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G Protein Multiplicity in Eukaryotic Signal Transduction Systems†

Michael A. Lochrie and Melvin I. Simon*
Division of Biology, 147-75, California Institute of Technology, Pasadena, California 91125
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G proteins comprise a specific family of guanine nucleotide binding regulatory proteins that serve as intermediaries in a variety of transmembrane signaling processes in eukaryotic cells (Gilman, 1987). They are located on the cytoplasmic surface of membranes, where they physically couple ligand-bound receptors to the regulation of effector proteins which produce changes in intracellular metabolism. G proteins are heterotrimers composed of α (M_r 39000–54000), β (M_r 35000 or 36000), and γ (M_r 8000–10000) subunits. The α subunit interacts with guanine nucleotides. The β and γ subunits form a tightly associated $\beta\gamma$ complex. Different G proteins contain different α and probably different γ subunits but only one or two types of β subunits.

G proteins function by utilizing a guanine nucleotide binding and hydrolysis cycle. In this process ligand-bound receptors induce a nucleotide exchange reaction on the α subunit in which GDP can be exchanged for GTP. In the best characterized G protein coupled systems α-GTP directly regulates effector proteins.[1] The regulatory activity of α-GTP is terminated by an intrinsic GTPase activity of the α subunit, and the α-GDP that results can then initiate another GTP binding and hydrolysis cycle. Through multiple cycling events, a large amplification of the original stimulus can be obtained. Two bacterial toxins can interrupt the signal transduction cycle at specific stages through covalent ADP-ribosylation of the α subunit. Pertussis toxin uncouples receptors from G proteins to abolish signaling, while cholera toxin inhibits GTPase activity to enhance signaling.

G_α Protein Diversity. Over the past few years it has become evident that the number of G proteins is larger than previously suspected. The best studied G proteins are transducin, G_s and G_i. Transducin (T_r) is the retinal *rod* photoreceptor G protein that couples photolyzed rhodopsin to the activation of a cGMP phosphodiesterase. G_s and G_i are found in many cell types and function to couple various receptors to a *stimulation* or *inhibition* of adenylyl cyclase activity. Less well characterized proteins that have been studied more recently include G_o, the *other* G protein, that is abundant in brain and heart (Sternweis & Robishaw, 1984), $T_c\alpha$, which is found in *cone* photoreceptors (Lerea et al., 1986), various novel G_i-like pertussis toxin substrates such as those isolated from brain (Katada et al., 1987), neutrophils (Dickey et al., 1987), and erythrocytes (Iyengar et al., 1987) and a protein called G_p (Waldo et al., 1987). The existence of several more G proteins from yeast [SCG1 (Dietzel & Kurjan, 1987); GP1 (Nakafuku et al., 1987); GP2 (Nakafuku et al., 1988)], the fruit fly *Drosophila* (J. Hurley, personal communication), the slime mold *Dictyostelium* [G1, G2 (R. Firtel and P. Devreotes, personal communication)], and humans [G_z (Fong et al., 1988)] has been inferred from isolation of the corresponding α-subunit gene or cDNA.

Diversity in signal transducing systems is not just restricted to G proteins but is being uncovered at all levels. For example, the mas oncogene product, the substance K receptor (Masu et al., 1987), the *a* and α factor yeast pheromone receptors, and a protein called G-21 (Kobilka et al., 1987) all exhibit homology to receptors that are known to interact with G proteins (Dohlman et al., 1987). The list of effectors that may be regulated by G proteins has expanded from enzymes involved in cyclic nucleotide metabolism to include calcium and potassium channels (Dunlap et al., 1987) and phospholipases A_2 and C (Jelsema & Axelrod, 1987; Taylor & Merritt, 1986). Protein kinase C, which is activated by breakdown products of phospholipase C metabolism, consists of at least seven distinct species (Ono et al., 1987). Even a "simple" single cell eukaryote such as yeast may have at least three G proteins (Dietzel & Kurjan, 1987).

This review focuses on information that has been obtained as a result of the recent isolation and characterization of several cDNAs and genes encoding different G protein subunits. The emphasis will be on discussing α subunits since much of the knowledge that has emerged about G protein similarity and diversity is a result of analyzing their sequences. To date over

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* Address correspondence to this author.

[1] Recent data indicate that $\beta\gamma$ may also regulate certain effectors (Katada et al., 1986; Jelsema & Axelrod, 1987; Logothetis et al., 1987).

