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G PROTEINS IN SIGNAL TRANSDUCTION

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SIGNAL TRANSDUCTION PATHWAYS MEDIATED BY G PROTEINS

The last five years have seen major advances in our understanding of the central and generalized role of G-proteins as transducers of receptor signals into effector responses as outlined in Scheme 1. Signal-transducing G proteins, in contrast to other GTP-binding proteins, are heterotrimers formed of one GTP-binding (and hydrolyzing) α subunit, one β subunit, and one γ subunit.[1] This increase in knowledge is perhaps best illustrated by the fact that

[1]*Acronyms:* ACTH, adrenocorticotropic hormone; Ang. II, angiotensin II; C3a, complement fragment 3a; C5a, complement fragment 5a; CCK, cholcystokinin; CGRP, calcitonin gene related peptide; CRF, corticotrpin releasing factor; CTX, cholera toxin; DRG, dorsal root ganglion; EGF, epidermal growth factor FSH, follicle stimulating hormone; G, G protein; GABA, gamma-amino butyric acid; GnRH, gonadotropin releasing hormone; GTPγS, guanosine 5' (γ-thio)thriphosphate; GRF, growth hormone releasing factor; GRP, gastrin releasing peptide; 5HT, serotonin; Iso, isoproterenol: LDL, low density lipoprotein LH, luteinizing hormone; Lt, leukotriene (types: A_4, B_4, C_4); MSH, melanocyte stimulating hormone; NE, norepineprine; NPY, neuropeptide Y; PAF, platelet activating factor; PG, prostaglandin (types: E1, E2, F2α, D, I2); PMI, peptide with N-terminal methionine and carboxyterminal isoleucine amide; PHI, peptide with N-terminal histidine and carboxyterminal isoleucine amide; PTH, parathyroid stimulating hormone; PTX, pertussis toxin; PYY, peptide YY; R, receptor; SRIF, (SST), somatostatin; TRH, thyrotropin releasing hormone; TSH, thyroid stimulating hormone (thyrotropin); VIP, vasoactive intestinal peptide.

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	input	transduction			output
Location	Extracellular Milieu	Plasma Membrane			Intracellular Milieu or Plasma Membrane
		G-Protein Coupled Receptor		G-Protein Regulated Effector	
Functional Elements	Primary Messenger →	R →	G Protein →	E →	Secondary Messenger; Membrane Potential
Molecular Diversity					
# known # estimated	ca.30 ?	75 ca.100	12 up to 15	6 ca. 12	cAMP, IP's, DAG, Ca^{2+}, cGMP, AA

Scheme 1 Flow of information through G protein dependent signal transduction pathways.

five years ago only two systems were known to be regulated through a G-protein: adenylyl cyclase, originally discovered in 1971 to be stimulated in a GTP-dependent manner (1, 2) and then to be inhibited in a GTP-dependent manner (3–7), and retinal phosphodiesterase, discovered by Wheeler & Bitensky to require GTP for activation by light (8). However, from 1985 onward, many other membrane functions were found to be regulated by a GTP-dependent process. These include receptor-mediated activation of phospholipases C and A_2 [the first papers on GTP involvement in phospholipase C regulation appeared in mid-1985 (9, 10)] and a number of hormone- and neurotransmitter-regulated ionic channels [the first indirect data supporting a G protein regulation of ionic channels appeared in late 1985 (11, 12), and reports on the nucleotide regulation under cell-free conditions appeared in late 1986 (13) and early 1987 (14; reviewed in 15)]. More types of membrane functions may have to be added in the not so distant future (Table 1).

The increased awareness of G protein involvement in receptor action was due to two major developments: (*a*) the application of molecular biology (reviewed in 16, 17) and generation of peptide-directed antibodies (18, 19) to study the components involved, and (*b*) the cooperative interactions between two fields that had not interacted before, namely, biochemistry and electrophysiology (reviewed in 15, 20). Whereas the latter development led to the rather dramatic broadening of the functions of G-protein action in signal transduction, the former allowed unequivocal description of the structures of the components involved. Together, these methodologies made it possible to rapidly and convincingly establish that G proteins are not just G_s, G_i, and G_t (transducin), but constitute a rather large family of homologous proteins (the α subunit of G_t was cloned in 1985, and those of G_s, an incomplete G_o, and two of the three G_i's were cloned in 1986), and that the same transduction mechanism exists not only in animal cells (21, 22), but also in yeasts (23, 24),

Table 1 Classes of G protein effector functions

Effector class[a]	G Protein(s)[b]	Effect	Comment
Proven in cell-free assays			
Adenylyl cyclases	G_s/"G_i"	Stimulation/ inhibition	Table 3
Retinal cGMP-specific PDE	G_t	Stimulation	Table 3
Phospholipase C	"G_p" (G_o?)	Stimulation	c
Phospholipase A_2	"G_p"	Stimulation	d
Ionic channels (six types)	G_k = G_i's/ G_o's; G_s	Stimulation/ inhibition	Table 6
Suggested by indirect or intact cell studies[e]			
Na/H antiport	"G_p" (?)	Stimulation	f
Voltage-gated Ca^{2+} channels	G_i's, G_o's (?)	Inhibition	Table 7
Insulin-sensitive glucose transporter	G_s (?)	Stimulation	g
Liver Ca^{2+} pump	G_s (?)	Inhibition	h
Renal Na/K ATPase	?	Inhibition	i
More ionic channels (?)	?	Stimulation/ inhibition	Table 7
Phospholipase D	"G_p" (?)	Stimulation	j
Nonretinal PDEs	?	Stimulation	k

[a] The number of distinct gene products in each class may be large. For example, work from the laboratories of Gilman and Randall Reed shows that there are at least four adenylyl cyclases; the atrial, GH_3, and hippocampal neuron G_k-gated K^+ channels differ in conductance and G protein specificity; the heart and skeletal muscle dihydropyridine-sensitive Ca^{2+} channels stimulated by G_s are products of separate genes.

[b] Except in the cases of adenylyl cyclase inhibition ("G_i") and phospholipase C activation ("G_p"), the G protein involved is known. In the cases of "G_i" and "G_p", in vitro experiments with activated purified α subunits have failed. Although some preliminary data indicate that the PTX-sensitive "G_p" may be one of the G_o variants, confirmation is needed.

[e] These are systems in which mediation by phosphorylation has not been ruled out but indications are strong that a direct G protein regulation is possible.

[c] Litosch et al (9); Cockroft & Gomperts (10); for discussion and references, see Birnbaumer et al (16).

[d] Murayama & Ui (150); Burch et al (151); for further discussion and references, see Birnbaumer et al (16).

[f] Inferred from persistent PTX-sensitive thrombin response in phorbolester-desensitized fibroblasts (152).

[g] Inferred from kinetics and toxin sensitivity of agonist-mediated regulation of glucose transport in fat cells (153).

[h] Inferred from effect of glucagon peptide 19-29 on liver Ca pump (154).

[i] Inferred from dopamine-mediated regulation of (Na,K) ATPase (155).

[j] Inferred from the fact that receptors that increase phospholipase D activity are coupled by G proteins and that GTPγS stimulates phospholipase D activity in isolated membranes (156).

[k] Inferred from agonist-induced PTX-insensitive increased rates of cAMP hydrolysis (157).

Drosophila melonogaster (25), *Dictyostelium discoideum* (26, 27), and higher plants (carrots), in which stimulation of phospholipid breakdown by iodoindoleacetic acid is dependent on the presence of GTP (28).

Twenty-four separate G-protein–coupled receptors have been cloned (reviewed in 29), and it is now possible to count at least 86 molecularly and/or pharmacologically defined distinct receptors (Table 2) that are coupled to a large number of effectors by a family of 9 to possibly 12–15 G proteins in mammalian cells (Tables 3 and 4) and an as yet undisclosed number in nonmammalian cells (Table 5).

Table 2 Ligands acting through G protein–coupled receptors

Ligand(s)	Minimum molecular diversity of receptors Pharmacological criteria	Cloned
Neurotransmitters and autacoids		
Catecholamines	8 (α_1a,b; α_2a,b,c, β_1, β_2, β_3)	7
Acetylcholine (M type)	3–4 (M1, M2, M3)	5 (M1–M5)
Dopamine	2 (D1, D2)	1 (D2)
Serotonin	5 (5HT-1a,b,c, 5HT-2, D)	3 (1a, 1c, 2)
Histamine	3 (H1, H2, H3)	None
GABA	1 (type B)	None
Glutamic acid	1 (quisqualate)	None
Purines	4 [P1 (Ado1, Ado2); P2x, P2y]	None
Peptide and glycoprotein hormones		
ACTH	1	None
Opioids (> 5 ligands)	3 (μ, δ, κ)	None
MSH, CRF, GRF, TRH, GnRH, SRIF	6 (1 each)	None
Vasopressin/oxytocin	4 (V1a, V1b, V2, OT)	None
Glucagon	2 (G1, G2)	
Glucagon 19–29	1	None
CCK, PTH, Ang. II, GRP, calcitonin, CGRP, NPY, PYY, secretin, galanin, kyotorphin	12 (1 each)	None
VIP	2 (VIP-1, VIP-2)	None
PHI (PMI)	1	None
Bradykinin	2 (BK1, BK2)	None
LH, FSH, TSH	4 (2 for LH, 1 each for TSH and FSH)	2 (LH2, TSH)
Neurokinins [substance P (NK1), substance K (NK2)]	3 (NK1, NK2, NK3)	2 (NK1, NK2)
Arachidonic acid metabolites		
PGE1/2	2 (PGE-s, PGE-i)	None
PGD, PGI_2, thromboxane, LtA_4, LtB_4, LtC_4, $PGF_{2\alpha}$	7 (1 each)	None
Other		
Chemoattractant (fMet-Leu-Phe or fMLP), C5a, C3a, thrombin	4 (1 each)	None
Phosphatidic acid	1	None
PAF	1	None
Sensory		
Light	4 (low, red, green, blue)	4
Odor	?	None
Taste (?)	?	None

Detailed referencing of this table can be found in (29).

Table 3 Diversity of mammalian G protein α subunits

Name	Purified[a]	Cloned[a]	Function(s) identified[a]
α_s (1 gene, 4 splice variants)	Yes	Yes	Yes: 3[b]
3 α_i's (3 genes: α_{i1}, α_{i2}, α_{i3})[c]	Yes	Yes	Yes
α_{o1} (1 gene: α_{o1})	Yes	Yes	Yes (?)[d]
α_{o2} (splice variant of α_o gene?)	Yes	No	No
α_t-rod	Yes	Yes	Yes
α_t-cone	No	Yes	Inferred
α_{olf}	No	Yes	Inferred
$\alpha_{z/x}$	Yes	Yes	No
"α_p"-PhLC[e]	?, no[f]	?, no[f]	Yes
"α_p"-PhLA$_2$[e]	?, no[f]	?, no[f]	Yes

[a] For detailed referencing, see (29).
[b] See Table 5.
[c] Named in chronologic order of cloning (158).
[d] Stimulatory roles in both K^+ channel (65) and phospholipase C (159) regulation have been reported.
[e] PTX-sensitive and PTX-insensitive activities have been reported; the heterotrimeric nature of the G protein involved is inferred from PTX sensitivity and from inhibition of "G_p"-mediated activities by $\beta\gamma$ dimers (160). For detailed discussion, see (16).
[f] The PTX-sensitive form may have been purified and/or cloned, but the PTX-insensitive form is unknown.

Table 4 Diversity in subunit composition of G protein $\beta\gamma$ dimers[a]

Subunits	References
β Subunits: β_1 (β_{36}), β_2 (β_{35}), β_3 (migration ?), β_4 (migration)	
4 cloned	
β_1	109, 161, 162
β_2	163–165
B_3	166
B_4	_[b]
2 seen on SDS-PAGE	165, 167–169
γ Subunits	
γ_T-1 (10 kDa); γ_t-2 (6 kDa)	170, 171
One cloned (74 amino acids long)	110, 111
Two seen on SDS-PAGE	169, 171
Antigenicity distinct from that of γ_G's	172
Silver staining distinct from that of γ_G's	169
γG-a (6 kDa), γ_G-b (10 kDa)	107, 169
Peptide map of 6-kDa form distinct from that of the 6-kDa γ_T	108
Antigenicity distinct from that of γ_T's	168, 172
One cloned (71 amino acids long)	112
At least two identified on SDS-PAGE	169
γG-c (7 kDa)	168
No cloned (?)	_[c]
Migration on SDS-PAGE distinct from that of other γ_G's	169
Antigenicity distinct from that of other γ_G's	168

[a] β and γ subunits are purified as dimers composed of mixtures of the subunits described in this table.
[b] M. Simon, personal communication.
[c] At the time of this writing, the protein correlate to the cloned γ subunits has not been identified, but because of its length (71 amino acids), it is likely to be of the smaller class (6–7 kDa).

