Molecular Biology of the Cell
Vol. 5, 1–5, January 1994

Essay

Guanylyl Cyclase Receptors

David L. Garbers,* Doris Koesling,† and Günter Schultz†

*Howard Hughes Medical Institute and Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas 75235; and †Institut für Pharmakologie, Freie Universität Berlin, D-14195, Berlin, Germany

INTRODUCTION

For about two decades, the enzymes (guanylyl cyclases) that synthesize cyclic GMP have been described as soluble or particulate, depending upon where they are found in a cell homogenate (Kimura and Murad, 1974; Chrisman *et al.*, 1975). The particulate forms have been often further separated into those that are easily solubilized with nonionic detergents and those that are not. Early studies suggested that the enzymes found in the soluble and particulate fractions were different proteins, and the cloning of the mRNA not only confirmed this, but demonstrated that multiple subtypes existed within both compartments. The particulate forms appear to contain a single transmembrane domain, a protein kinase homology domain, and a single putative catalytic site, whereas the soluble forms contain two subunits, each of which exhibits a putative consensus cyclase catalytic domain (Figure 1).

Four different cDNA clones (α_1, α_2, β_1, β_2) encoding homologous proteins have been obtained for subunits of the soluble guanylyl cyclases (Koesling *et al.*, 1991; Garbers, 1992; Yuen and Garbers, 1992), but only the heterodimer, $\alpha_1\beta_1$, has been purified from tissue extracts and shown to contain associated heme (Figure 1) (Garbers, 1979; Gerzer *et al.*, 1981; Humbert *et al.*, 1990). Therefore, the pairing of the remaining subunits, whether they are normally found as heteromers and whether they are actually present within the cytoplasm, is not known. Coexpression of α_2/β_1 results in the formation of an active enzyme (Harteneck *et al.*, 1991), but it is not known if $\alpha 2\beta 1$ exists normally. Nitric oxide appears to be a ligand for $\alpha 1\beta 1$ and activates the heterodimer by as much as 170-fold (Humbert *et al.*, 1990).

The known particulate guanylyl cyclases contain a single putative transmembrane domain that separates an extracellular ligand binding domain from intracellular protein kinase-like and cyclase catalytic domains (Figure 1). Four unique cDNA clones have been isolated from various mammalian cDNA libraries for members of this part of the family and have been designated as GC-A(NPR-A), GC-B(NPR-B), GC-C(STaR), and RetGC (Koesling *et al.*, 1991; Garbers, 1992; Shyjan *et al.*, 1992). Atrial natriuretic peptide (ANP), C-type natriuretic peptide (CNP), and heat-stable enterotoxins/guanylin appear to represent the natural ligands for GC-A, GC-B, and GC-C, respectively (Koesling *et al.*, 1991; Garbers, 1992). The ligands are synthesized in many different tissues and likely function both locally and as circulating hormones. The RetGC has the same signature domains as the other particulate forms but has been suggested to function as the photoreceptor guanylyl cyclase, not responding to extracellular ligands (Shyjan *et al.*, 1992); however, a limited number of potential ligands have been examined, and whether the enzyme is localized to rod outer segments has not been confirmed. A guanylyl cyclase purified from rod outer segments (Margulis *et al.*, 1993), in fact, could be encoded by a different mRNA than the one cloned from retina (Shyjan *et al.*, 1992). The ANP-clearance receptor (Fuller *et al.*, 1988) is another member of this family; it binds ANP and other natriuretic peptides but contains neither a protein kinase-like nor a cyclase catalytic domain (Figure 1). There has been controversy about the function of this receptor. Some have suggested that it functions as a clearance receptor, whereas others have argued that it is a signaling molecule.

The requirements for basal cyclase activity or for ligand activation of the cyclases are just beginning to be understood, and although principally at a speculative stage, this will be area of focus for the essay.

IS A DIMER THE MINIMUM CATALYTIC ELEMENT OF CYCLASES?