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Table I: G-Protein Gene and cDNA Characterization

gene[a] (protein)	chromosome location[b]	organism	mRNA size[c]	tissue distribution[d]	references
GNAI1 ($G_{i-1}\alpha$)	7	human	2.2, 3.9	Ad, Fi, He, Ki, Lu, Pa, Sp; not HL-60, Ly	Kim et al., 1988
		human	2.2	Br ≫ Li	Bray et al., 1987
		bovine		Pi	Michel et al., 1986
		rat	3.9	Br	Nukada et al., 1986a
		rat	3.5	Br, Ki > Lu, In > 01 > Li, He	Jones & Reed, 1987
GNAI2 ($G_{i-2}\alpha$)	3, 12	human	2.6	Br = Ki; Ly, Me, EBV; not SKN	Beals et al., 1987
		human		Mo	Didsbury et al., 1987
		human	2.7	Ad, Fi, He, HL-60, Ht, Ki, Li, Lu, Ly, Pa, Sp	Kim et al., 1988
		bovine		Pi	Michel et al., 1986
		rat		C6	Itoh et al., 1986
		rat	2.35	Lu > He, In, Ki > Br > 01, Li	Jones & Reed, 1987
	9	mouse	2.7	Ma, S49	Sullivan et al., 1986
GNAI3 ($G_{i-3}\alpha$)	1	human	2.6, 2.4	Ki ≫ Br; Ly, SKN; not Me, EBV	Beals et al., 1987
		human		HL-60	Didsbury & Synderman, 1987
		human		Li	Suki et al., 1987
		human	2.8	Ad, Fi, HL-60, Ht, Ki, Li, Lu, He, Pa, Sp	Kim et al., 1988
		rat	3.5	He, In, Ki, Li, Lu, 01 > Br	Jones & Reed, 1987
GNAS1 ($G_{s-L}\alpha$) ($G_{s-S}\alpha$)	20	human	1.9	Fe, Br	Bray et al., 1986
		human		Ad, Ht, Ki, Li, Ov, Te	Mattera et al., 1986
		bovine		Br	Nukada et al., 1986b
		bovine	1.9	Ad, Br, Li, COS	Robishaw et al., 1986a,b
		rat		C6	Itoh et al., 1986
		rat	1.85	He, Ki > Br, In, Li, Lu, 01	Jones & Reed, 1987
		mouse		S49	Rall & Harris, 1987
	2	mouse	1.9	Ma, S49; not S49 cyc-	Sullivan et al., 1986
GNAO1 ($G_o\alpha$)		bovine		Br	Ovchinnikov et al., 1987b
		bovine	4.0	Br, Re > He; Li, Lu	Van Meurs et al., 1987; Price et al., 1987
		rat		C6	Itoh et al., 1986
		rat	4.5, 4.1	Br, Ki, In > He, Li, Lu, 01	Jones et al., 1987
GNAZ1 ($G_z\alpha$)	22	human			Fong et al., 1988
		bovine	3.0, 2.5, 2.2	Br, Re > Ad, Ki, Li > Lu, Sp > He, Te	Fong et al., 1988
GNAT1 ($T_r\alpha$)	3	human			
		bovine	2.6	Re; not Br, He, Li	Medynski et al., 1985
		bovine	2.9	Re	Tanabe et al., 1985
		bovine	2.45	Re	Yatsunami & Khorana, 1985
	9	mouse			
GNAT2 ($T_c\alpha$)	1	human			
		bovine		Re	Lochrie et al., 1985
		bovine	6.0, 8.0	Re; not Ad, He, Br	R. Miake-Lye, personal communication
	17	mouse			
SCG1 (SCG1)		yeast		haploid; not diploid	Dietzel & Kurjan, 1987
GPA1 (GP1α)	8	yeast	1.7	haploid; not diploid	Nakafuku et al., 1987; Miyajima et al., 1987
GPA2 (GP2α)	5	yeast	1.9	haploid and diploid	Nakafuku et al., 1988
GNB1 (β36)	1	human	3.0	Li	Codina et al., 1986
		human	3.4, 1.9	ubiquitous	Fong et al., 1987; unpublished results
		bovine	3.0–3.3, 1.5–1.7	ubiquitous	Fong et al., 1986; unpublished results
		bovine	3.2, 1.7	Br, Re, Li	Sugimoto et al., 1985
	19	mouse			
GNB2 (β35)	7	human	1.9	ubiquitous	Fong et al., 1987; unpublished results
		bovine	1.7	ubiquitous	Fong et al., 1987; unpublished results
		bovine	1.8	Ad	Gao et al., 1987a
GNGT1 ($T_r\gamma$)		bovine		Re	Hurley et al., 1984
		bovine	0.6	Re	Yatsunami et al., 1985

