mediated via isoprenylated/methylated cysteine residues have yet to be identified, it is interesting to note that farnesyl cysteine analogs can have profound pharmacological effects on signal transducing systems in a variety of cell types (Scheer & Gierschik, 1993, 1995; Ding et al., 1994; Philips et al., 1993; Ma et al., 1994; Huzoor-Akbar et al., 1993; Marom et al., 1995). Whether these effects are specifically related to isoprenylated/methylated protein function is currently unknown, but this possibility must be kept in mind pending the identification of putative pharmacological targets for these analogs.

REFERENCES

Anant, J. S., Ong, O. C., Xie, H., Clarke, S., & O'Brien, P. J. (1992) *J. Biol. Chem. 267*, 687−690.
Anderegg, R. J., Betz, R., Carr, S. A., Crabb, J. W., & Duntze, W. (1988) *J. Biol. Chem. 263*, 18236−18240.
Baldwin, P. A., & Hubbell, W. L. (1985) *Biochemistry 24*, 2633−2639.
Beranger, F., Paterson, H., Powers, S., de Gunzberg, J., & Hancock, J. F. (1994) *Mol. Cell. Biol. 14*, 744−758.
Bigay, J., Faurobert, E., Franco, M., & Chabre, M. (1994) *Biochemistry 33*, 14081−14090.
Buss, J. E., Solski, P. A., Schaeffer, J. P., MacDonald, M. J., & Der, C. J. (1989) *Science 243*, 1600−1603.
Cheng, H., Parish, C. A., Gilbert, B. A., & Rando, R. R. (1995) *Biochemistry 34*, 16662−16671.
Clapham, D. E., & Neer, E. J. (1993) *Nature 365*, 403−406.
Clarke, S. (1992) *Annu. Rev. Biochem. 61*, 355−386.
Dietrich, A., Meister, M., Brazil, D., Camps, M., & Gierschik, P. (1994) *Eur. J. Biochem. 219*, 171−178.
Ding, J., Lu, D. J., Pérez-Sala, D., Ma, Y. T., Maddox, J. F., Gilbert, B. A., Badwey, J. A., & Rando, R. R. (1994) *J. Biol. Chem. 269*, 16837−16844.
Farnsworth, C. C., Wolda, S. L., Gelb, M. H., & Glomset, J. A. (1989) *J. Biol. Chem. 264*, 20422−20429.
Farnsworth, C. C., Kawata, M., Yoshida, Y., Takai, Y., Gelb, M. H., & Glomset, J. A. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6196−6200.
Fukada, Y., Takao, T., Ohguro, H., Yoshizawa, T., Akino, T., & Shimonishi, Y. (1990) *Nature 346*, 658−660.
Fukada, Y., Matsuda, T., Kokame, K., Takao, T., Shimonishi, Y., Akino, T., & Yoshizawa, T. (1994) *J. Biol. Chem. 269*, 5163−5170.
Glenn, J. S., Watson, J. A., Havel, C. M., & White, J. M. (1992) *Science 256*, 1331−1333.