All cyclases contain at least one region homologous to an area known to contain the catalytic domain in the guanylyl cyclase-A receptor (Figure 2) (Chinkers *et al.*, 1989; Thorpe and Morkin, 1990). Each of the subunits of the soluble forms of guanylyl cyclase contain one such domain (Koesling *et al.*, 1991; Yuen and Garbers, 1992), and mammalian adenylyl cyclases contain two homologous regions (Krupinski *et al.*, 1989). We will refer to these regions of homology as consensus catalytic domains, even though they may not always function as cyclase catalytic sites. A single consensus catalytic domain may not be sufficient for production of cyclic GMP from guanylyl cyclases or of cyclic AMP from adenylyl cyclases, although studies have not yet ruled out the possibility of low rates of cyclic nucleotide formation by a monomeric site. (A monomeric site in this case is considered as a single consensus catalytic site. There are two such sites in a single mammalian adenylyl

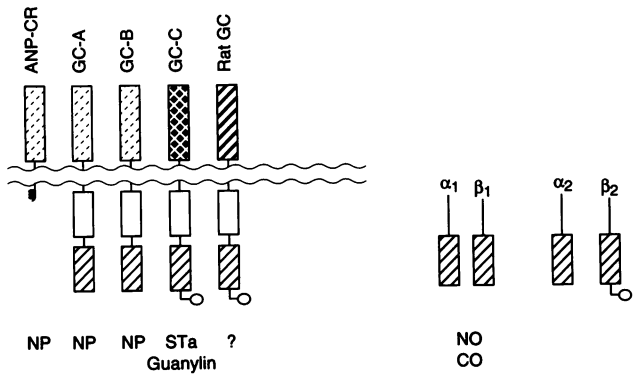

Figure 1. Members of the guanylyl cyclase receptor family. NP, natriuretic peptide; STa, heat stable enterotoxin; NO, nitric oxide; CO, carbon monoxide.

cyclase polypeptide chain). The observations that support a dimer as the minimum catalytic unit include:

1) Expression of individual α or β subunits, each of which contain a consensus cyclase catalytic domain, results in no detectable soluble guanylyl cyclase activity, whereas coexpression of the subunits in various cultured cell lines leads to high cyclase activity (Harteneck *et al.*, 1990; Buechler *et al.*, 1991).
2) Deletion mutants of the GC-A receptor, where essentially only the consensus cyclase catalytic domain are expressed, possess enzyme activity, but all activity migrates coincident with homodimers (Thorpe *et al.*, 1991; Thompson and Garbers, unpublished data).
3) Mammalian adenylyl cyclases contain two consensus cyclase catalytic domains, but constructs where each putative catalytic domain are expressed separately lead to negligible enzyme activity. However, activity is regained when the separated proteins are cotransfected (Tang *et al.*, 1991).
4) Certain point mutations within the consensus catalytic domain of guanylyl cyclases act as dominant negative mutations by virtue of their ability to form inactive homomers or heteromers with wild-type subunits (Yuen *et al.*, 1994; Thompson and Garbers, unpublished data).

In the latter studies, both the soluble ($\alpha_1\beta_1$) and particulate (GC-A) cyclases have been inactivated in a dominant negative manner by point mutations within the putative catalytic domain. Taken together, the data provide support for an argument that dimerization is required for expression of high cyclase catalytic activity; dominant negative point mutations also raise the question of whether a single catalytic site is formed during the dimerization process, although conceivably the dimerization may be required for shared GTP binding sites or for the appropriate folding of each subunit. Determination of the number of GTP (guanylyl cyclases) or ATP (adenylyl cyclases) molecules bound per dimer, nucleotide cross-linking studies, activity measurements at high concentrations of monomeric sites to determine whether low rates of catalysis occurs, and ultimately the crystal structure of the catalytic domain are now required to determine whether cyclases form a single catalytic site per dimer.

HOMOMERS OR HETEROMERS?