[a] Gene names are according to McAlpine et al. (1987). The name of the corresponding protein is given in parentheses. The yeast SCG1 and GP1α genes are the same, but the two reported protein sequences differ by five amino acids possibly because of strain polymorphisms. As many as four $G_s\alpha$ proteins, two long (L) and two short (S), may be encoded by a single gene (Bray et al., 1986). Also there are three sequences that have been named G_i solely on the basis of their close sequence homology. It remains to be determined which cDNA corresponds to which purified protein and which of these inhibits adenylyl cyclase. [b] Mammalian chromosome locations are taken from Ashley et al. (1987), Blatt et al. (1988), and Neer et al. (1987). [c] Messenger RNA sizes are given in kilobases for only the most prevalent species. [d] Tissue distribution of mRNAs was taken from Northern blot data, the cDNA library source, or other reported experiments. Relative levels of expression are indicated (>, greater than; = , equal to; not, low or undetectable). Abbreviations used are as follows: Ad, adrenal; Br, brain; C6, C6 rat glioma cell line; COS, monkey kidney cell line; EBV, Epstein–Barr transformed polyclonal B cell; Fi, fibroblast; He, heart; HL-60, human myeloid leukemia cell line; Ht, hepatoma; In, intestine; Ki, kidney; Li, liver; Lu, lung; Ly, lymphocytes; Ma, PU-5 murine macrophage cell line; Me, melanoma; Mo, human U937 monocyte cell line; 01, olfactory epithelium; Ov, ovary; Pa, pancreas; Pi, pituitary; Sp, spleen; Re, retina; S49 or S49 cyc-, S49 or S49 cyc- murine lymphoma cell line; SKN, neuroblastoma cell line; Te, testes.

30 amino acid sequences corresponding to 10 different classes of α subunits are available (Table I). In contrast to the diversity of α subunits there are probably only two β subunits. The sequences of two cDNA clones, β1 and β2, have been

