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G Proteins and Dual Control of Adenylate Cyclase

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Recent studies have revealed that the hormone-sensitive adenylate cyclase system is far more complicated than originally suspected—consisting of at least three types of proteins embedded in the lipids of the plasma membrane. This complex of proteins has the ability to receive information from several sources and generate a single, integrated response. The decision-making process begins when receptors for a large number of hormones, neurotransmitters, and other regulatory molecules interact with appropriate endogenous ligands and with drugs at the cell surface. These interactions ultimately result in stimulation or inhibition of adenylate cyclase activity; there are subsequent alterations of intracellular phosphorylation as a consequence of the actions of cAMP-dependent protein kinases and counter-regulatory phosphoprotein phosphatases. Stimulatory receptors include those for β-adrenergic agonists, ACTH, gonadotropins, and many others, while inhibitory control is exerted by such agents as α_2-adrenergic and muscarinic agonists and opioids.

Receptors communicate with a pair of homologous guanine-nucleotide-binding regulatory proteins—one (G_s) mediates stimulation of adenylate cyclase activity, while the other (G_i) is responsible for inhibition. The G proteins control the activity of the actual catalyst of the enzyme system (C) in a complex series of reactions discussed below. Notable in this scheme is the fact that the two G proteins share a common subunit, and the action of this subunit appears to be crucial for the integrative capacity of the system. The G proteins that regulate adenylate cyclase activity are members of a larger family of homologous guanine-nucleotide-binding proteins that includes transducin, a regulatory protein of the outer segment of the retinal rod. The products of the *ras* genes may also be relatives.

Progress in dissection of the hormone-sensitive adenylate cyclase system was slow through the 1960s and much of the 1970s for a number of valid, and for some time unsuspected, reasons. These include the hydrophobic nature of the components; their multiplicity, lability, and extremely low concentration (1 part in 10^5 of cell protein is typical); and the need for proper orientation of the components in an appropriate membrane for hormonal regulation of enzymatic activity. However, a number of advances have now led to a successful path of experimentation. It is clear that detailed understanding of the mechanism of regulation of cAMP synthesis will soon be achieved from study of the interactions of purified components that have been reconstituted in lipid bilayers of defined composition.

G_s and G_i

The two guanine-nucleotide-binding regulatory components of the adenylate cyclase system have been purified and studied in some detail—particularly in detergent-containing solutions (Sternweis et al., JBC *256*, 11517-11526, 1981; Bokoch et al., JBC *258*, 2072-2075, 1983). The table and two figures are included to facilitate the following discussion.

The catalyst of adenylate cyclase is essentially inactive with its physiological substrate, MgATP, in the absence of G_s. G_s is thus most conveniently assayed by its ability to stimulate adenylate cyclase activity; the best source of C for such an assay is obviously one that is free of G_s. Such resolved preparations of C can be made biochemically. Alternatively, the membrane of the cyc$^-$ S49 lymphoma cell mutant is a superb assay vehicle. This mutant is deficient in G_s activity, but retains C, and was particularly useful for elucidating the importance and activity of G_s. It was originally assumed that cyc$^-$ was devoid of adenylate cyclase, since it had essentially undetectable levels of this enzymatic activity. However, addition of G_s to cyc$^-$ membranes fully restores adenylate cyclase activity stimulated by hormone, guanine nucleotide, and fluoride.

G_s has an oligomeric structure with 45,000 and 35,000 dalton subunits, while G_i has a similar structure with 41,000 and 35,000 dalton subunits. A third subunit, M_r 10,000, also appears to be present in both proteins, although this has not been proved rigorously. The larger (α) subunit of each protein contains a site for NAD-dependent ADP-ribosylation catalyzed by a bacterial toxin. Cholera toxin ADP-ribosylates $G_{s\alpha}$ in the presence of a membrane-bound protein cofactor (ARF), while islet-activating protein (IAP; one of the toxins of Bordetella pertussis) ADP-ribosylates $G_{i\alpha}$. Such ADP-ribosylation results in characteristic modifications of the function of each regulatory protein. The α subunits of each G protein also contain a site with high-affinity for guanine-nucleotide binding. The 35,000 dalton (β) subunits of G_s and G_i are indistinguishable from each other functionally (see below) and by analysis of amino acid composition and maps of proteolytic peptides.