Table 5 Nonmammalian G protein subunits as of mid-1989[a]

Subunits		Reference(s)
Saccharo-Myces cerevisiae		
2 α subunits:	*GPA1* (pheromone receptor responsive)	23, 24
	GPA2 (adenylyl cyclase ?)	173
1 β subunit: *STE 4*		113
1 γ subunit: *STE 18*		113
Dictyostelium discoideum		26, 27, 174
2 α subunits:	Gα-1 (adenylyl cyclase stimulation?)	27, 175
	Gα-2 (phospholipase C and guanylyl cyclase stimulation)	27, 175
1 β subunit		26
Drosophila melanogaster		
4 α subunits:	1 α_i-like	176
	1 α_s-like	177
	2 α_o-like (splice variants of same gene)	178, 179
1 β subunit		180
Xenopus oocyte		
4 α subunits:	1 α_o-like	181
	1 α_s-like	Olate et al, unpublished
	1 α_{i2}-like	Olate et al, unpublished
	1 α_{i1}-like	Olate et al, unpublished

[a] All identified by cloning, none purified.

Table 6 Ionic channels regulated under cell-free conditions by pure G protein and/or GTPγS

Channel type	G Protein	Effect[a]	Tissue or cell	Ref.
K_{Gk} (40 pS)	G_i (1,2,3)	+	Heart	13, 14, 62, 65, 93
K_{Gk} (50 pS)	G_{i3}	+	GH_3 cells	63, 98
K_{Gk} (4 types)	G_{ol}	+	Hippocampal neurons	102
K_{ATP}	G_{i3}	+	RIN cells, heart, skeletal muscle	182, 183,
$Na(K)_{[Amil]}$	G_{i3}	+	Renal medullary collecting tubule	66
$Ca_{[DHP]}$ (L type)	G_s (all 4)	+	Heart, skeletal muscle	33, 67, 68, 184
$Na_{[TTX]}$	G_s	−	Heart	185
$K_{Ca[Charyb]}$	? (Iso/GTP)	+	Uterine smooth muscle	186
$Ca_{[Ni]}$ (T type)	? (GTPγS)	−	Rat dorsal root ganglion cells	187

[a] +, stimulation; −, inhibition

Effectors are also being cloned and characterized at the molecular level. At the time of this writing, these include two of the three transducin-responsive retinal phosphodiesterase (30–32), the G_s- and calmodulin-responsive adenylyl cyclases (33, 34), a G_s-sensitive voltage gated Ca^{2+} channel (35, 36), a G protein–regulated *Drosophila* phospholipase (25, 37), and its mammalian congeners (38, 39). However, it is clear that our knowledge of the molecular structures of effectors is lagging behind that of receptors and the G proteins proper, possibly because of the scarcity and complexity of effectors. Despite this, the discovery of new effector systems regulated by G proteins, as seen in functional assays, is at an all-time high, (Tables 1, 6, and 7).

MECHANISM OF G PROTEIN ACTIVATION AND ACTION

Roles of GTPase and Subunit Dissociation

G proteins undergo cyclical activation/deactivation cycles under the influence of GTP and receptors. These cycles involve two chemical reactions: (*a*) a subunit dissociation, which follows as a corollary to the activation of the G protein from GTP-**G** to GTP-**G*** and which results in the formation of GTP-α* plus $\beta\gamma$; and (*b*) the hydrolysis of GTP by α* to give the deactivated GDP-α complex, which then reassociates with $\beta\gamma$ to give GDP-**G*** (Figure 1). Release of GDP and the Mg^{2+}-dependent transition GTP-**G** to GTP-**G*** are the slow steps accelerated by ligand-occupied receptors [for a review, see Birnbaumer et al (40)].

Receptors catalyze the activation of G proteins by GTP (41–44). This requires that the receptor be dissociated from the G protein before the G protein is deactivated by the GTPase reaction. Individual reactions affected by

Table 7 Ionic channels that may be under direct regulation by G proteins as inferred from whole-cell recordings

Channel type	Agonist	G protein[a]	Effect	Cell	Mimicked by TPA	References
Ca (N type?)	Opioid	$G_o > G_i$	Inhibition	NG108-15	?	103
Ca (L type)	Ang II	G_i	Stimulation	YI Adrenal	?	188
Ca (L type)	GnRH	G_i/G_o	Stimulation	GH_3	?	189
Ca (L type)	SST	? GTPγS	Inhibition	AtT-20	Yes	190
Ca (?)	GABA(B)	? GTPγS	Inhibition	Chick DRG	Yes (total)	191, 192
	NE (αAR)	? GTPγS	Inhibition	Chick DRG	Yes (total)	191, 192
Ca (?)	GABA(B)	? GTPγS	Inhibition	Rat DRG	No	193–195
Ca (?)	NPY	$G_o > G_i$	Inhibition	Rat DRG	Yes (partial)	104
	Bradykinin	$G_o = G_i$	Inhibition	Rat DRG	Yes (partial)	105, 196

[a] All the G proteins involved are PTX sensitive.

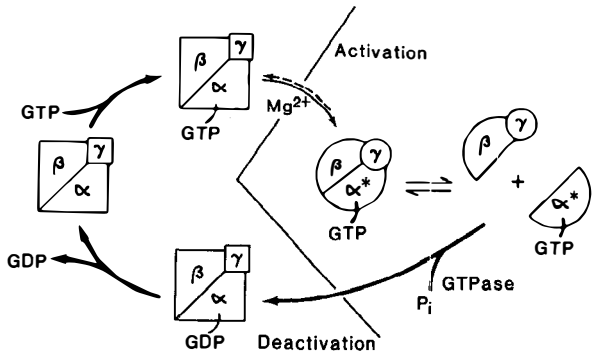


Figure 1 Regulatory cycle of a G protein. Squares and semisquares represent inactive conformations as they relate to modulation of effector functions. Circles and semicircles represent activated forms of the G protein. Activation is both GTP and Mg^{2+} dependent and is stabilized by subunit dissociation to give an activated $G\alpha^*$-GTP complex plus the $G\beta\gamma$ dimer. Hydrolysis of GTP by the $G\alpha$ subunit deactivates it, increases its affinity for $G\beta\gamma$, and leads to reassociation to give an inactive holo-G protein with GDP bound to it. Reinitiation of the activation system requires release of GDP and renewed binding of GTP. Specificity of action is encoded in $G\alpha$. Different $G\alpha$ subunits associate with a common pool of $G\beta\gamma$ dimers.

receptors are (*a*) stimulation of GDP release and facilitation of GTP binding (45), (*b*) facilitation of the transition from inactive to active conformation of the GTP-liganded G protein (46–48), and (*c*) facilitation of G protein subunit dissociation. Stimulation of GDP release was originally shown to occur in turkey erythrocyte membranes (45) and was confirmed in other systems. Facilitation of the activation reaction proper was shown to occur in rat liver membranes in which nonhydrolyzable analogs of GTP activate adenylyl cyclase with distinct time constants (indicating that GDP release is not rate limiting for the slower of the two) and in which hormonal stimulation accelerates the activation by all GTP analogs and hence affects the activation reaction proper (46–48). Facilitation of subunit dissociation by the receptor has not been shown experimentally, even though it is clear that activated G proteins tend to dissociate in solution (49–51) as well as in membranes (52–54) and that this reaction is the most likely candidate accelerated by hormonal stimulation.

Figure 2 illustrates our view of the reaction sequences that make up the cyclical activation/deactivation process that a G protein undergoes in the presence of hormone receptor **R**. It should be noted that G protein subunit dissociation must occur before receptor separation if the receptor is to act as a catalyst, i.e. separate from the G protein prior to GTP hydrolysis. The subunit dissociation reaction releases the **R-G*** complex from the thermodynamic microscopic reversibility constraint, which is imposed by the fact that the receptor participates in the "activation reaction" and which dictates that the

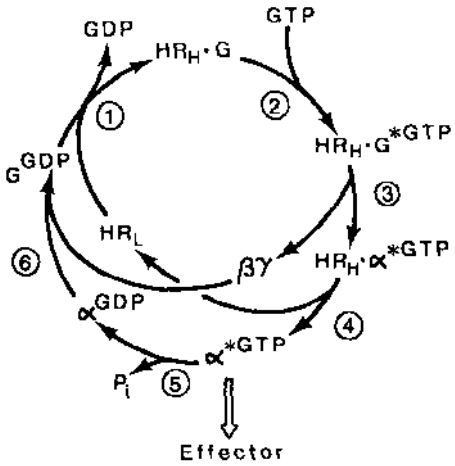


Figure 2 Integrated view of receptor-mediated catalytic activation of a G protein in the context of the dual subunit dissociation and GTPase cycles of G proteins. The role of receptors is to promote nucleotide exchange and to stabilize a GTP-dependent "activated" form of the G protein, and the G protein undergoes a cyclical dissociation-reassociation reaction and oscillates between GDP, nucleotide-free, and GTP states. The cycles are driven energetically forward by the capacity of the G protein to hydrolyze GTP (43, 45, 197, 198) and kinetically by the dissociation of $\beta\gamma$ dimer from the activated receptor-G protein complex. The receptor has high affinity for agonist ($\mathbf{R_H}$) when it is associated with the nucleotide-free trimeric $\alpha\beta\gamma$ form of the G protein and has low affinity for the agonist ($\mathbf{R_L}$) when it is free. Furthermore, the receptor has higher affinity for the trimeric $\alpha\beta\gamma$ form of **G** than for the **G**-GDP, thus accounting for the finding that GDP and GDP analogs promote the $\mathbf{R_H}$-to-$\mathbf{R_L}$ transition. The $\beta\gamma$ dimers are required for the interaction of α with **R**, and after formation of **G**-GTP, no **G*** forms unless it is "aided" by receptor. Thus, receptor has an even higher affinity for the **G***-GTP state than for the nucleotide-free state of **G**. As a consequence, receptor dissociation is absolutely dependent on reaction 3 (subunit dissociation). Thermodynamic reasons do not allow **R** both to stabilize the **G*** state and increase the likelihood to dissociate from it. Reaction 4 states, further, that the α*GTP loses its ability to stay associated with receptor and decomposes further into free activated α*GTP plus free receptor, thus accounting for the fact that under "working" conditions (saturation by both GTP and hormone and hence sustained regulation of effector), only a small proportion of receptors are found in their high-affinity, G protein–associated state. It follows that the G protein cycle is driven forward not only by the GTPase but also, and obligatorily so, by the subunit dissociation reaction. Although not shown explicitly, reactions 1, 2, and 3 are assumed to be reversible. The scheme accounts for the experimental findings that receptors act catalytically and therefore need to dissociate from the G protein at one time or another (41) and that receptors accelerate the transition from inactive **G**-GTP$_\gamma$S to active **G***-GTPγS transition and therefore must have higher intrinsic affinity for the activated than the inactive state (46–48).

affinity of **R** for **G*** has to be higher than for **G**. Once the **G*** has transformed into $\beta\gamma$ plus α^* (reaction products of the catalytic action of **R**), **R** is free to separate and proceed to interact with other **G**-GDP complexes. Although the reaction products are shown as $\beta\gamma$ plus **R**-α^*, they could also be α^* plus **R**-$\beta\gamma$. However, the point of the Figure 2 diagram is that it could not be **R**

plus **G*** and that free **R** has to arise secondary to the formation of **R**-α* or **R**-$\beta\gamma$.

The GTPase reaction is clearly the turnoff mechanism of the G protein. α-GDP complexes are inactive in regulating effector functions (55, 56). The GTPase activity of the α subunit is depicted in Figure 2 as being operative only after formation of free α*-GTP, but it is not known whether a trimeric G protein hydrolyzes GTP. As assessed with purified proteins, the GTPase operates with a k_{cat} of 1–4 min^{-1} (56–58).

Roles of G Protein Subunits in Signal Transduction

ROLES OF α SUBUNITS The α subunits of G proteins, activated by GTP or GTP analogs and resolved from $\beta\gamma$ dimers, are undoubtedly the mediators of the receptor signals. This has been shown for stimulation of the retinal cyclic GMP (cGMP)-specific phosphodiesterase by α_t (59), for stimulation of adenylyl cyclase by α_s (50–61), and for stimulation of a variety of ionic channels including the atrial and GH_3 cell inwardly rectifying K^+ channels by α_{i1}, α_{i2}, α_{i3} (62–65); an amiloride-sensitive cation channel by α_{i3} (66); and dihydropyridine-sensitive Ca^{2+} channels by the same α_s that stimulates adenylyl cyclase (36, 67, 68).