If a dimer is the fundamental unit for catalysis, are the monomeric sites within each dimer the same or different? At least one form of soluble guanylyl cyclase exists as a heterodimer ($\alpha_1\beta_1$), but whether or not soluble homodimers also form is not yet known. If they exist, the homodimers of α_1 or β_1 apparently possess low enzyme activity relative to that of the heterodimer. GC-A is known to form highly active homodimers, but an epitope-tagged and truncated form of GC-A has been recently shown capable of forming heteromers with GC-B in overproducing cells (Chinkers and Wilson, 1992). Furthermore, Koller *et al.* (1992) have demonstrated that the protein kinase homology domains of GC-A and GC-B can be interchanged with retention of the signaling function of either receptor, suggesting that various regions can function as elements in other receptors. Because mammalian adenylyl cyclases contain two internal consensus catalytic regions, they could form intramolecular heteromers or intermolecular hetero- or homodimers dependent on the alignment of two cyclase polypeptide chains and on whether or not different adenylyl cyclase subtypes interact. If the folding of two monomeric sites represents the necessary structure of an active guanylyl cyclase (or adenylyl cyclase) and cyclases exist as tetramers or higher-ordered structures, the potential diversity in ligand binding or signaling properties of the cyclases could be significantly expanded in the face of but a relatively few enzyme genes.

HOW ARE CYCLASES ACTIVATED?

Conceivably conformational changes that may occur within the catalytic domain upon activation are the same for all cyclases independent of the activating agent.

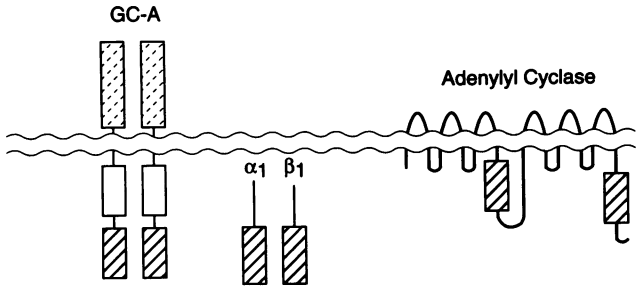

Figure 2. Two consensus catalytic domains are found in active guanylyl or adenylyl cyclases. The consensus catalytic regions are indicated by the stippled bar.

Studies have not concentrated on this question but instead on the state of oligomerization and on the requirements for activation of the cyclases.

The general conclusion from three groups studying GC-A has been that the receptor exists as an oligomer in the absence or presence of its ligand, ANP (Iwata *et al.*, 1991; Chinkers and Wilson, 1992; Lowe, 1992). However, the size of the oligomer, the site(s) of dimerization, and whether ANP causes significant changes in the state of oligmerization remain unclear (Iwata *et al.*, 1991; Chinkers and Wilson, 1992; Lowe, 1992). There are no substantial data on the size of GC-A as predicted from hydrodynamic measurements, although Chinkers and Wilson (1992) have suggested that it migrates as a tetrameric protein on gel filtration columns. The above studies also failed to address the relationship between aggregation state and relative enzyme activity.

ATP appears to potentiate, or be required for, transduction of the ANP binding signal to activation of the GC-A catalytic domain (Yuen *et al.*, 1992; Garbers, 1992); the site of ATP binding is not certain but is most likely the protein kinase domain. It also has been suggested, however, that ATP binds to regulatory proteins other than guanylyl cyclase (Chang *et al.*, 1990). The evidence for the latter hypothesis was not substantial, resting on the observation that ANP sensitivity is lost upon enzyme purification. However, in recent work (Wong, Foster, and Garbers, unpublished data), an ANP/ATP sensitive cyclase receptor has been purified to apparent homogeneity, and although these experiments do not rule out the possibility of another ATP-binding regulatory protein, they demonstrate that such a hypothetical protein is not necessary for receptor activation.