Hancock, J. F., Magee, A. I., Childs, J. E., & Marshall, C. J. (1989) *Cell 57*, 1167−1177.
Hrycyna, C. A., Sapperstein, S. K., Clarke, S., & Michaelis, S. (1991) *EMBO J. 10*, 1699−1709.
Huzoor-Akbar, Wang, W., Kornhauser, R., Volker, C., & Stock, J. B. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 868−872.
Inglese, J., Koch, W. J., Caron, M. G., & Lefkowitz, R. J. (1992) *Nature 359*, 147−150.
Kisselev, O. G., Ermolaeva, M. V., & Gautam, N. (1994) *J. Biol. Chem. 269*, 21399−21402.
Kisselev, O. G., Ermolaeva, M. V., & Gautam, N. (1995) *J. Biol. Chem. 270*, 25356−25358.
König, B., Welte, W., & Hofmann, K. P. (1989) *FEBS Lett. 257*, 163−166.
Kort, E. N., Goy, M. F., Larsen, S. H., & Adler, J. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3939−3943.
Lai, R. K., Pérez-Sala, D., Cañada, F. J., & Rando, R. R. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7673−7677.
Lerner, E. C., Qian, Y., Hamilton, A. D., & Sebti, S. (1995) *J. Biol. Chem. 270*, 26770−26773.
Ma, Y.-T., Shi, Y.-Q., Lim, Y. H., McGrail, S. H., Ware, J. A., & Rando, R. R. (1994) *Biochemistry 33*, 5414−5420.
Marom, M., Haklai, R., Ben-Baruch, G., Marciano, D., Egozi, Y., & Kloog, Y. (1995) *J. Biol. Chem. 270*, 22263−22270.
Marshall, C. J. (1993) *Science 259*, 1865−1866.
Matsuda, T., Takao, T., Shimonishi, Y., Murata, M., Asano, T., Yoshizawa, T., & Fukada, Y. (1994) *J. Biol. Chem. 269*, 30358−30363.
Musha, T., Kawata, M., & Takai, Y. (1992) *J. Biol. Chem. 267*, 9821−9825.
Neer, E. J., Lok, J. M., & Wolf, L. G. (1984) *J. Biol. Chem. 259*, 14222−14229.
Ohguro, H., Fukada, Y., Yoshizawa, T., Saito, T., & Akino, T. (1990) *Biochem. Biophys. Res. Commun. 167*, 1235−1241.
Ohguro, H., Fukada, Y., Takao, T., Shimonishi, Y., Yoshizawa, T., & Akino, T. (1991) *EMBO J. 10*, 3669−3674.
Omer, C. A., & Gibbs, J. B. (1994) *Mol. Microbiol. 11*, 219−225.
Parish, C. A., & Rando, R. R. (1994) *Biochemistry 33*, 9986−9991.
Parish, C. A., Smrcka, A. V., & Rando, R. R. (1995) *Biochemistry 34*, 7722−7727.
Parish, C. A., Smrcka, A. V., & Rando, R. R. (1996) *Biochemistry 35* (in press).
Pérez-Sala, D., Tan, E. W., Cañada, F. J., & Rando, R. R. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 3043−3046.
Pérez-Sala, D., Gilbert, B. A., Tan, E. W., & Rando, R. R. (1992) *Biochem. J. 284*, 835−840.
Pfeffer, S. R., Dirac-Svejstrup, A. B., & Soldati, T. (1995) *J. Biol. Chem. 268*, 17057−17059.
Philips, M. R., Pillinger, M. H., Staud, R., Volker, C., Rosenfeld, M. G., Weissmann, G., & Stock, J. B. (1993) *Science 259*, 977−980.
Reuveny, E., Slesinger, P. A., Inglese, J., Morales, J. M., Iñiguez-Lluhi, J. A., Lefkowitz, R. J., Bourne, H. R., Jan, Y. N., & Jan, L. Y. (1994) *Nature 370*, 143−146.
Schafer, W. R., & Rine., J. (1992) *Annu. Rev. Genet. 30*, 209−237.
Scheer, A., & Gierschik, P. (1993) *FEBS Lett. 319*, 110−114.
Scheer, A., & Gierschik, P. (1995) *Biochemistry 34*, 4952−4961.
Shahinian, S., & Silvius, J. R. (1995) *Biochemistry 34*, 3813−3822.
Shapiro, M. J., Chakrabarti, I., & Koshland, D. E. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 1053−1056.
Silvius, J. R., & l'Heureux, F. (1994) *Biochemistry 33*, 3014−3022.
Sinensky, M., & Lutz, R. J. (1992) *BioEssays 14*, 25−31.
Smeland, T. E., Seabra, M. C., Goldstein, J. L., & Brown, M. S. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 10712−10716.
Smrcka, A. V., & Sternweis, P. C. (1993) *J. Biol. Chem. 268*, 9667−9774.
Stephens, L., Smrcka, A., Cooke, F. T., Jackson, T. R., Sternweis, P. C., & Hawkins, P. T. (1994) *Cell 77*, 83−93.
Sternweis, P. C. (1994) *Curr. Opin. Cell Biol. 6*, 198−203.
Stryer, L. (1991) *J. Biol. Chem. 266*, 10711−10714.
Takai, Y., Kaibuchi, K., Kikuchi, A., & Kawata, M. (1992) *Int. Rev. Cytol. 133*, 187−230.
Tan, E. W., & Rando, R. R. (1992) *Biochemistry 31*, 5572−5578.
Tang, W.-J., & Gilman, A. G. (1991) *Science 254*, 1500−1503.
Thomason, P. A., James, S. R., Casey, P. J., & Downes, C. P. (1994) *J. Biol. Chem. 269*, 16525−16528.
Ueda, N., Iñiquez-Lluhi, J. A., Lee, E., Smrcka, A. V., Robishaw, J. D., & Gilman, A. G. (1994) *J. Biol. Chem. 269*, 4388−4395.
Wickman, K. D., Iñiguez-Lluhi, J. A., Davenport, P. A., Taussig, R., Krapivinsky, G. B., Linder, M. E., Gilman, A. G., & Clapham, D. E. (1994) *Nature 368*, 255−257.
Yamane, H. K., Farnsworth, C. C., Xie, H., Howald, W., Fung, B. K.-K., Clarke, S., Gelb, M. H., & Glomset, J. A. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 5868−5872.
Yamane, H. K., Farnsworth, C. C., Xie, H., Evans, T., Howland, W. N., Gelb, M. H., Glomset, J. H., Clarke, S., & Fung, B. K.-K. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 286−290.

BI960603G