ROLES OF $\beta\gamma$ DIMERS Three roles for $\beta\gamma$ dimers emerge from Figure 2 and from experiments on inhibitory effects of $\beta\gamma$'s (Table 8): 1. as mentioned above, by dissociating from R-G*, $\beta\gamma$'s allow R's to act as catalysts (41, 42); 2. because GDP-α's are not recognized by receptors (69–71), $\beta\gamma$'s are essential for reactivation of the G protein; and 3. by inhibiting ligand-independent (i.e. unoccupied receptor-mediated) activation of the G protein at lower concentrations than ligand-stimulated (i.e. occupied receptor-mediated) G protein activation, $\beta\gamma$'s act as noise suppressors (72–75). Because the final consequence of receptor-mediated activation is the same, roles 2 and 3 remain the same if the intermediary states are α* plus $\beta\gamma$-R instead of $\beta\gamma$ plus α*-R; role 1 would then be ascribed to the dissociation of α*.

Figure 2 assumes that effectors are regulated by the activated α* and not the $\beta\gamma$ dimer. There are, however, two systems in which $\beta\gamma$ dimers, rather than

Table 8 Intramembrane roles of G protein $\beta\gamma$ dimers

Reaction	Product	Role
Reassociation with α^{GDP}	$\mathbf{G}^{GDP}$	Activation by **R**
Dissociation from $\mathbf{HR.G}^{*GTP}$	$\mathbf{HR.}\alpha^{*GTP}$	Signal amplification
Reassociation with $\mathbf{R.}\alpha^{*GTP}$	$\mathbf{R.G}^{GTP}$	Noise reduction

the α subunits, have been proposed to be the mediators of the receptor message. One is hormonal inhibition of adenylyl cyclase (c.f. 76), and the other is activation of phospholipase A_2 in nonretinal tissues (77). Both are subjects of disputes, which are intimately related to definition of G proteins by function, cloning, and toxin sensitivity (78).

G_i and inhibition of adenylyl cyclase: reduction of basal activity by $\beta\gamma$ and mediation of hormonal inhibition by α_i. The term G_i (formerly also N_i) is currently used for several pertussis toxin (PTX) substrates. Originally, however, it defined a functional entity, i.e. the putative GTP-binding regulatory component of adenylyl cyclase that mediates hormonal inhibition of adenylyl cyclase (reviewed in 21, 40, and 79). The putative regulatory component became a molecular entity when Katada & Ui demonstrated a direct correlation between PTX mediated block of hormonal inhibition and ADP-ribosylation of a substrate of approximately 40 kilodalton (kda) (80). The finding that inhibitory regulation of adenylyl cyclase by guanine nucleotides was unimpaired in α_s-negative S49 *cyc*$^-$ cells confirmed the existence of a G_i separate from G_s (81). In 1983, the purification of the "major PTX substrate" and the "putative inhibitory regulatory component of adenylyl cyclase" was published (82, 83), with the clear speculation that this was indeed G_i. Indeed, addition of the G_i trimer activated with the GTP analog GTPγS inhibited platelet adenylyl cyclase activity (84). However, upon resolution of activated G_i-α (α_i*) from G_i-$\beta\gamma$, the inhibition by the activated G_i correlated with an autonomous and at the beginning surprising but nevertheless bona fide and reproducible inhibitory effect of the $\beta\gamma$ dimer and not the α_i* (84, 85). Two schools of thought developed. One is espoused by those who discovered the effect of $\beta\gamma$ dimers, which, in addition to inhibiting membrane adenylyl cyclase, delay the activation of purified G_s in solution by nonhydrolyzable GTP analogs and accelerate the deactivation of aluminum fluoride–activated G_s. It proposed that the $\beta\gamma$ dimer, and not α_i, is the mediator of hormonal inhibition of adenylyl cyclase and that the dimer does this through a mass action effect by combining with GTP-activated α_s and deactivating it (84, 85; for a review see 86). However, the data are circumstantial and may be represented as follows: hormonal inhibition does exist, $\beta\gamma$ dimers inhibit G_s, and α_i, as tested, does not inhibit membrane adenylyl cyclase; ergo, $\beta\gamma$ mediates hormonal inhibition. The other school of thought (reviewed in 40 and 78; see 87 for additional data) proposes that even though the $\beta\gamma$ dimer can inhibit adenylyl cyclase by the mechanism described above, it is not the mediator of hormonal inhibition of adenylyl cyclase such as seen with α_2-adrenergic or somatostatin receptors. The reasonings for this are mainly threefold: 1. Guanine nucleotide and hormonal (somatostatin) inhibition of adenylyl cyclase is not impaired in the S49 *cyc*$^-$ cell (81, 88–91), a

mutant that lacks G_s (92). 2. Hormonal stimulation of adenylyl cyclase does not exhibit competitive kinetics with respect to hormonal inhibition as would be predicted if the two pathways shared a common $\beta\gamma$ intermediate (75, 87). 3. Although stimulatory hormones do not interfere with the action of inhibitory hormones (75, 87), they do interfere with the inhibitory action of $\beta\gamma$ dimers, the very effect that gave rise to the theory that $\beta\gamma$ dimers mediate hormonal inhibition of adenylyl cyclase (72, 75). It is worth noting that the effect of stimulatory hormones in interfering with the inhibitory actions of $\beta\gamma$ dimers is abolished by forskolin, a drug that was present in all of the initial experiments on $\beta\gamma$-mediated inhibition of adenylyl cyclase. Most probably, many of the conclusions drawn from studies in which forskolin was used in the assays (e.g. 84, 85) will need reevaluation. In the meantime, it will be necessary to contend with two independent mechanisms of inhibition of adenylyl cyclase: one due to $G_i\alpha$, mediating hormonal inhibition and operating regardless of activation of other G proteins; and the other due to $G\beta\gamma$, unrelated to physiologic inhibitory hormonal control of cAMP formation and operating primarily on agonist-independent (basal) adenylyl cyclase activity (noise suppression). Further studies are necessary to resolve this issue.

Despite the "nondefinition" of G_i, the above studies coined historically the name G_i for a group of three related PTX substrates, regardless of whether any one of the identified or cloned molecules is a true G_i mediating hormonal inhibition of adenylyl cyclase.

Effect of $\beta\gamma$ dimers on K^+ channel activity: stimulation or inhibition? In contrast to the situation with adenylyl cyclase inhibition, in which all the laboratories involved are able to reproduce each other's findings and the differences in opinion are based on alternative modes of interpretation of the same sets of data, there is no consensus about the sense of the effect of $\beta\gamma$ dimers on K^+ channel activity. Opposing effects are being reported, depending on the laboratory carrying out the experiments. Thus, one group of researchers report that although addition of $\beta\gamma$ dimers to quiescent membrane patches has no effect on K^+ channel activity (62, 63, 93), addition to membrane patches with K^+ channels stimulated either by GTP alone or by GTP plus the muscarinic agonist carbachol inhibits this activity (62, 75). This is interpreted as occurring by the same mechanisms by which $\beta\gamma$ dimers inhibit adenylyl cyclase activity, i.e. as a reflection of the noise suppressor activity of $\beta\gamma$ dimers (see above). Another group of researchers reports that addition of $\beta\gamma$ dimers to quiescent patches stimulates K^+ channel activity (77, 94–96) and that this stimulation occurs in the absence of GTP and does not require Mg^{2+} (94). The difference is irreconcilable. The stimulatory effect of $\beta\gamma$ dimers was originally assumed to be direct, but more recently it has been proposed to be secondary to stimulation of phospholipase A_2 (77). Supporting arguments for $\beta\gamma$ stimulation of phospholipase A_2 were that the effect can be

blocked by both an antibody to phospholipase A_2 and an inhibitor of arachidonic acid metabolism and that the effect could be mimicked by supramicromolar concentrations of arachidonic acid or one of its metabolites. In these studies, neither the effects of $\beta\gamma$ dimers nor those of the arachidonic acid or its metabolites required either GTP or Mg^{2+} (77). In partial contradiction to these results, those from another laboratory show that although arachidonic acid and a leukotriene derived from it stimulate K^+ channels, they do so through a GTP-dependent step (97). Studies showing that a monoclonal anti-α subunit antibody blocks G_k activity when added to inside-out membrane patches under activating conditions are consistent with mediation of effects of receptors by the α subunit and a lack of activity of $\beta\gamma$ dimers (98). However, as is the case for the role of $\beta\gamma$ in hormonal inhibition of adenylyl cyclase, the true effect of $\beta\gamma$ dimers on K^+ channel activity, in terms of both mechanism and sense of effect, requires further investigation, possibly by laboratories other than those currently engaged in this research.

MOLECULAR DIVERSITY OF PTX SUBSTRATES

Biochemical purification and molecular cloning have led to the molecular identification of seven PTX-sensitive Gα subunits, of which six are the products of separate genes and the seventh, $G_{o2}\alpha$, has not been cloned as yet (Table 3). Of these, the function of retinal rod and cone cell transducins (T-r and T-c) as stimulators of cGMP-phosphodiesterase is well established, but the function or functions of all the other PTX substrates, three G_i's and two G_o's, are still being unraveled. Although hormonal inhibition of adenylyl cyclase is blocked by PTX and PTX substrates have been purified, cloned, and named G_i-1, G_i-2, G_i-3 (in order of cloning), and G_o, none has yet shown the expected adenylyl cyclase–inhibitory effect. In contrast, the three G_i's have G_k activity, being equipotent in stimulating the inwardly rectifying K^+ channel involved in the bradycardic effects of vagal stimulation (65). Since G_i-3 stimulates the analogous channel involved in inhibition of pituitary hormone secretion by somatostatin (98), it is likely that G_i-1 and G_i-3 do the same in this tissue as in the heart and that one or all G_i-type PTX substrates are responsible for PTX-sensitive slow inhibitory postsynaptic potentials in the central nervous system. Type B gamma amino butyric acid ($GABA_B$), type M2 muscarinic, type 1a serotonin (5HT-1a) and D2 dopamine receptors are receptors that mediate such slow inhibitory postsynaptic potentials (99–101). Like $G_i\alpha$, $G_o\alpha$ stimulates ionic channels in central nervous neurons. However, the channels are different and of more than one type. They include nonselective monovalent cation channels and a K^+ selective but nonrectifying channel, all of which are insensitive to any of the $G_i\alpha$'s (102). In addition to its monovalent cation–stimulatory activity, G_o is directly or indirectly involved in the receptor-triggered inhibition of neuronal presynaptic Ca^{2+}

channels, such as elicited by opioid (103) and neuropeptide Y and bradykinin (104, 105) receptors.

It is interesting that all of the receptors that stimulate K^+ channels via G_i and inhibit Ca^{2+} channels with involvement of G_o and/or G_i also inhibit adenylyl cyclase. It may be that the PTX substrates of the G_i and G_o type all have multiple effects providing for a coordinate cellular response in which cAMP levels are lowered (inhibition of adenylyl cyclase), Ca^{2+} levels are lowered (inhibition of Ca^{2+} channels), and the cell is hyperpolarized (K^+ channel activation).

G_p-TYPE G PROTEINS: HYDROLYSIS OF MEMBRANE PHOSPHOLIPIDS

The G proteins that mediate the activation of membrane phospholipases have not yet been biochemically identified. They are referred to as G_p and, depending on whether the phospholipase is of the C or A_2 type, also as G_{plc} and G_{pla} (Table 1). Functional studies that are beyond the scope of this article but have been reviewed previously (16) indicate that they should be $\alpha\beta\gamma$ trimers and should participate in the receptor- and GTPase-driven regulatory cycle shown in Figures 1 and 2. Studies with PTX indicate that there are at least two types of G_p: one sensitive to PTX and the other not. Responses to vasopressin, angiotensin II, and α_1-adrenergic receptors in the liver and to thyrotropin- and gonadotropin-releasing hormones in the pituitary are due to stimulation of a phospholipase C by an PTX-insensitive G_p. Responses of neutrophils to chemoattractants are due to stimulation of a phospholipase C by a PTX-sensitive G_p. In addition, receptor-stimulated arachidonic acid release from cells, such as is seen on stimulation of mast cells and macrophages by activating substances or on stimulation of thyroid cells by α_1-adrenergic receptors, is due to activation of a phospholipase A_2 and occurs with the participation of a PTX-sensitive G_p. It is not known whether any of the cloned PTX substrates have G_p activity. Molecular cloning has recently provided a $G\alpha$, called Z/X, of unknown function (Table 3). $G_{z/x}\alpha$ lacks a PTX ADP-ribosylation site and is found only in the brain and spleen. A homolog should exist in other tissues. $G_{z/x}$ may be G_p or may have as yet unknown functions.

MOLECULAR BASIS OF SIGNAL TRANSDUCTION: RECEPTOR-Gα INTERACTIONS AT THE AMINO ACID LEVEL

The Point of View of the Receptor

Cloning of receptors [the first nonopsin receptor, the β-adrenergic receptor, was cloned in 1986 (106)] and of the subunits of G proteins, construction of

chimeras, and prolonged searching of the primary amino acid sequences for messages on structure-function relationships have resulted in several surprises as well as novel insights into molecular aspects of ligand-receptor and receptor-G protein interactions.