Incubation of either G_s or G_i with nonhydrolyzable guanine nucleotides (e.g. GTPγS) or with fluoride (in the presence of Mg^{2+} and Al^{3+}; see Sternweis and Gilman, PNAS *79*, 4888-4891, 1982) results in "activation" of the protein. By activation I mean a state in which the protein is capable of either stimulating or inhibiting the activity of C. In fact, activation of either G_s or G_i by GTPγS is essentially irreversible (in the presence of Mg^{2+}); free GTPγS can be removed, and the activated state is stable. Activation of either G_s or G_i has been shown to be the result of or associated with ligand-promoted dissociation of the protein's subunits, as follows (Northup et al., JBC *258*, 11369-11376, 1983; Katada et al., JBC, in press):

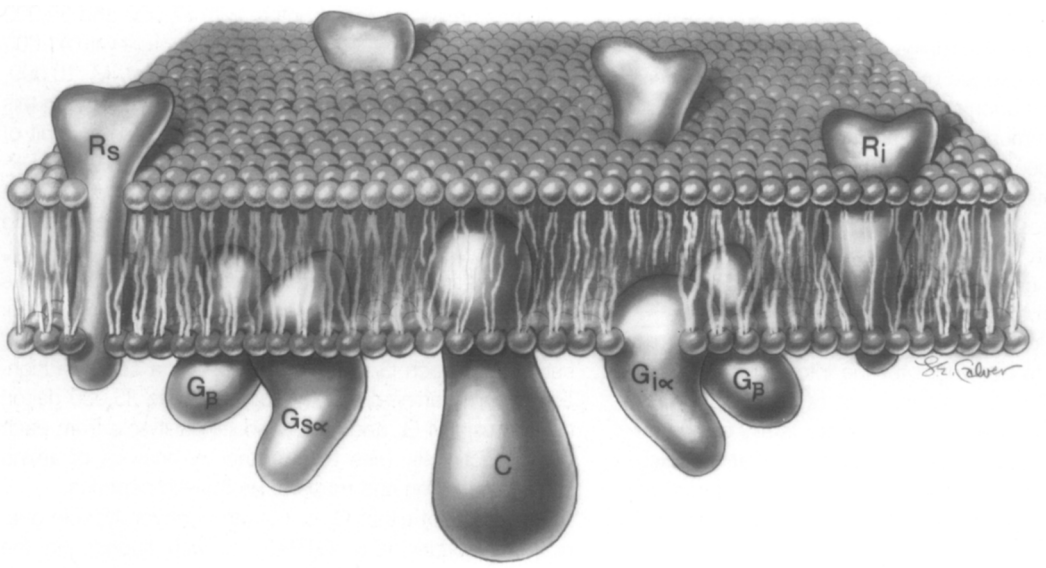
$$G_{s\alpha}\cdot\beta + \text{GTP}\gamma\text{S} \rightleftharpoons \text{GTP}\gamma\text{S}\cdot G_{s\alpha}\cdot\beta \xrightleftharpoons{Mg^{2+}} \text{GTP}\gamma\text{S}\cdot G_{s\alpha} + \beta$$

$$G_{i\alpha}\cdot\beta + \text{GTP}\gamma\text{S} \rightleftharpoons \text{GTP}\gamma\text{S}\cdot G_{i\alpha}\cdot\beta \xrightleftharpoons{Mg^{2+}} \text{GTP}\gamma\text{S}\cdot G_{i\alpha} + \beta$$

Components of the Hormone-Sensitive Adenylate Cyclase System and the Analogy with Transducin