The mechanism by which ATP functions in the guanylyl cyclase receptors has been studied principally with GC-A, although there are recent studies on GC-C. The concentrations of ATP required for one-half maximal stimulation are in the mM range (Chinkers *et al.*, 1989, 1991; Vaandrager *et al.*, 1993b), contrasting to low μM concentrations of GTP required for activation of G-proteins. There is, as of yet, no evidence for associated protein kinase or ATPase activity. In GC-A, deletion of the protein kinase-like domain results in a constitutively active cyclase that is no longer regulated by ANP or ATP (Chinkers *et al.*, 1989), and mutations within the protein kinase-like domain destroy ATP regulation of the enzyme (Koller *et al.*, 1993). Therefore, it has been suggested that the protein kinase homology domain functions as a negative regulatory element (Chinkers *et al.*, 1989). In GC-C, monomers have been detected on reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) but homodimers on nonreducing SDS-PAGE. In the absence of ATP at temperatures >30°C, the size of the apparent dimer increases to that of a large aggregate, and heat-stable enterotoxin (STa)-stimulated activity is lost (Vaandrager *et al.*, 1993a,b). ATP, therefore, appears to stabilize the STa-sensitive homomer (Vaandrager *et al.*, 1993b). It is possible that ATP functions in the same manner in both GC-A and GC-C, stabilizing a ligand-sensitive conformation. In GC-C, this conformation may predominate in the basal state, whereas in GC-A, ATP may drive the equilibrium towards the ligand-sensitive conformation.

ANP has been reported to bind in the ratio of 1 mol ANP/mol cyclase monomer based on equilibrium binding studies. If the stoichiometry of binding is 1 mol ANP/mol subunit, the number of ANP molecules required for activation of the cyclase oligomer becomes critical to understanding signaling. A rather general observation among the guanylyl cyclase receptors has been an apparent Kd for ligand binding that is significantly lower than the concentrations required to one-half maximally elevate cyclic GMP. Therefore, cyclic GMP is often one-half maximally elevated when a majority of the receptor binding sites appear occupied. One explanation for these observations could be that the cyclase is not activated until the oligomer (possibly a tetramer or higher-ordered species) is completely occupied by ligand.

A shift from high affinity to low affinity ANP binding also may occur as a function of time after ANP addition to GC-A and appears to require the presence of the protein kinase homology domain (Jewett *et al.*, 1993). Given that Potter and Garbers (1992) have shown that ANP-induced dephosphorylation of GC-A coincides with desensitization, it is tempting to suggest that the affinity state change is because of dephosphorylation. In GC-A overproducing cells such as 3T3 fibroblasts, phosphorylation is principally on serine residues but also occurs on threonine. The mechanisms by which dephosphorylation desensitizes is not known; the site(s) of phosphorylation, the number of phosphorylation sites/subunit, and whether dephosphorylation of specific sites on all subunits is required for desensitization remain major unanswered questions. Phosphorylation may also regulate subunit/subunit interactions because sea urchin sperm guanylyl cyclase purified to apparent homogeneity, shifts from positive cooperative to linear kinetic behavior as a function of GTP concentration upon dephosphorylation (Ramarao and Garbers, 1988).

Although models for adenylyl cyclase and guanylyl cyclase regulation contain common features, such as a hormone receptor and a nucleotide-binding regulatory component (Figure 3), there appear to be major differences in addition to a separation of the protein components in the adenylyl cyclase pathway. The guanine nucleotide binding protein, Gs, can be activated by low (μM) concentrations of GTP whereas mM concentrations of ATP are required for activation of the guanylyl cyclase. No ATPase activity has been found associated with GC-A, although the appropriate stimulus may not have been applied. For example, possibly the cyclase

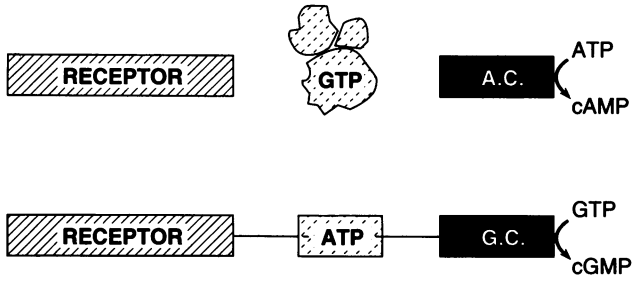

Figure 3. Comparison of models for adenylyl cyclase and guanylyl cyclase regulation through a hormone receptor.

requires an ATPase activating protein and possesses no detectable ATPase activity in the absence of such a factor. ATP analogues also do not appreciably activate the cyclase in the absence of ligand, although this too has not been extensively studied. With the sea urchin guanylyl cyclase, for example, ATP has been shown to activate the enzyme at low GTP concentrations (Garbers *et al.*, 1974).