Thus, with the cloning of the first nonopsin gene, the β-adrenergic receptor, and since then 20 more, it has become apparent that G protein–coupled receptors all belong to a superfamily of opsinlike proteins, spanning the plasma membrane seven times and displaying an extracellular, often glycosylated amino terminus and an intracellular carboxyl terminus of variable lengths. On comparing structures predicted by computer analysis and the direct amino acid sequences, two facts became apparent: the molecules cannot be divided like the low-density lipoprotein or epidermial growth factor receptors into three discrete domains coding for ligand binding, membrane anchor, and G protein regulation. Mutational studies from the laboratories of Dixon at Merck & Co. and Lefkowitz and Caron at Duke University, as well as previous studies on rhodopsin, indicate that both ligand binding and ligand-mediated "activation" of receptors occur as a response of ligand interaction with a pocket formed from the transmembrane regions proper. In the recently cloned luteinizing hormone (LH) receptor there is an additional attachment site on a much larger amino-terminal domain. However, it is to be expected that "activation" will be due to interaction of a portion of the LH molecule with the hypothetical pocket formed by the transmembrane regions. It follows that one cannot cut off the ligand-binding and activation domain from one receptor and transfer it into another. It is also unclear which part of the receptor is responsible for G protein activation. Even though mutations in the third cytoplasmic domain close to attachments to the transmembrane regions lead to loss of receptor activity, suggesting this to be a critical area of receptor–G protein interaction, mutations of any one of the other cytoplasmic loops, or of the carboxyl terminus, also close to the transmembrane domains, also interfere with G protein activation by the receptor. It may be that, like the ligand, the G protein simultaneously contacts several of the transmembrane segments at their points of turning into cytoplasmic sequences. Thus, receptors may have both a "ligand pocket" (opening toward the extracellular space), and a "G protein pocket" (opening toward the intracellular space), both involving the general structure of the receptor, as opposed to a few individual amino acids or a discrete domain. By definition, this G protein pocket must be complex, having elements that provide for recognition of the $\beta\gamma$ dimer aspect of the G proteins and elements that vary from receptor to receptor depending on the G protein it is designed to activate upon ligand binding. Sequence comparisons among four receptors that act by activating the same phospholipase C signal transduction pathway, namely, the muscarinic M1, the adrenergic α_1, the serotonin 5HT-1c, and the substance K

receptors, fail to suggest which areas or portions of the receptors might be involved in G protein activation.

The Point of View of the G Protein

The same lack of clearly definable domains also applies to the G proteins. These proteins are heterotrimers whose α subunits do not interact with receptors unless they are associated with $\beta\gamma$ dimers. There is no information about structure-function relationships for β subunits, although three mammalian, one yeast, and one *Dictyostelium discoideum* β subunits have now been cloned (Tables 4 and 5). It is also not known whether γ subunits play a role in the interaction of the G protein trimer with receptors. We have proposed that the γ subunits serve to anchor the complexes in the plasma membrane because $\beta\gamma$'s from erythrocytes are hydrophobic and insoluble in the absence of detergents, whereas those from transducin, which differ only in their γ subunits (107–109), are water soluble. However, this does not rule out a role for γ subunits in interaction with receptor. γ subunits have been cloned from retinal rod, brain, and yeast cells (110–113). Structurally they are related but also quite diverse, inviting mutational analysis to establish structure-function relationships.

In contrast to β and γ subunits, structure-function studies are beginning to define the regions of Gα involved in its interaction with the receptor and effector. Thus, receptor–G protein interactions have been found to be impaired both in a functional mutation, UNC, which is at position -6 from the α subunit carboxyl terminus (114), and upon ADP-ribosylation of the α subunits by PTX (115), which occurs on the Cys at position -4 from the carboxyl terminus (116). As these changes did not affect GTPase or GTP analog activation, it seems safe to conclude that the carboxyl termini of Gα's must be part of the "receptor interaction" domains of Gα's. Surprisingly, a study of the effector specificity of a chimera made by Masters et al, in which the replacement of 60% of the amino-terminal portion of α's with that of an α_i showed no loss of effector (or receptor) specificity (117). This finding places one of the effector specificity determinants of the Gα subunit on the 40% carboxy-terminal portion of the molecule. These studies do not rule out interaction of either receptor or effector with the remainder of the Gα molecule. The reported effects of carboxylterminal antibodies in blocking receptor-mediated activation of various G proteins, such as the effect of monoclonal antibody 4A interfering with rhodopsin activation of transducin (118, 119) and with muscarinic receptor–mediated activation of atrial G_k (120) and the effects of peptide-directed carboxy-terminal antibodies interfering with receptor-mediated activation (122) and inactivation of adenylyl cyclase (121), are in agreement with the conclusion that the carboxyl terminus of a Gα should be

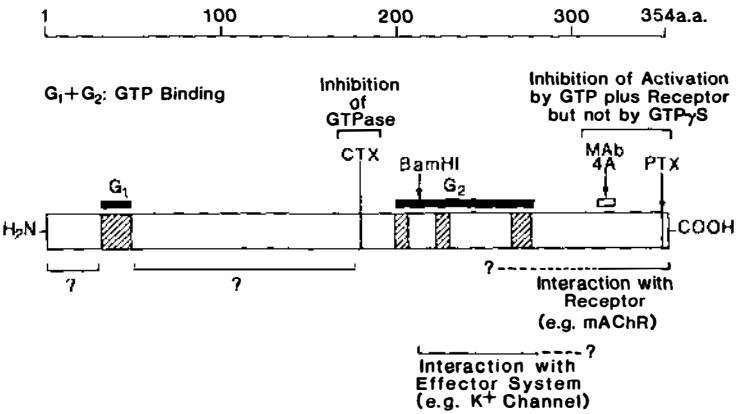


Figure 3 Summary of assignments of functional domains to structural domains of Gα subunits. G1 and G2: regions of contact of α subunits with guanine nucleotide. BamHI: position of restriction site used to create 60/40 chimeras between $G\alpha_s$ and $G\alpha_i$. For more details, see (29).

one of the points of contact of Gα with receptors. Figure 3 summarizes these findings.

Homologous Signal Transduction System in Yeast: Pheromone-Induced Mating

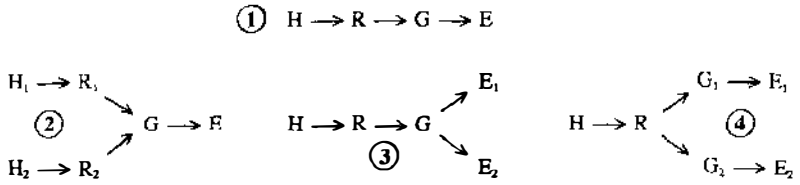
Haploid cells of *Saccharomyces cerevisiae* are of either the **a** or the α type, secreting, respectively, **a** or α mating factors (pheromones), which interact on α cells with **a** pheromone receptor *(STE3)* and on **a** cells with α pheromone receptor *(STE2)* to trigger, among other responses, cell arrest in G_1 and mating between the opposite types to give **a**/α diploid cells (cf 123 and 124). The STE2 and STE3 pheromone receptors belong to the opsin family of membrane proteins (125) and couple to a Gαβγ protein (G_m for mating) encoded by the *GPA1*(α), *STE4*(β), and *STE18*(γ) genes (113, 126). G_m is the same in α and **a** cells, and its α, β, and γ subunits are highly homologous to mammalian Gα, Gβ and Gγ, respectively. Disruption of the *GPA1* gene results in a phenotype equal to pheromone stimulation, i.e. growth arrest (23, 24). This phenotype can be interrupted by disruption of *STE4* or *STE18*, indicating that in contrast to signal transduction in diploid animal cells, signal transduction in haploid yeast cells, destined to trigger responses leading to diploidy, is mediated by the βγ and not the α portion of the G protein (113). *GPA1*, *STE4*, and *STE18* genes are expressed only in haploid cells (23, 24, 113).

The structural homology between the mammalian α subunits and yeast α_m, found a functional correlate when it was shown that substitution of mamma-

lian α_s for yeast α_m *(GPA1)* in *gpa1* cells suppresses the *gpa1* mutation (127). This indicates that mammalian $G_s\alpha$ interacts with yeast $G\beta\gamma$. A response to pheromone in *gpa1* cells expressing $G_s\alpha$ was not restored, however, indicating that the mammalian α subunit does not interact with STE gene product. Presumably, for restoration of pheromone to happen, it will be necessary to supply the yeast cell with a $G_s\alpha$-compatible receptor (e.g. β-adrenergic receptor). This would make yeast cells mating competent in response to isoproterenol and convert them into an interesting system to analyze, via isolation of suppressor mutations, the sites of G-R interaction.

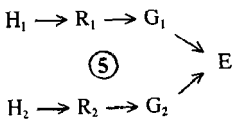
SPECIFICITIES IN RECEPTOR–G PROTEIN–EFFECTOR INTERACTION

Evidence for the notion that single G proteins are designed to interact with classes of receptors as opposed to single receptor subtypes comes from three sets of observations: (*a*) discoveries in the late 1960s that up to five different hormone receptors can activate a single adenylyl cyclase system in an isolated membrane (128, 129) (*b*) the discoveries in the early and middle 1970s that receptors can be transferred from one cell to another (130) and (*c*) that there are no species and/or tissue specificity restrictions as to the source of G_s for reconstitution of a hormonally stimulable adenylyl cyclase system in *cyc*− membranes (131, 132). The discovery that the same splice variant of G_s that activates adenylyl cyclase is also able to regulate Ca^{2+} channel activity (68) indicates that one G protein can interact with more than one effector. The discovery that three different G_i proteins all activate the same K^+ channel (65) indicates that several G proteins may regulate a single effector. Ashkenazi et al (133) showed that single receptors may affect more than one G protein. It follows that transmembrane wiring diagrams are in fact combinations of the following four basic configurations:



The complexity that may exist in the wiring of transmembrane signal transmission was well illustrated by the findings of Ewald et al with PTX-treated rat sensory neurons (104, 105). On studying the efficacy with which brain G_i and G_o reconstitute Ca^{2+} current regulation by neuropeptide (NPY) and bradykinin, they discovered that the effect of NPY could be fully recon-

stituted with G_o, with G_i being much less potent, whereas the effect if bradykinin could be only partially reconstituted by G_o, requiring G_i to achieve full reconstitution:



The important notion that emerges from these findings is that the wiring diagrams describing signal transduction by G proteins must be determined individually and separately for each cell or tissue of interest. This includes the determination not only of the receptors that are present but also of the G proteins and effectors needed to process the receptor signals.

ALTERED G PROTEIN FUNCTIONS: CLINICAL PERSPECTIVE

There are disease states which are due to or associated with altered G protein function. The classical example is, of course, the severe diarrhea associated with *Vibrio cholerae* infections, which is due to ADP-ribosylation of mucosal G_s, elevating cAMP levels and promoting serosal-to-luminal water transport in the small intestine colonized by the bacteria. Coli bacteria secrete a heat-labile toxin, enterotoxin, that is homologous to choleratoxin (CTX) and is very probably responsible for most of the symptoms associated with diarrheas that afflict travelers in less developed areas of the world.

Most of the symptoms associated with whooping cough (caused by *Bordetella pertussis*) are due to PTX, including "sensitization to histamine," hypoglycemia, and the cough of neurological origin that persists after the infective stage of the disease has passed and airways no longer show signs of local irritation. The hypoglycemia, which also afflicts some children after vaccination against pertussis, is due to an abnormal increase in insulin levels in plasma, which, in turn, is due to the action of the PTX of the vaccine. The toxin ADP-ribosylates cell G_i and, in so doing, uncouples the insulin release control system of the cell from a tonic G_i-dependent α_2-adrenergic inhibition. Indeed, one of the groups that first purified PTX used this as a bioassay and had given it the name "islet-activating protein" (IAP) (134).

In one genetic disease the levels of $G_s\alpha$ subunits are 50% of control levels in all somatic cells (135, 136). This disease, which is autosomal dominant, is clinically most noticeable as a lack of parathyroid hormone (PTH) action in the kidneys, which gave it the clinical name pseudohypoparathyroidism, (type Ia). As might be expected, patients with this condition exhibit other

abnormalities, unrelated to PTH action, which may also relate to the assumed primary $G_s\alpha$ defect. These include hypothyroidism, impaired prolactin secretion in response to thyrotropin-releasing hormone, and decreased antidiuresis in response to vasopressin. Under normal circumstances, target cells for PTH and vasopressin are likely to have more limiting amounts of $G_s\alpha$ than the remainder of the cells of the body. The reason for the reduced levels of $G_s\alpha$ is bound to be complex. Patients with type Ia pseudohypoparathyroidism also exhibit olfactory dysfunction (137). Thus, they not only have reduced classical $G_s\alpha$, but very probably also reduced level of $G_{olf}\alpha$, the independent gene product that is normally expressed in high concentrations in olfactory cilia, where it mediates stimulation of adenylyl cyclase (138).