Component	M_r	Ligand Binding Sites	Comment
Stimulatory receptors (R_s)	Various	β-adrenergic agents, adrenocorticotropin, gonadotropins, many others	
Inhibitory receptors (R_i)	Various	α-adrenergic agents, muscarinic agents, opioids, others	
Stimulatory G protein (G_s)	α: 45K[a] β: 35K γ: 10K[b]	GTP; AlF_4^-	α: Activator of C; site of ADP-ribosylation by cholera toxin β: Deactivates $G_{s\alpha}$
Inhibitory G protein (G_i)	α: 41 K β: 35 K γ: 10K[b]	GTP; AlF_4^-	α: Site of ADP-ribosylation by IAP β: Deactivates $G_{s\alpha}$
Catalytic unit (C)	?	Forskolin[c] Adenosine[c]	Converts ATP → cAMP
ADP-ribosylation factor (ARF)	21K	None known	Substrate for cholera toxin is ARF·G_s·GTP
Transducin	α: 39K β: 35K γ: 10K	GTP; AlF_4^-	α: Activates a cGMP phosphodiesterase; independently ADP-ribosylated by cholera toxin and IAP β: Deactivates α

[a] G_s from certain sources contains a 52,000 dalton α subunit in addition to the 45,000 dalton polypeptide. It is likely that they are products of the same gene.
[b] A 10,000 dalton peptide is usually found in preparations of G_i and G_s. It is probable that it is a third (γ) subunit. A similar subunit exists in transducin. Its function is unknown.
[c] Since the catalyst has not been purified, it is not certain that these ligands bind to this polypeptide.



The obvious next question was which subunit is responsible for the characteristic effects of the "activated" G protein. Resolution of the subunits can be achieved by high performance gel filtration, and it was then determined that GTPγS·$G_{s\alpha}$ was the necessary and sufficient activator of C. The β subunit of G_s thus inhibits activation and stimulates deactivation by promoting formation of the G_s oligomer (Northup et al., JBC *258*, 11361–11368, 1983).

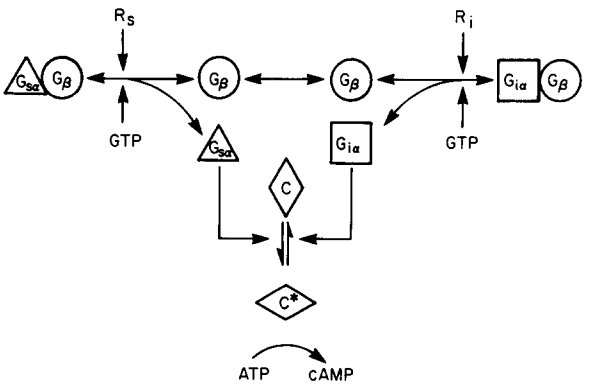
Logic suggests that GTPγS·$G_{i\alpha}$ would act analogously as an inhibitor of C, either directly or competitively. Although in fact GTPγS·$G_{i\alpha}$ does appear to have such activity, it seems to be relatively weak. When the resolved subunits of G_i were tested for their ability to inhibit adenylate cyclase activity in platelet membranes, the inhibitory activity of the "activated" protein was found to reside largely with the β subunit. This was an important observation, and it forms the basis for the statement made above—that the β subunits of G_s and G_i are functionally indistinguishable (Katada et al., JBC, in press). The inhibitory activity of the β subunit of G_i is not exerted on C, but rather at the level of the stimulatory coupling protein

$$\beta + G_{s\alpha} \rightleftharpoons G_{s\alpha} \cdot \beta,$$

particularly in the relative absence of guanine nucleotides and Mg^{2+}. Resolved $G_{i\alpha}$ actually stimulates adenylate cyclase activity in the presence of GTP by virtue of its ability to bind to β.

Effects of the Agonist–Receptor Complex

Studies of the purified G proteins have given important clues about the functions of the system. However, these proteins must be reconstituted with purified receptors to allow study of the intricacies of the receptor–G protein



interaction and, thus, the mechanism of hormonal regulation of adenylate cyclase activity. Progress has been made by Citri and Schramm (Nature *287,* 297–300, 1980; JBC *257,* 13257–13262, 1982), who demonstrated the feasibility of the approach with crude preparations of receptor and G_s, and by Ross and coworkers, who have utilized purified components (Pedersen and Ross, PNAS *79,* 7228–7232, 1982; Brandt et al., Biochem. *22,* 4357–4362, 1983).