Purified soluble guanylyl cyclase ($\alpha_1\beta_1$) contains heme as a prosthetic group (Gerzer *et al.*, 1981, Humbert *et al.*, 1990), and it is the heme that has been proposed as the receptor for nitric oxide and more recently for carbon monoxide (Verma *et al.*, 1993). Although it has been suggested that binding of NO to the sixth coordination position of the heme iron disrupts the proximal base-iron bond and that release of the proximal base then results in a conformational change in the enzyme (Traylor and Sharma, 1992), little is understood with respect to the molecular basis of nitric oxide activation of soluble cyclases. Recently, Wedel and colleagues (1993) discovered that mutation of His-105 of the β_1 subunit caused a loss of NO sensitivity and of heme content without substantial losses in basal cyclase activity. Various other His mutations within the α_1 or β_1 subunit failed to alter responsiveness to NO. His-105 is located within the amino terminal regions of the β_1 subunit, well in front of the consensus catalytic domain, and thus the binding of heme may occur within this region. Another gas, CO, which was reported in the past to stimulate soluble guanylyl cyclases several-fold (Brune *et al.*, 1990), also has now been suggested as a physiological activator (Verma *et al.*, 1993; Zhou *et al.*, 1993). The arguments for CO as a normal regulatory molecule have rested principally on the colocalization of soluble guanylyl cyclase and of heme oxygenase-2 (an enzyme that forms CO in the process of heme degradation) in the brain (Verma *et al.*, 1993). It has not been determined whether heme oxygenase is regulated in a manner consistent with potential physiological regulation of the cyclase (Maines, 1988), but even if not, possibly other pathways of CO synthesis will be discovered.

CONCLUSIONS

Guanylyl cyclase receptors exist in virtually all cells, and cGMP concentrations therefore can be markedly altered; yet in only a few instances are there descriptions of a distinct behavioral change in response to cGMP elevations. The cyclase receptors may serve as effective reagents in searches aimed at uncovering the functions of cGMP in the many cells where its importance remains unknown. That cyclases appear to require at least dimerization for activity has led to the development of dominant negative subunits that can specifically interrupt a cGMP signaling pathway. The cloning of new receptors and the discovery of new ligands has and will lead to the introduction of foreign cyclase receptors into given cells, to not only aid in defining the function of cGMP, but in determining whether the cyclase-coupled receptors also signal through pathways not involving cGMP.

ACKNOWLEDGMENTS

We would like to dedicate this essay to Dr. Eycke Böhme who published many pioneering studies on the soluble forms of guanylyl cyclase. This study was supported in part by an Alexander von Humboldt Foundation U.S.A. Senior Scientist Award.

REFERENCES

Brune, B., Schmidt, K.-U., and Ullrich, V. (1990). Activation of soluble guanylate cyclase by carbon monoxide and inhibition by superoxide anion. Eur. J. Biochem. *192*, 683–688.

Buechler, W.A., Nakane, M., and Murad, F. (1991). Expression of soluble guanylate cyclase activity requires both enzyme subunits. Biochem. Biophys. Res. Commun. *174*, 351–357.

Chang, C.H., Kohse, K.P., Chang, B., Hirata, M., Jiang, B., Douglas, J.B., and Murad, F. (1990). Characterization of ATP-stimulated guanylate cyclase activation in rat lung membranes. Biochim. Biophys. Acta *1052*, 159–165.