Another disease affecting G_s is a particular type of mitral valve prolapse which is associated with hyperresponsiveness to β-adrenergic stimulation. The level of G_s in erythrocyte membranes of these patients is unaffected when tested for by CTX labeling but, on reconstitution with adenylyl cyclase G_s from these membranes, is 32% more active than that from control membranes (139). The molecular basis of the increased specific activity of the G_s from the patients could be of a genetic nature, but need not be at the level of G_s.

Although details still need further clarification, it is clear that heart failure is associated not only with alterations in β-adrenergic receptor function but also with changes in G protein function. Thus, in human heart failure a diminished contractile response to β-adrenergic agonists is correlated with a decrease in measurable β-adrenergic receptor density and an increase in PTX-sensitive G protein levels without changes in G_s function as assessed by either cholera toxin labeling or a complementation assay (140). On the other hand, a decrease in G_s function was reported in ventricular failure induced by pressure overload in dogs (141). This is a model system that is associated with a decrease in the proportion of β-adrenergic receptors in the high-affinity agonist–binding state in the face of an increase of total receptor density. In both situations the heart failure is preceded by compensatory hyperstimulation by catecholamines, and it is likely that the changes in receptor and its coupling are secondary to primary overstimulation of the receptor, i.e. are the reflections of a complex desensitization process. Interestingly, long-term treatment of human patients with β_1-selective antagonists results in an enhancement in the coupling of and action of catecholamines through β_2- versus α_1-adrenergic receptors (142). Whether this is associated with correlative changes in one or the other of the $G_s\alpha$ splice variants, is a matter of speculation.

G proteins are altered in experimental diabetes as well: decreases in both liver G_s activity and liver PTX substrates, relieved by insulin injection, have been reported in response to streptozotocin-induced diabetes (143, 144).

A superactive G_s was found in some pituitary growth hormone–secreting adenomas with elevated cAMP levels (145). This raised the possibility that G proteins may, under certain circumstances, acquire oncogenic capacities. Proof for the causal role of G_s in the generation of this type of benign tumor was obtained by DNA sequence analysis. α_s genes from four independent tumors with high basal adenylyl cyclase activity, but not from tumors with normal adenylyl cyclase activity or from non-tumor tissues, were found to be mutated. Two of the mutations were from Arg^{201} to Cys^{201}, one was from Arg^{201} to His^{201} and the fourth was Gln^{227} to Arg^{227} (146). Arg^{201} is the site of ADP-ribosylation by CTX, which, like mutations of Gln^{227}, leads to loss of the GTPase activity of α_s and concomittant superactivation by GTP.

Lithium ion has been proposed to exert its anti–manic-depressive effects by acting at the level of G proteins (147; see also discussion in 148). However, lithium is known to interfere profoundly with G protein–independent polyphosphoinositide breakdown, and, as proposed by another research group (149), inhibition of inositolphosphate hydrolysis, rather than alteration of G protein function, may be the cause of the therapeutic action of lithium.

CONCLUSION

Signal transduction by G proteins is a fundamental and widespread mechanism used by a wide variety of hormones, neurotransmitters, and autocrine and paracrine factors to regulate cellular functions. G proteins modulate not only cAMP formation, but also intracellular Ca^{2+} mobilization, arachidonic acid release, and, very importantly, membrane potential. Membrane potential is not only a trigger for neurotransmitter release and conduction of nerve impulses. In tissues such as secretory cells, it is the main regulator of Ca^{2+} entry. In the heart, the frequency of action potentials determines the frequency of contraction, and the duration of the depolarized state determines Ca^{2+} entry and the force of contraction. More subtle changes in resting membrane potential alter the predisposition of the cell to be stimulated by other factors and hormones. It is easy to imagine that persistent changes in membrane potential may acutely and chronically affect the proliferative properties of the cell.

The mechanism by which G proteins are activated provides for amplification, reversal of action, and continued monitoring of hormone. It provides for amplification because few receptor molecules may act catalytically to activate many G proteins molecules; it provides for reversal of action because they have an internal turnoff mechanism whereby the $G\alpha$ subunit hydrolyzes GTP to GDP; and it provides for continued monitoring of the primary messenger level because each activation cycle requires not only GTP but also occupied receptor.

Not all G proteins are known, and some are known whose functions are still unknown. More G proteins and more effector functions affected by them will surely be found. Work is in progress to unravel a complicated network of interactions among receptors, G proteins, and effector systems, which affects regulation not only of metabolic activities of organs such as liver, heart, and fat, but also of the integrative functions of the central nervous system.

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Literature Cited

1. Rodbell, M., Krans, H. M. J., Pohl, S. L., Birnbaumer, L. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. III. Binding of glucagon: Method of assay and specificity. *J. Biol. Chem.* 246:1861–71
2. Rodbell, M., Krans, H. M. J., Pohl, S. L., Birnbaumer, L. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. IV. Binding of glucagon: Effect of guanyl nucleotides. *J. Biol. Chem.* 246: 1872–76
3. Birnbaumer, L. 1973. Hormone-sensitive adenylyl cyclases: Useful models for studying hormone receptor functions in cell-free systems. *Biochim. Biophys. Acta* 300:129–58
4. Birnbaumer, L., Nakahara, T., Yang, P.-Ch. 1974. Studies on receptor-mediated activation of adenylyl cyclases. II. Nucleotide and nucleoside regulation of the activities of the beef renal medullary adenylyl cyclase and their stimulation by neurohypophyseal hormones. *J. Biol. Chem.* 249:7857–66
5. Yamamura, H., Lad, P. M., Rodbell, M. 1977. GTP stimulates and inhibits adenylate cyclase in fat cell membranes through distinct regulatory processes. *J. Biol. Chem.* 252:7964–66
6. Londos, C., Cooper, D. M. F., Schlegel, W., Rodbell, M. 1978. Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: Basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis. *Proc. Natl. Acad. Sci. USA* 75:5362–66
7. Jakobs, K. H., Saur, W., Schultz, G. 1978. Inhibition of platelet adenylate cyclase by epinephrine requires GTP. *FEBS Lett.* 85:167–70
8. Wheeler, G. L., Bitensky, M. W. 1977. A light-activated GTPase in vertebrate photoreceptors: Regulation of light-activated cyclic GMP phosphodiesterase. *Proc. Natl. Acad. Sci. USA* 74: 4238–42
9. Litosch, I., Wallis, C., Fain, J. N. 1985. 5-Hydroxytryptamine stimulates inositol phosphate production in a cell-free system from blowfly salivary glands. Evidence for a role of GTP in coupling receptor activation to phosphoinositide breakdown. *J. Biol. Chem.* 260:5464–71
10. Cockroft, S., Gomperts, B. D. 1985. Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. *Nature* 314: 534–36
11. Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M., Hille, B. 1985. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature* 317:536–38
12. Breitwieser, G. E., Szabo, G. 1985. Uncoupling of cardiac muscarinic and beta-adrenergic receptors from ion channels by a guanine nucleotide analogue. *Nature* 317:538–40
13. Kurachi, Y., Nakajima, T., Sugimoto, T. 1986. On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: Involvement of GTP-binding proteins. *Pflügers Arch.* 407:264–74
14. Yatani, A., Codina, J., Brown, A. M., Birnbaumer, L. 1987. Direct activation

of mammalian atrial muscarinic potassium channels by GTP regulatory protein G_k. *Science* 235:207–11

15. Brown, A. M., Birnbaumer, L. 1988. Direct G protein gating of ion channels. *Am. J. Physiol.* 23:H401–10
16. Birnbaumer, L., Codina, J., Mattera, R., Yatani, A., Scherer, N. M., et al. 1987. Signal transduction by G proteins. *Kidney Int.* 32(Suppl. 23): S14–37
17. Lochrie, M. A., Simon, M. I. 1988. G Protein multiplicity in eukaryotic signal transduction systems. *Biochemistry* 17: 4957–65
18. Mumby, S. M., Kahn, R. A., Manning, D. R., Gilman, A. G. 1986. Antisera of designed specificity for subunits of guanine nucleotide-binding regulatory proteins. *Proc. Natl. Acad. Sci. USA* 83:265–69
19. McKenzie, F. R., Mullaney, I., Unson, C. G., Spiegel, A. M., Milligan, G. 1988. The use of anti-peptide antibodies to probe interactions between receptors and guanine nucleotide binding proteins. *Biochem. Soc. Trans.* 16:434–37
20. Birnbaumer, L., VanDongen, A. M. J., Codina, J., Yatani, A., Mattera, R., et al. 1989. Identification of G protein-gated and G protein-modulated ionic channels. Molecular basis for G protein action. In *Secretion and Its Control,* ed. C. M. Armstrong, G. S. Oxford, pp. 18–54. New York: Rockefeller Univ. Press.
21. Rodbell, M. 1980. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* 284:17–22
22. Gilman, A. G. 1984. G Proteins and dual control of adenylate cyclase. *Cell* 36: 577–79
23. Dietzel, C., Kurjan, J. 1987. The yeast SCG1 Gene: A G_α-like protein implicated in the a- and α-factor response pathway. *Cell* 50:1001–10
24. Miyajima, I., Nakafuku, M., Nakayama, N., Brenner, C., Miyajima, A., et al. 1987. GPA1, A haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. *Cell* 50:1011–19
25. Selinger, Z., Minke, B. 1988. Inositol lipid cascade of vision studied in mutant flies. *Cold Spring Harbor Symp. Quant. Biol.* 53:333–41
26. Pupillo, M., Klein, P., Vaughan, R., Pitt, G., Lilly, P., et al. 1988. cAMP receptor and G-protein interactions control development of *Dictyostelium. Cold Spring Harbor Symp. Quant. Biol.* 53:656–65
27. Kumagai, A., Mann, S. K. O., Pupillo, M., Pitt, G., Devreotes, P. N., Firtel, R. A. 1988. A molecular analysis of G protein and control of early gene expression by the cell-surface cAMP receptor in *Dictyostelium. Cold Spring Harbor Symp. Quant. Biol.* 53:675–85
28. Zbell, B., Schwendmann, I., Hohenadel, H., Walter-Back, C. 1989. In *Proc. 9th Int. Washington Spring Symp. Biology of Cellular Transducing Signals,* May 8–12. ed. J. Y. Vanderhoek. Washington, DC: Geo. Washington Univ.
29. Birnbaumer, L., Abramowitz, J., Brown, A. M. 1990. Signal transduction by G proteins. *Biochem. Biophys. Acta.* In press
30. Ovchinnikov, Y. A., Lipkin, V. M., Kumarev, V. P., Gubanov, V. V., Khramtsov, N. V., 1986. Cyclic GMP phosphodiesterase from cattle retina. Amino acid sequence of the γ-subunit and nucleotide sequence of the corresponding cDNA. *FEBS. Lett.* 204:288–92
31. Ovchinnikov, Y. A., Gubanov V. V., Khramtsov, N. V., Ischenko, K. A., Zagranichny, V. E., et al. 1987. Cyclic GMP phosphodiesterase from bovine retina. Amino acid sequence of the α-subunit and nucleotide sequence of the corresponding cDNA. *FEBS Lett.* 223:169–73
32. Pittler, S. J., Baehr, W., Dryja, T. P., Wasmuth, J. J., McConnell, D. G., et al. 1989. Molecular characterization of human and bovine rod photoreceptor cGMP phosphodiesterase α-subunit and chromosomal localization of the human gene. *Genomics.* In press
33. Krupinski, J., Coussen, F., Bakalyar, H. A., Tang, W.-J., Feinstein, P. G., et al. 1989. Adenyl cyclase amino-acid sequence possible channel-like or transporter-like structure. *Science* 244:1558–64
34. Smigel, M. D. 1986. Purification of the catalyst of adenylate cyclase. *J. Biol. Chem.* 261:1976–82
35. Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., et al. 1987. Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 328:313–18
36. Yatani, A., Imoto, Y., Codina, J., Hamilton, S. L. Brown, A. M., Birnbaumer, L. 1988. The stimulatory G protein of adenylyl cyclase, G_s, directly stimulates dihydropyridine-sensitive skeletal muscle Ca^{2+} channels. Evidence for direct regulation independent