The isoproterenol–β-adrenergic receptor complex activates G_s in a reconstituted system composed of lipids, partially purified receptor, and purified G_s. The receptor acts catalytically to activate G_s and presumably facilitates a conformational change of G_s; this change may be synonymous with subunit dissociation, and it results in high-affinity binding of guanine nucleotide analogs. If GTP rather than GTPγS is utilized, the agonist–receptor complex promotes the hydrolysis of GTP to GDP by G_s. This appears to be the counterpart of the hormone-stimulated GTPase activity described by Cassel and Selinger (BBA *452,* 538–551, 1976). Even when stimulated, the rate of turnover of GTP by G_s is spectacularly slow (1/min). It is possible that there is a long-lived $G_s \cdot$GTP (or very possibly $G_{s\alpha} \cdot$GTP) complex that activates C in the membranes.

G Protein–Catalyst Interactions

Details of these interactions are limited, beyond those implied above, since purified preparations of C are not available. The protein is hydrophobic and very labile; hope for its purification comes from Pfeuffer and Metzger (FEBS Lett. *145,* 369–375, 1982), who have had apparent success with a forskolin affinity column. Purification and characterization of C remain major hurdles.

Other Functions; Other G Proteins

More speculative issues involve consideration of other functions of the G proteins. There are reasons to suspect that G proteins represent branch points for transduction of information across the plasma membrane and that they regulate proteins other than the catalytic unit of adenylate cyclase. Maguire and Erdos (JBC *255,* 1030–1035, 1980), for example, have defined a Mg^{2+} transport system in S49 lymphoma cells that is homone-sensitive (β-adrenergic and prostaglandin E_1). The effect of isoproterenol to inhibit Mg^{2+} influx appears to be dependent on β-adrenergic receptors and G_s, but to be independent of C or of cAMP.

The 41,000 dalton subunit of G_i has largely (for the moment) been assigned the role of an anti-inhibitor of adenylate cyclase activity; this polypeptide may thus be an excellent candidate as a mediator of other functions that are regulated by α-adrenergic or muscarinic receptors. Processes such as Ca^{2+} gating and phosphatidylinositol metabolism spring to mind.

One also needs to consider the possibility of analogous proteins and mechanisms in other systems. This is simple to do, since knowledge of a light-activated cGMP-specific phosphodiesterase in the retina has evolved in parallel with that of adenylate cyclase (reviewed by Stryer et al., Curr. Top. Mem. Transp. *15,* 93–108, 1981). The analogy is overwhelming. The receptor is rhodopsin, the effector is the phosphodiesterase, and a G protein, termed transducin by some, stands in the middle. Transducin has an $\alpha \cdot \beta \cdot \gamma$ subunit structure. The α subunit (M_r 39,000) binds GTP and can be ADP-ribosylated by either cholera toxin or IAP (at independent sites). Binding of guanine nucleotide analogs causes dissociation of α from $\beta \cdot \gamma$, and α is the activator of the phosphodiesterase. The β subunit of transducin is essentially indistinguishable from that of G_s or G_i, and it can deactivate $G_{s\alpha}$. The three G proteins are thus close relatives indeed; it seems likely that the family is larger.

The products of the *ras* genes might be distant relatives. Like G_s, G_i, and transducin, the *ras* gene products are membrane-bound proteins that bind GTP. Their molecular weights are about half those of the α subunits of the G proteins discussed above. However, the finding of two sites of ADP-ribosylation in the α subunit of transducin hints at some type of duplication of a smaller precursor. Sequence information on the G proteins is unfortunately limited at the moment to that of an ADP-ribosylated tryptic peptide from transducin (the IAP site) (Manning et al., JBC *259,* 749–756, 1984). It is provocative, however, to find an analogous sequence in v-K-*ras* (Taparowsky et al., Cell *34,* 581–586, 1983):

Transducin:	Lys/Arg-Glu-Asn-Leu-Lys-Asn(ADP-ribose)-Gly-Leu-Phe
v-K-*ras*:	Arg-Glu-Gln-Leu-Lys-Arg-

While the obvious analogy is lost after five residues, it is intriguing that Arg, the residue known to be ADP-ribosylated by cholera toxin, replaces the Asn in transducin that is ADP-ribosylated by IAP. Additional sequence information, which should be available shortly, may point to exciting relationships between these systems.

Acknowledgments

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