Chinkers, M., and Garbers, D.L. (1989). The protein kinase domain of the ANP receptor is required for signaling. Science *245*, 1392–1394.

Chinkers, M., Singh, S., and Garbers, D.L. (1991). Adenine nucleotides are required for activation of rat atrial natriuretic peptide receptor/guanylyl cyclase expressed in a baculovirus system. J. Biol. Chem. *266*, 4088–4093.

Chinkers, M., and Wilson, E.M. (1992). Ligand-independent oligomerization of natriuretic peptide receptors. Identification of heteromeric receptors and a dominant negative mutant. J. Biol. Chem. *267*, 18589–18597.

Chrisman, T.D., Garbers, D.L., Parks, M.A., and Hardman, J.G. (1975). Characterization of particulate and soluble guanylate cyclases from rat lung. J. Biol. Chem. *250*, 374–381.

Fuller, F., Porter, J.G., Arfsten, A.E., Miller, J., Schilling, J.W., Scarborough, R.M., Lewicki, J.A., and Schenk, D.B. (1988). Atrial natriuretic peptide clearance receptor. Complete sequence and functional expression of cDNA clones. J. Biol. Chem. *263*, 9395–9401.

Garbers, D.L., Hardman, J.G., and Rudolph, F.B. (1974). Kinetic analysis of sea urchin sperm guanylate cyclase. Biochemistry *13*, 4166–4171.

Garbers, D.L. (1979). Purification of soluble guanylate cyclase from rat lung. J. Biol. Chem. *254*, 240–243.

Garbers, D.L. (1992). Guanylyl cyclase receptors and their endocrine, paracrine, and autocrine ligands. Cell *71*, 1–4.

Gerzer, R., Böhme, E., Hofmann, F., and Schultz, G. (1981). Soluble guanylate cyclase purified from bovine lung contains heme and copper. FEBS Lett. *132*, 71–74.

Harteneck, C., Koesling, D., Söling, A., Schultz, G., and Böhme, E. (1990). Expression of soluble guanylate cyclase: catalytic activity requires two enzyme subunits. FEBS Lett. *272*, 221–223.

Harteneck, C., Wedel, B., Koesling, D., Malkewitz, J., Böhme, E., and Schultz, G. (1991). Molecular cloning and expression of a new α-subunit of soluble guanylyl cyclase. Interchangeability of the α-subunits of the enzyme. FEBS Lett. *292*, 217–222.

Humbert, P., Niroomand, F., Fischer, G., Mayer, B., Koesling, D., Hinsch, K.-D., Gausepohl, H., Frank, R., Schultz, G., and Böhme, E. (1990). Purification of soluble guanylyl cyclase from bovine lung by a new immunoaffinity chromotographic method. Eur. J. Biochem. *190*, 273–278.

Iwata, T., Uchida-Mizuno, K., Katafuchi, T., Ito, T., Hagiwara, H., and Hirose, S. (1991). Bifunctional atrial natriuretic peptide receptor (type A) exists as a disulfide-linked tetramer in plasma membranes of bovine adrenal cortex. J. Biochem. *110*, 35–39.

Jewett, J.R.S., Koller, K.J., Goeddel, D.V., and Lowe, D.G. (1993). Hormonal induction of low affinity receptor guanylyl cyclase. EMBO J. *12*, 769–777.

Kimura, H., and Murad, F. (1974). Evidence for two different forms of guanylate cyclase in rat heart. J. Biol. Chem. *249*, 6910–6916.

Koesling, D., Böhme, E., and Schultz, G. (1991). Guanylyl cyclases, a growing family of signal-transducing enzymes. FASEB J. *5*, 2785–2791.

Koller, K.J., deSauvage, F.J., Lowe, D.G., and Goeddel, D.V. (1992). Conservation of the kinaselike regulatory domain is essential for activation of the natriuretic peptide receptor guanylyl cyclases. Mol. Cell. Biol. *12*, 2581–2590.