of phosphorylation by cAMP-dependent protein kinase. *J. Biol. Chem.* 263: 9887–95
37. Bloomquist, B. T., Shortridge, R. D., Schneuwly, S., Perdew, M., Montell, C., et al. 1988. Isolation of a putative phospholipase C gene of *drosophila, norpA,* and its role in phototransduction. *Cell* 54:723–33
38. Suh, P.-G., Ryu, S. H., Moon, K. H., Suh, H. W., Rhee, S. G. 1988. Cloning and sequence of multiple forms of phospholipase C. *Cell* 54:161–69
39. Katan, M., Kriz, R. W., Totty, N., Philp, R., Meldrum, E., et al. 1988. Determination of the primary structure of PLC-154 demonstrates diversity of phosphoinositide-specific phospholipase C activities. *Cell* 54:171–77
40. Birnbaumer, L., Hildebrandt, J. D., Codina, J., Mattera, R., Cerione, R. A., et al. 1985. Structural basis of adenylyl cyclase stimulation and inhibition by distinct guanine nucleotide regulatory proteins. In *Molecular Mechanisms of Signal Transduction,* ed. P. Cohen, M. D. Houslay, pp. 131–82. Amsterdam: Elsevier
41. Tolkovsky, A. M., Levitzki, A. 1978. Mode of coupling between the β-adrenergic receptor and adenylate cyclase in turkey erythrocytes. *Biochemistry* 17:3795–3810
42. Asano, T., Pedersen, S. E., Scott, C. W., Ross, E. M. 1984. Reconstitution of catecholamine-stimulated binding of guanosine 5'-O-(3-thiotriphosphate) to the stimulatory GTP-binding protein of adenylate cyclase. *Biochemistry* 23: 5460–67
43. Brandt, D. R., Ross, E. M. 1986. Catecholamine-stimulated GTPase cycle. Multiple sites of regulation by beta-adrenergic receptor and Mg^{2+} studied in reconstituted receptor-G_s vesicles. *J. Biol. Chem.* 261:1656–64
44. May, D. C., Ross, E. M. 1988. Rapid binding of guanosine 5'-O-(3-thiotriphosphate) to an apparent complex of β-adrenergic receptor and the GTP-binding regulatory protein G_s. *Biochemistry* 27:4888–93
45. Cassel, D., Selinger, Z. 1978. Mechanism of adenylate cyclase activation through the *beta*-adrenergic receptor: Catecholamime-induced displacement of bound GDP by GTP. *Proc. Natl. Acad. Sci. USA* 75:4155–59
46. Birnbaumer, L., Swartz, T. L., Abramowitz, J., Mintz, P. W., Iyengar, R. 1980. Transient and steady state kinetics of the interaction of guanyl nucleotides with the adenylyl cyclase system from rat liver plasma membranes. Interpretation in terms of a simple two-state model. *J. Biol. Chem.* 255:3542–51
47. Iyengar, R., Abramowitz, J., Bordelon-Riser, M. E., Birnbaumer, L. 1980. Hormone receptor-mediated stimulation of adenylyl cyclase systems: Nucleotide effects and analysis in terms of a two-state model for the basic receptor-affected enzyme. *J. Biol. Chem.* 255:3558–64
48. Iyengar, R., Birnbaumer, L. 1982. Hormone receptors modulate the regulatory component of adenylyl cyclases by reducing its requirement for Mg ion and increasing its extent of activation by guanine nucleotides. *Proc. Natl. Acad. Sci. USA* 79:5179–83
49. Howlett, A. C., Gilman, A. G. 1980. Hydrodynamic properties of the regulatory component of adenylate cyclase. *J. Biol. Chem.* 260:2861–66
50. Northup, J. K., Smigel, M. D., Sternweis, P. C., Gilman, A. G. 1983. The subunits of the stimulatory regulatory component of adenylate cyclase. Resolution of the activated 45,000-Dalton (alpha) subunit. *J. Biol. Chem.* 258: 11369–76
51. Codina, J., Hildebrandt, J. D., Birnbaumer, L., Sekura, R. D. 1984. Effects of guanine nucleotides and Mg on human erythrocyte N_i and N_s, the reulatory components of adenylyl cyclase. *J. Biol. Chem.* 259:11408–18
52. Skorecki, K. L., Verkman, A. S., Jung, C. H., Ausiello, D. A. 1986. Evidence for vasopressin activation of adenylate cyclase by subunit dissociation. *Am. J. Physiol.* 250:C115–C23
53. Ransnäs, L. A., Insel, P. A. 1988. Subunit dissociation is the mechanism for hormonal activation of the G_s protein in native membranes. *J. Biol. Chem.* 263:17239–42
54. Iyengar, R., Rich, K. A., Herberg, J. T., Premont, R. T., Codina, J. 1988. Glucagon receptor-mediated activation of G_s is accompanied by subunit dissociation. *J. Biol. Chem.* 263:15348–53
55. Fung, B. K.-K. 1983. Characterization of transducin from bovine retinal rod outer segments. I. Separation and reconstitution of the subunits. *J. Biol. Chem.* 256:10495–502
56. Graziano, M. P., Freissmuth, M., Gilman, A. G. 1989. Expression of $G_{s\alpha}$ in *Escherichia coli:* Purification and properties of two forms of the protein. *J. Biol. Chem.* 264:409–18
57. Pedersen, S. E., Ross, E. M. 1982. Functional reconstitution of β-

adrenergic receptors and the stimulatory GTP-binding protein of adenylate cyclase. *Proc. Natl. Acad. Sci. USA* 79: 7228–32

58. Navon, S. E., Fung, B. K.-K. 1984. Characterization of transducin from bovine retinal rod outer segments. Mechanism and effects of cholera toxin-catalyzed ADP-ribosylation. *J. Biol. Chem.* 259:6686–93
59. Fung, B. K.-K., Hurley, J. B., Stryer, L. 1981. Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc. Natl. Acad. Sci. USA* 78:152–56
60. Graziano, M. P., Casey, P. J., Gilman, A. G. 1987. Expression of cDNAs for G proteins in *Escherichia coli*. Two forms of $G_{s\alpha}$ stimulate adenylate cyclase. *J. Biol. Chem.* 262:11375–81
61. Olate, J., Mattera, R., Codina, J., Birnbaumer, L. 1988. Reticulocyte lysates synthesize an active *alpha* subunit of the stimulatory G protein G_s. *J. Biol. Chem.* 263:10394-400
62. Codina, J., Yatani, A., Grenet, D., Brown, A. M., Birnbaumer, L. 1987. The alpha subunit of the GTP binding protein G_k opens atrial potassium channels. *Science* 236:442–45
63. Codina, J., Grenet, D., Yatani, A., Birnbaumer, L., Brown, A. M. 1987. Hormonal regulation of pituitary GH_3 cell K^+ channels by G_k is mediated by its alpha subunit. *FEBS Lett.* 216:104–6
64. Mattera, R., Yatani, A., Kirsch, G. E., Graf, R., Olate, J., et al. 1989. Recombinant α_i-3 subunit of G protein activates G_k-gated K^+ channels. *J. Biol. Chem.* 264:465–71
65. Yatani, A., Mattera, R., Codina, J., Graf, R., Okabe, K., 1988. The G protein-gated atrial K^+ channel is stimulated by three distinct $G_i\alpha$-subunits. *Nature* 336:680–82
66. Light, D. B., Ausiello, D., Stanton, B. A. 1989. A GTP binding protein, α_i^*-3, directly activates a sodium-conducting ion channel in a renal epithelium. *J. Clin. Invest.* 84:345–56
67. Yatani, A., Codina, J., Imoto, Y., Reeves, J. P., Birnbaumer, L., Brown, A. M. 1987. A G protein directly regulates mammalian cardiac calcium channels. *Science* 238:1288–92
68. Mattera, R., Graziano, M. P., Yatani, A., Zhou, Z., Graf, R., et al. 1989. Individual splice variants of the α subunit of the G protein G_s activate both adenylyl cyclase and Ca^{2+} channels. *Sciemce* 243:804–7
69. Kanaho, Y., Tsai, S.-C., Adamik, R., Hewlett, E. L., Moss, J., Vaughan, M. 1984. Rhodopsin-enhanced GTPase activity of the inhibitory GTP-binding protein of adenylate cyclase. *J. Biol. Chem.* 259:7378–81
70. Navon, S. E., Fung, B. K.-K. 1987. Characterization of transducin from bovine retinal rod outer segments. Participation of the amino-terminal region of T_α in subunit interaction. *J. Biol. Chem.* 262:15746–51
71. Florio, V. A., Sternweis, P. C. 1989. Mechanism of muscarinic receptor action on G_o in reconstituted phospholipid vesicles. *J. Biol. Chem.* 264:3909–15
72. Cerione, R. A., Staniszewski, C., Caron, M. G., Lefkowitz, R. J., Codina, J., Birnbaumer, L. 1985. A role for N_i in the hormonal stimulation of adenylate cyclase. *Nature* 318:293–95
73. Cerione, R. A., Gierschik, P., Staniszewski, C., Benovic, J. L., Codina, J., et al. 1987. Functional differences in the beta-gamma complexes of transducin and the inhibitory guanine nucleotide regulatory protein. *Biochemistry* 26:1485–91
74. Okabe, K., Yatani, A., Evans, T., Ho, Y.-K, Codina, J., et al. 1989. Inhibition of G_k-gated K^+ channel by $\beta\gamma$ dimers of G proteins. Effect of agonists. *J. Biol. Chem.* In press
75. Hildebrandt, J. D., Kohnken, R. E. 1989. Two mechanisms mediating inhibition of adenylyl cyclase. *J. Biol. Chem.* In press
76. Gilman, A. G. 1987. G proteins: Transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56:615–49
77. Kim, D., Lewis, D. L., Graziadei, L., Neer, E. J., Bar-Sagi, D., Clapham, D. E. 1989. G Protein $\beta\gamma$-Subunits activate the cardiac muscarinic K^+ channel via phospholipase A_2. *Nature* 337:557–60
78. Birnbaumer, L. 1987. Which G protein subunits are the active mediators in signal transduction. *Trends Pharmacol. Sci.* 8:209–11
79. Cooper, D. M. F. 1982. Bimodal regulation of adenylate cyclase. *FEBS Lett.* 138:157–63
80. Katada, T., Ui, M. 1982. Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. *Proc. Natl. Acad. Sci. USA* 79:3129–33
81. Hildebrandt, J. D., Sekura, R. D., Codina, J., Iyengar, R., Manclark, C. R., Birnbaumer, L. 1983. Stimulation and inhibition of adenylyl cyclases is mediated by distinct proteins. *Nature* 302:706–9

82. Bokoch, G. M., Katada, T., Northup, J. K., Hewlett, E. L., Gilman, A. G. 1983. Identification of the predominant substrate for ADP-ribosylation by islet-activating protein. *J. Biol. Chem.* 258:2072–75
83. Codina, J., Hildebrandt, J. D., Iyengar, R., Birnbaumer, L., Sekura, R. D., Manclark, C. R. 1983. Pertussis toxin substrate, the putative N_i of adenylyl cyclases, is an alpha/beta hetertodimer regulated by guanine nucleotide and magnesium. *Proc. Natl. Acad. Sci. USA* 80:4276–80
84. Katada, T., Bokoch, G. M., Northrup, J. K., Ui, M., Gilman, A. G. 1984. The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. Properties and function of the purified protein. *J. Biol. Chem.* 259:3568–77
85. Katada, T., Bokoch, G. M., Smigel, M. D., Ui, M., Gilman, A. G. 1984. The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. Subunit dissociation and the inhibition of adenylate cyclase in S49 lymphoma cyc^- and wild type membranes. *J. Biol. Chem.* 259:3586–95
86. Casey, P. J., Gilman, A. G. 1988. G protein involvement in receptor-effector coupling. *J. Biol. Chem.* 263:2577–80
87. Toro, M.-J., Montoya, E., Birnbaumer, L. 1987. Inhibitory regulation of adenylyl cyclases. Evidence against a role for beta/gamma complexes of G proteins as mediators of G_i-dependent hormonal effects. *Mol. Endocrinol.* 1:669–76
88. Hildebrandt, J. D., Hanoune, J., Birnbaumer, L. 1982. Guanine nucleotide inhibition of cyc^- S49 mouse lymphoma cell membrane adenylyl cyclase. *J. Biol. Chem.* 257:14723–25
89. Hildebrandt, J. D., Birnbaumer, L. 1983. Inhibitory regulation of adenylyl cyclase in the absence of stimulatory regulation. Requirements and kinetics of guanine nucleotide induced inhibition of the cyc^- S49 adenylyl cyclase. *J. Biol. Chem.* 258:13141–47
90. Hildebrandt, J. D., Codina, J., Risinger, R., Birnbaumer, L. 1984. Identification of a *gamma* subunit associated with the adenylyl cyclase regulatory proteins N_s and N_i. *J. Biol. Chem.* 259:2039–42
91. Jakobs, K. H., Aktories, K., Schultz, G. 1983. A nucleotide regulatory site for somatostatin inhibition of adenylate cyclase in S49 lymphoma cells. *Nature* 303:177–78
92. Harris, B. A., Robishaw, J. D., Mumby, S. M., Gilman, A. G. 1985. Molecular cloning of complementary DNA for the alpha subunit of the G protein that stimulates adenylate cyclase. *Science* 229:1274–77
93. Kirsch, G., Yatani, A., Codina, J., Birnbaumer, L., Brown, A. M. 1988. The *alpha* subunit of G_k activates atrial K^+ channels of chick, rat and guinea pig. *Am. J. Physiol.* 254:H1200–5
94. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., Clapham, D. E. 1987. The $\beta\gamma$ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. *Nature* 325:321–26
95. Logothetis, D. E., Kim, D., Northrup, J. K., Neer, E. J., Clapham, D. E. 1988. Specificity of action of guanine nucleotide-binding regulatory protein subunits on the cardiac muscarinic K^+ channel. *Proc. Natl. Acad. Sci. USA* 85:5814–18
96. Kurachi, Y., Ito, H., Sugimoto, T., Katada, T., Ui, M. 1989. Activation of atrial muscarinic K^+ channels by low concentrations of $\beta\gamma$ subunits of G rat brain protein. *Pflügers Arch.* 413:325–27
97. Kurachi, Y., Ito, H., Sugimoto, T., Shimizu, T., Miki, I., Ui, M. 1989. Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel. *Nature* 337:555–57
98. Yatani, A., Codina, J., Sekura, R. D., Birnbaumer, L., Brown, A. M. 1987. Reconstitution of somatostatin and muscarinic receptor mediated stimulation of K^+ channels by isolated G_k protein in clonal rat anterior pituitary cell membranes. *Mol. Endocrinol.* 1:283–89
99. Andrade, R., Malenka, R. C., Nicoll, R. A. 1986. A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. *Science* 234:1261–65
100. Sasaki, K., Sato, M. 1987. A single GTP-binding protein regulates K channels coupled with dopamine, histamine and acetylcholine receptors. *Nature* 325:259–62
101. Thalmann, R. H. 1988. Evidence that guanosine triphosphate (GTP)-binding proteins control a synaptic response in brain: Effect of pertussis toxin and GTP-γ-S on the late inhibitory postsynaptic potential of hippocampal CA_3 neurons. *J. Neurosci.* 8:4589–602
102. VanDongen, A., Codina, J., Olate, J., Mattera, R., Joho, R., et al. 1988. Newly identified brain potassium channels gated by the guanine nucleotide binding (G) protein G_o. *Science* 242:1433–37

103. Heschelcr, J., Rosenthal, W., Trautwein, W., Schultz, G. 1986. The GTP-binding protein, N_o, regulates neuronal calcium channels. *Nature* 325:445–47
104. Ewald, D. A., Sternweis, P. C., Miller, R. J. 1988. Guanine nucleotide-binding protein G_o-induced coupling of neuropeptide Y receptors to Ca^{2+} channels in sensory neurons. *Proc. Natl. Acad. Sci. USA* 85:3633–37
105. Ewald, D. A., Pang, I.-H., Sternweis, P. C., Miller, R. J. 1989. Differential G protein-mediated coupling of neurotransmitter receptors to Ca^{2+} channels in rat dorsal root ganglion neurons in vitro. *Neuron* 2:1185–93
106. Dixon, R. A. F., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. C., et al. 1986. Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. *Nature* 321:75–79
107. Hildebrandt, J. D., Codina, J., Birnbaumer, L. 1984. Interaction of the stimulatory and inhibitory regulatory proteins of the adenylyl cyclase system with the catalytic component of *cyc*$^-$ S49 cell membranes. *J. Biol. Chem.* 259:13178–85
108. Hildebrandt, J. D., Codina, J., Rosenthal, W., Birnbaumer, L., Neer, E. J., et al. 1985. Characterization by two-dimensional peptide mapping of the subunit composition of the regulatory N_s and N_i proteins of adenylyl cyclase, and transducin, the guanine nucleotide binding protein of retinal rod outer segments. *J. Biol. Chem.* 260:14867–72
109. Codina, J., Stengel, D., Woo, S. L. C., Birnbaumer, L. 1986. Beta subunits of the human liver G_s/G_i signal transducing proteins and those of bovine retinal rod cell transducin are identical. *FEBS Lett.* 207:187–92
110. Hurley, J. B., Fong, H. K. W., Teplow, D. B., Dreyer, W. J., Simon, M. I. 1984. Isolation and characterization of a cDNA clone for the gamma subunit of bovine retinal transducin. *Proc. Natl. Acad. Sci. USA* 81:6948–52
111. Yatsunami, K., pandya, B. V., oprian, D. D., khorana, H. G. 1985. cDNA derived amino acid sequence of the gamma subunit of GTPase from bovine rod outer segments. *Proc. Natl. Acad. Sci. USA* 82:1936–40
112. Gautam, N., Baetscher, M., Aebersold, R., Simon, M. I. 1989. A G protein gamma subunit shares homology with *ras* proteins. *Science* 244:971–74
113. Whiteway, M., Hougan, L., Dignard, D., Thomas, D. Y., Bell, L., et al. 1989. The STE4 and STE18 genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. *Cell* 56:467–77
114. Sullivan, K. A., Miller, R. T., Masters, S. B., Beiderman, B., Heideman, W., Bourne, H. R. 1987. Identification of receptor contact site involved in receptor-G protein coupling. *Nature* 330:758–60
115. Sunyer, T., Monastirsky, B., Codina, J., Birnbaumer, L. 1989. Effect of pertussis toxin on properties of regulatory G proteins. *Mol. Endocrinol.* 3:1115–24
116. West, R. E. Jr., Moss, J., Vaughan, M., Liu, T., Liu, T.-Y. 1985. Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cystein 347 is the ADP-ribose acceptor site. *J. Biol. Chem.* 260:14428–30
117. Masters, S. B., Sullivan, K. A., Miller, R. T., Beiderman, B., Lopez, N. G., et al. 1988. Carboxy terminal domain of G_s-*alpha* specifies coupling of receptors to stimulation of adenylyl cyclase. *Science* 241:448–51
118. Hamm, H. E., Deretic, D., Hofmann, K. P., Schleicher, A., Kohl, B. 1987. Mechanism of action of monoclonal antibodies that block the light activation of the guanyl nucleotide-binding protein, transducin. *J. Biol. Chem.* 262:10831–38
119. Hamm, H. E., Deretic, D., Arendt, A., Hargrave, P. A., Koenig, B., Hofmann, K. P. 1988. Site of G protein binding to rhodopsin mapped using synthetic peptides of G-alpha sequences. *Science* 241:832–35
120. Simonds, W. F., Goldsmith, P. K., Woodard, C. J., Unson, C. G., Spiegel, A. M. 1989. Receptor and effector interactions of G_s: Functional studies with antibodies to the α_s carboxyl terminal decapeptide. *FEBS Lett.* 249:189–94
121. Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C., Spiegel, A. M. 1989. G_{i2} mediates α-adenergic inhibition of adenylyl cyclase in platelet membranes: *in situ* identification with Giα C-terminal antibodies. *Proc. Natl. Acad. Sci. USA*. 86:7809–13
122. Yatani, A., Hamm, H., Codina, J., Mazzoni, M. R., Birnbaumer, L., Brown, A. M. 1988. A monoclonal-antibody to the alpha-subunit of G_k blocks muscarinic activation of atrial K^+ channels. *Science* 241:828–31
123. Marsh, L., Herskowitz, I. 1988. From membrane to nucleus: The pathway of signal transduction in yeast and its ge-

netic control. *Cold Spring Harbor Symp. Quant. Biol.* 53:557–65
124. Matsumoto K., Nakafuku, M., Nakayama, N., Miyajima, I., Kaibuchi, K., et al. 1988. The role of G proteins in yeast signal transduction. *Cold Spring Harbor Symp. Quant. Biol.* 53:567–75
125. Blumer, K. J., Reneke, J. E., Courchesne, W. E., Thorner, J. 1988. Functional domains of a peptide hormone receptor: The α-factor receptor (STE2 gene product) of yeast *Saccharomyces cerevisiae*. *Cold Spring Harbor Symp. Quant. Biol.* 53:591–603
126. Kurjan, J., Dietzel, C. 1988. Analysis of the role of SCG1 (GPA1), a Gα homolog, and SST2 in pheromone response and desensitization in yeast. *Cold Spring Harbor Symp. Quant. Biol.* 53: 577–84
127. Blinder, D., Spatrick P. Bouvier, S., Sullivan, C., Jenness, D. 1988 Recovery from pheromone-induced arrest of the yeast cell cycle: α-factor binding and mutants that show pheromone-independent arrest of cell division. *Cold Spring Harbor Symp. Quant. Biol.* 53: 605–10
128. Birnbaumer, L., Rodbell, M. 1969. Adenyl cyclase in fat cells. II. Hormone receptors. *J. Biol. Chem.* 244:3477–82
129. Rodbell, M., Birnbaumer, L., Pohl, S. L. 1970. Adenyl cyclase in fat cells. III. Stimulation by secretin and the effects of trypsin on the receptors for lipolytic hormones. *J. Biol. Chem.* 245:718–22
130. Citri, Y., Schramm, M. 1980. Resolution, reconstitution and kinetics of the primary action of hormone receptor. *Nature* 287:297–300
131. Ross, E. M., Howlett, A. C., Ferguson, K. M., Gilman, A. G. 1978. Reconstitution of hormone-sensitive adenylate cyclase activity with resolved components of the enzyme. *J. Biol. Chem.* 253:6401–12
132. Kaslow, H. R., Farfel, Z., Johnson, G. L., Bourne, H. R. 1979. Adenylate cyclase assembled *in vitro:* Cholera toxin substrates determine different patterns of regulation by isoproterenol and guanosine 5'-triphosphate. *Mol. Pharmacol.* 15:472–83
133. Ashkenazi, A., Winslow, J. W., Peralta, E. G., Peterson, G. L., Schimerlik, M. I., et al. 1987. An M2 muscarinic receptor subtype coupled to both adenylyl cyclase and phosphoinositide turnover. *Science* 238:672–75
134. Ui, M. 1984. Islet-activating protein, pertussis toxin: A probe for functions of the inhibitory guanine nucleotide regulatory component of adenylate cyclase. *Trends Pharmacol. Sci.* 5:277–79
135. Farfel, Z., Brickman, A., Kaslow, H. R., Brothers, V. M., Bourne, H. R. 1980. Defect of receptor-cyclase coupling protein in pseudohypoparathyroidism. *New Engl. J. Med.* 303:237–42
136. Levine, M. A., Ahn, T. G., Klupt, S. F., Kaufman, K. D., Smallwood, P. M., et al. 1988. Genetic deficiency of the α subunit of the guanine nucleotide-binding protein G_s as the molecular basis for Albright hereditary osteodystrophy. *Proc. Natl. Acad. Sci. USA* 85:617–21
137. Weinstock, R. S., Wright, H. N., Spiegel, A. M., Levine, M. A., Moses, A. 1986. Olfactory dysfunction in humans with deficient guanine nucleotide-binding protein. *Nature* 322:635–37
138. Pace, U., Hanski, E., Salomon, Y., Lancet, D. 1985. Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature* 316:255–58
139. Davies, A. O., Su, C. J., Balasubramanyam, A., Birnbaumer, L. 1989. Abnormal guanine nucleotide regulatory protein in patients with the dysautonomia of mitral valve prolapse: Evidence from reconstitution of G_s. Submitted
140. Feldman, A. M., Cates, A. E., Veazey, W. B., Hershberger, R. E., Bristow, M. R., et al. 1988. Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. *J. Clin. Invest.* 82:189–97
141. Longabough, P. J., Vatner, D. E., Vatner, S. F., Homcy, C. J. 1988. Decreased stimulatory guanosine triphosphate binding protein in dogs with pressure-overloaded left ventricular failure. *J. Clin. Invest.* 81:420–24
142. Hall, J. A., Kaumann, A. J., Brown, M. J. 1989. Selective β1 adrenoreceptor blockade enhances positive inotropic responses to endogenous catecholamines mediated through β2 adrenoreceptors in human atrial myocardium. *Circ. Res.* Submitted
143. Dighe, R. R., Rojas, F. J., Birnbaumer, L., Garber, A. J. 1984. The impact of streptozotocin-induced diabetes mellitus on the glucagon-stimulable adenylyl cyclase system in rat liver. *J. Clin. Invest.* 73:1013–23
144. Gawler, D., Milligan, G., Spiegel, A. M., Unson, C. G., Houslay, M. D. 1987. Abolition of the expression of inhibitory guanine nucleotide regulatory protein G_i activity in diabetes. *Nature* 327:229–32
145. Vallar, L., Spada, A., Giannattasio, G. 1987. Altered G_s and adenylate cyclase