Koller, K.J., Lipari, M.T., and Goeddel, D.V. (1993). Proper glycosylation and phosphorylation of the type A natriuretic peptide receptor are required for hormone-stimulated guanylyl cyclase activity. J. Biol. Chem. *268*, 5997–6003.

Krupinski, J., Coussen, F., Bakalyar, H.A., Tang, W.-J., Feinstein, P.G., Orth, K., Slaughter, C., Reed, R.R., and Gilman, A.G. (1989). Adenylyl cyclase amino acid sequence: possible channel- or transporter-like structure. Science *244*, 1558–1564.

Lowe, D.G. (1992). Human natriuretic peptide receptor-A guanylyl cyclase is self-associated prior to hormone binding. Biochemistry *31*, 10421–10425.

Maines, M.D. (1988). Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J. *2*, 2557–2568.

Margulis, A., Goraczniak, R.M., Duda, T., Sharma, R.K., and Sitaramayya, A. (1993). Structural and biochemical identity of retinal rod outer segment membrane guanylate cyclase. Biochem. Biophys. Res. Commun. *194*, 855–861.

Potter, L.R., and Garbers, D.L. (1992). Dephosphorylation of the guanylyl cyclase-A receptor causes desensitization. J. Biol. Chem. *267*, 14531–14534.

Ramarao, C.S., and Garbers, D.L. (1988). Purification and properties of the phosphorylated form of guanylate cyclase. J. Biol. Chem. *263*, 1524–1529.

Shyjan, A.W., deSauvage, F.J., Gillett, N.A., Goeddel, D.V., and Lowe, D.G. (1992). Molecular cloning of a retina-specific membrane guanylyl cyclase. Neuron *9*, 727–737.

Tang, W.-J., Krupinski, J., and Gilman, A.G. (1991). Expression and characterization of calmodulin-activated (type I) adenylylcyclase. J. Biol. Chem. *266*, 8595–8603.

Thorpe, D.S., and Morkin, E. (1990). The carboxyl region contains the catalytic domain of the membrane form of guanylate cyclase. J. Biol. Chem. *265*, 14717–14720.

Thorpe, D.S., Niu, S., and Morkin, E. (1991). Overexpression of dimeric guanylyl cyclase cores of an atrial natriuretic peptide receptor. Biochem. Biophys. Res. Commun. *180*, 538–544.

Traylor, T.G., and Sharma, V.S. (1992). Why NO? Biochemistry *31*, 2847–2849.

Vaandrager, A.B., Schulz, S., DeJonge, H.R., and Garbers, D.L. (1993a). Guanylyl cyclase C is an N-linked glycoprotein receptor that accounts for multiple heat-stable enterotoxin-binding proteins in the intestine. J. Biol. Chem. *268*, 2174–2179.

Vaandrager, A.B., van der Wiel, E., and deJonge, H.R. (1993b). Heat-stable enterotoxin activation of immunopurified guanylyl cyclase C. Modulation by adenine nucleotides. J. Biol. Chem. *268*, 19598–19603.

Verma, A., Hirsch, D.J., Glatt, C.E., Ronnett, G.V., and Synder, S.H. (1993). Carbon monoxide: a putative neural messenger. Science *259*, 381–384.

Wedel, B., Humbert, P., Harteneck, C., Foerster, J., Malkewitz, J., Böhme, E., Schultz, G., and Koesling, D. (1994). Mutation of His-105 of the β1-subunit yields a nitric oxide-sensitive form of soluble guanylyl cyclase. Proc. Natl. Acad. Sci. U.S.A. *(in press)*.

Yuen, P.S.T., Doolittle, L.K., and Garbers, D.L. (1994). Dominant negative mutants of nitric oxide-sensitive guanylyl cyclase. J. Biol. Chem. *(in press)*.

Yuen, P.S.T., and Garbers, D.L. (1992). Guanylyl cyclase-linked receptors. Annu. Rev. Neurosci. *15*, 193–225.

Zhuo, M., Small, S.A., Kandel, E.R., and Hawkins, R.D. (1993). Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus. Science *260*, 1946–1950.