activity in human GH-secreting pituitary adenomas. *Nature* 330:566–69
146. Bourne, H. 1987. Discovery of a new oncogene in pituitary tumours? *Nature* 330:517–18
147. Avissar, S., Schreiber, G., Danon, A., Belmaker, R. H. 1988. Lithium inhibits adrenergic and cholinergic increases in GTP binding in rat cortex. *Nature* 331:440–42
148. Drummond, A. H. 1988. Lithium affects G-protein receptor coupling. *Nature* 331:388
149. Worley, P. F., Heller, W. A., Snyder, S. H., Baraban, J. M. 1988. Lithium blocks a phosphoinositide mediated cholinergic response in hippocampal Slices. *Science* 239:1428–29
150. Murayama, T., Ui, M. 1985. Receptor-mediated inhibition of adenylate cyclase and stimulation of arachidonic acid release in 3T3 fibroblasts. Selective susceptibility to islet-activating protein, pertussis toxin. *J. Biol. Chem.* 260:7226–33
151. Burch, R. M., Luini, A., Axelrod, J. 1986. Phospholipase A_2 and phospholipase C are activated by distinct GTP-binding proteins in response to alpha$_1$-adrenergic stimulation in FRTL-5 cells. *Proc. Natl. Acad. Sci. USA* 83:7201–5
152. Paris, S., Pouysségur, J. 1986. Pertussis toxin inhibits thrombin-induced activation of phosphoinositide hydrolysis and Na^+/H^+ exchange in hamster fibroblasts. *EMBO J.* 5:55–60
153. Kuroda, M., Honnor, R. C., Cushman, S. W., Londos, C., Simpson, I. A. 1987. Regulation of insulin-stimulated glucose transport in the isolated rat adipocyte. cAMP-independent effects of lipolytic and antipolytic agents. *J. Biol. Chem.* 262:245–53
154. Mallat, A., Pavione, C., Dufour, M., Lotersztajn, S., Bataille, D., Pecker, F. 1987. A glucagon fragment is responsible for the inhibition of the liver Ca^{2+} Pump by Glucagon. *Nature* 325:620–22
155. Bertorello, A., Aperia, A. 1989. Regulation of Na^+-K^+-ATPase activity in kidney proximal tubules: Involvement of GTP binding proteins. *Am. J. Physiol.* 256:F57–F62
156. Bocckino, S. B., Blackmore, P. F., Wilson, P. B., Exton, J. H. 1987. Phosphatidate accumulation in hormone-treated hepatocytes via a phospholipase D mechanism. *J. Biol. Chem.* 262: 15309–15
157. Hughes, A. R., Martin, M. W., Harden, T. K. 1984. Pertussis toxin differentiates between two mechanisms of attenuation of cyclic AMP accumulation by muscarinic cholinergic receptors. *Proc. Natl. Acad. Sci. USA* 81:5680–84
158. Suki, W., Abramowitz, J., Mattera, R., Codina, J., Birnbaumer, L. 1987. The human genome encodes at least three non-allelic G proteins with *alpha*$_i$-type subunits. *FEBS Lett.* 220:187–92
159. Kikuchi, A., Kozawa, O., Kaibuchi, K., Katada, T., Ui, M., Takai, Y. 1986. Direct evidence for involvement of a guanine nucleotide-binding protein in chemotactic peptide-stimulated formation of inositol bisphosphate and trisphosphate in differentiated human leukemic (HL-60) cells. Reconstitution with G_i or G_o of the plasma membranes ADP-ribosylated by pertussis toxin. *J. Biol. Chem.* 261:11558–62
160. Moriarty, T. M., Gillo, B., Carty, D. J., Premont, R. T., Landau, E. M., Iyengar, R. 1988. $\beta\gamma$ Subunits of GTP-binding proteins inhibit muscarinic receptor stimulation of phospholipase C. *Proc. Natl. Acad. Sci. USA* 85:8865–69
161. Sugimoto, K., Nukada, T., Tanabe, T., Takahashi, H., Noda, M., et al. 1985. Primary structure of the beta-subunit of bovine transducin deduced from the cDNA sequence. *FEBS Lett* 191:235–40
162. Fong, H. K. W., Hurley, J. B., Hopkins, R. S., Miake-Lye, R., Johnson, M. S., et al. 1986. Repetitive segmental structure of the transducin beta subunit: Homology with the CDC4 gene and identification of related mRNAs. *Proc. Natl. Acad. Sci. USA* 83:2162–66
163. Fong, H. K. W., Amatruda, T. T. III, Birren, B. W., Simon, M. I. 1987. Distinct forms of the β subunit of GTP-binding regulatory proteins identified by molecular cloning. *Proc. Natl. Acad. Sci. USA* 84:3792–96
164. Gao, B., Gilman, A. G., Robishaw, J. D. 1987. A second form of the β subunit of signal-transducing G proteins. *Proc. Natl. Acad. Sci. USA* 84:6122–25
165. Gao, B., Mumby, S., Gilman, A. G. 1987. The G protein β_2 complementary DNA encodes the β_{35} subunit. *J. Biol. Chem.* 262:17254–57
166. Levine, M. A., Smallwood, P. M., Moen, P., Halman, L. J., Modi, W. 1989. Identification of a cDNA encoding a third form of the β subunit of GTP-binding regulatory proteins. See Ref. 28, Abstr. 57
167. Amatruda, T. T. III, Gautam, N., Fong, H. K. W., Northrup, J. K., Simon, M. I. 1988. The 35- and 36-kDa β subunits of GTP-binding regulatory proteins are

products of separate genes. J. Biol. Chem. 263:5008–11
168. Evans, T., Fawzi, A., Fraser, E. D., Brown, M. L., Northrup, J. K. 1987. Purification of a beta$_{35}$ form of the *beta-gamma* complex common to G-proteins from human placental membranes. *J. Biol. Chem.* 262:176–81
169. Deleted in proof
170. Kühn, H. 1980. Light-induced, reversible binding of proteins to bovine photoreceptor membranes. Influence of nucleotides. *Neurochemistry* 1:269–85
171. Fukada, Y., Ohguro, H., Saito, T., Yoshizawa, T., Akino, T. 1989. $\beta\gamma$-Subunits of bovine transducin composed of two components with distinctive γ-subunits. *J. Biol. Chem.* 264:5937–43
172. Gierschik, P., Codina, J., Simons, C., Birnbaumer, L., Spiegel, A. 1985. Antisera against a guanine binding protein from retina cross-react with the *beta* subunit of the adenylyl cyclase associated guanine nucleotide binding proteins, N_s and N_i. *Proc. Natl. Acad. Sci. USA* 82:721–27
173. Nakafuku, M., Obara, T., Kaibuchi, K., Miyajima, I., Itoh, H., et al. 1988. Isolation of a second yeast *Saccharomyces cerevisiae* gene *(GPA2)* coding for guanine nucleotide-binding regulatory protein: Studies on its structure and possible functions. *Proc. Natl. Acad. Sci. USA* 85:1374–78
174. Pupillo, M., Kumagai, A., Pitt, G., Firtel, R., Devreotes, P. N. 1989. Multiple α-subunits of G proteins in *Dictyostelium. Proc. Natl. Acad. Sci. USA*. 86:4892–96
175. Kumagi, A., Pupillo, M., Gundersen R., Miake-Lye, R., Devreotes, P., Firtel, R. A. 1989. Regulation and function of G-alpha protein subunits in *Dictyostelium. Cell* 57:265–75
176. Provost, N. M., Somers, D. E., Hurley J. B. 1988. A *Drosophila melanogaster* G protein α subunit gene is expressed primarily in embryos and pupae. *J. Biol. Chem.* 263:12070–76
177. Quan, F., Wolfgang, W. J., Forte, M. A. 1989. The *Drosophila* gene coding for the α subunit of a stimulatory G protein is preferentially expressed in the nervous system. *Proc. Natl. Acad. Sci. USA*. 86:4321–25
178. Thambi, N. C., Quan, F., Wolfgang, W. J., Spiegel, A., Forte, M. 1989. Immunological and molecular characterization of Goα-like proteins in the *Drosophila* CNS. *J. Biol. Chem.* 264:18552–60
179. Yoon, J., Shortridge, R. D., Bloomquist, B. T., Schneuwly, S., Perdew, M. H., Pak, W. L. 1989. Molecular characterization of Drosophila gene encoding G_o α subunit. *J. Biol. Chem.* 264:18536–43
180. Yarfitz, S., Provost, N. M., Hurley, J. B. 1988. Cloning of a *Drosophila melanogaster* guanine nucleotide regulatory protein β-subunit gene and characterization of its expression during developmcnt. *Proc. Natl. Acad. Sci. USA* 85:7134–38
181. Olate, J., Jorquera, H., Purcell, P., Codina, J., Birnbaumer, L., Allende, J. E. 1989. Molecular cloning and sequence determination of a cDNA coding for the alpha subunit of a G_o type protein of *Xenopus laevis* oocytes. *FEBS Lett.* 244:188–92
182. Parent, L., Coronado, R. 1989. Reconstitution of the ATP-sensitive potassium channel of skeletal muscle. Activation of a G-protein dependent process. *J. Gen. Physiol.* In press
183. Ribalet, B., Ciani, S., Eddlestone, G. T. 1989. Modulation of ATP-scnsitive K channels in RINm5F cells by phosphorylation and G proteins. *Biophys. J.* 55:587A
184. Imoto, Y., Yatani, A., Reeves, J. P., Codina, J., Birnbaumer, L., Brown, A. M. 1988. α subunit of G_s directly activates cardiac calcium channels in lipid bilayers. *Am. J. Physiol.* 255:H722–28
185. Schubert, B., VanDongen, A. M. J., Kirsch, G. E., Brown, A. M. 1989. β-adrenergic inhibition of cardiac sodium channels by dual G protein pathways. *Science* 245:516–19
186. Toro, L., Ramos-Franco, J., Stephani, E. 1989. GTP-dependent regulation of myometrial K_{Ca} channels incorporated into lipid bilayers. *J. Gen. Physiol.* In press
187. Dolphin, A. C., Scott, R. H., Wooton, J. F. 1988. Photorelease of GTPγS inhibits the low threshold channel current in cultured rat dorsal root ganglion (DRG) neurons. *Pflügers Arch.* 411:19P
188. Hescheler, J., Rosenthal, W., Hinsch, K.-D., Wulfern, M., Trautwein, W., Schultz, G. 1988. Angiotensin II-induced stimulation of voltage-dependent Ca^{2+} currents in an adrenal cortical cell line. *EMBO J.* 7:619–24
189. Rosenthal, W., Hescheler, J., Hinsch, K.-D., Spicher, K., Trautwein, W., Schultz, G. 1988. Cyclic AMP-independent, dual regulation of voltage-dependent Ca^{2+} currents by LHRH and somatostatin in a pituitary cell line. *EMBO J.* 7:1627–33
190. Lewis, D. L., Weight, F. F., Luini, A. 1986. A guanine nucleotide-binding pro-

tein mediates the inhibition of voltage-dependen calcium current by somatostatin in a pituitary cell line. *Proc. Natl. Acad. Sci. USA* 83:9035–39

191. Holz, G. G., Rane, S. G., Dunlap, K. 1986. GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. *Nature* 319:670–72
192. Rane, S. O., Dunlap, K. 1986. Kinase C activator 1,2-oleylacetylglycerol attenuates voltage-dependent calcium current in sensory neurons. *Proc. Natl. Acad. Sci. USA* 83:184–88
193. Scott, R. H., Dolphin, A. C. 1986. Regulation of calcium currents by GTP analogue: Potentiation of (−)-baclofen-mediated inhibition. *Neurosci. Lett.* 69:59–64
194. Dolphin, A. C., Scott, R. H. 1987. Calcium channel currents and their inhibition by (−)-baclofen in rat sensory neurones: Modulation by guanine nucleotides. *J. Physiol.* 386:1–17
195. Dolphin, A. C., McGuirk, S. M., Scott, R. H. 1989. An investigation into the mechanisms of inhibition of calcium channel currents in cultured rat sensory neurones by guanine nucleotide analogues and (−)-baclofen. *Br. J. Pharmacol.* 97:263–73
196. Perney, T. M., Miller, R. J. 1989. Two different G-proteins mediate neuropeptide Y and bradykinin-stimulated phospholipid breakdown in cultured rat sensory neurons. *J. Biol. Chem.* 264:7317–27
197. Godchaux, W. III, Zimmerman, W. F. 1979. Membrane-dependent guanine nucleotide binding and GTPase activities of soluble protein from bovine rod cell outer segments. *J. Biol. Chem.* 254:7874–84
198. Cerione, R. A., Staniszewski, C., Caron, M. G., Lefkowitz, R. J., Codina, J., Birnbaumer, L. 1985. A role for N_i in the hormonal stimulation of adenylate cyclase. *Nature* 318:293–95