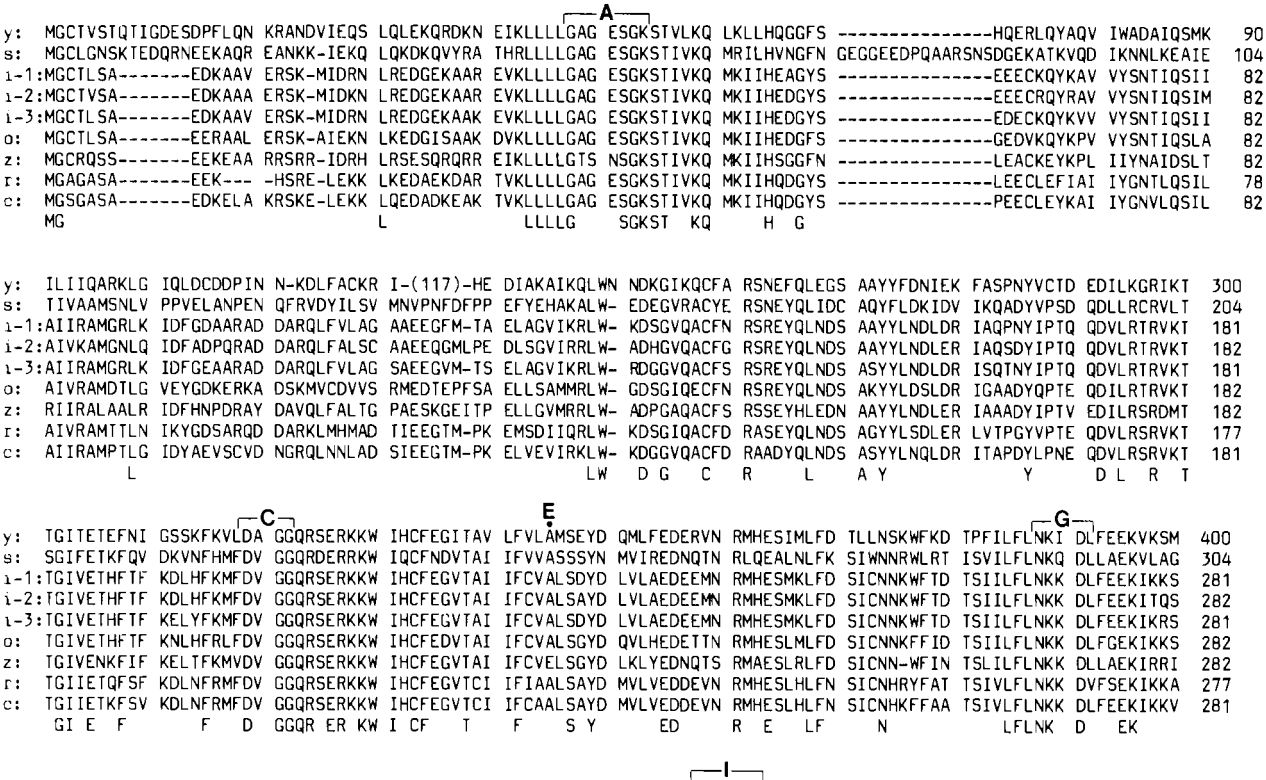

FIGURE 1: Amino acid sequence homologies among G protein α subunits. Sequences were aligned by using the FASTP program of Lipman and Pearson (1985). Hyphens indicate positions where gaps were introduced to achieve an optimal alignment. The α-subunit sequences compared are yeast GP1 (y), rat $G_{s\text{-}L}$ (s), $G_{i\text{-}1,2,3}$ (i-1, -2, -3), and G_o (o), human G_z (z), and bovine rod (r) and cone (c) transducin. A sequence of 117 nonhomologous amino acids from GP1α is not shown. The sources of the sequences are given in Table I. Letters beneath the sequences indicate amino acids that are invariant at that position. Regions marked A, C, E, G, and I are discussed in the text. The standard (IUPAC) one letter amino acid code is used.

determined. They have been shown to correspond to two β proteins, β36 and β35, respectively, which are 10% different in amino acid sequence (Gao et al., 1987b; Amatruda et al., 1988). However, the functional significance of these differences is unknown. With regard to γ-subunit diversity, only one γ-subunit sequence ($T_r\gamma$) is available (Hurley et al., 1984; Yatsunami et al., 1985), but it is clear that there are other γ subunits to be characterized (Hildebrandt et al., 1985).

A variety of other proteins exist that are known or postulated to specifically bind guanine nucleotides. Certain initiation and elongation cofactors of the protein synthesis machinery such as Ef-Tu clearly utilize a molecular switch similar to that of G proteins in which alternating GDP and GTP forms interact with distinct macromolecules. Members of the ras-like oncogene product family (Barbacid, 1987) are also thought to use a similar device since they bind GDP and GTP specifically and possess GTPase activity. However, the details of a putative nucleotide exchange reaction are lacking. Tubulins bind and hydrolyze GTP but appear to function by mechanisms that are fundamentally different from G proteins or protein synthesis cofactors. The LepA (March & Inouye, 1985) and ARF (Kahn & Gilman, 1986) proteins can bind GTP. The yeast *SEC4* protein (Salminen & Novick, 1987) and others are predicted to bind GTP on the basis of strong amino acid sequence homology to ras. However, little is known about the biochemical mechanisms involved in the function of these proteins. All of these proteins can be distinguished from G_α subunits, however, because (a) they are not susceptible to pertussis or cholera toxin modification, (b) they do not appear to interact with $\beta\gamma$ subunits, and (c) the G_α subunits have more extensive amino acid sequence similarity to each other. They can also be distinguished by size. In general, G_α proteins have 350–400 amino acids, while ras-like proteins have about 200 and proteins similar to Ef-Tu often have from about 400 to 900 amino acids. Therefore, while we will compare the other GTP binding proteins to G_α subunits particularly with regard to their shared structural features that presumably function in guanine nucleotide binding and hydrolysis, we also distinguish them from the G_α's. Except for the tubulins, all known GTP binding proteins share limited sequence homology motifs but the subset that is represented by G proteins shares a number of additional features that are unique to G proteins.

Guanine Nucleotide Interactions. The percent sequence identity between α subunits varies widely from 35 to 94% (Table II), but there are 82 invariant amino acids among them (Figure 1). A majority of the most highly conserved sequences are near regions predicted to participate in guanine nucleotide interactions. The basis for these predictions comes from biophysical studies on the crystal structures of the GDP form of Ef-Tu (Jurnak, 1985; la Cour et al., 1985) and genetic studies of mutations that affect the guanine nucleotide interactions of ras proteins. These two independent approaches

have yielded complementary data and identified five homologous regions that are critical for guanine nucleotide interactions. Analogous regions can be found in G proteins. These are indicated in Figure 1 as A, C, E, G, and I from the nomenclature of Halliday (1983–1984). Halliday regions have properties that allow them to be readily identified. Most importantly, each is characterized by an almost invariant consensus sequence. In the Ef-Tu crystal structure, these consensus sequences are preceded by short hydrophobic sequences that form β strands and are followed by longer, more polar sequences that form α helices. It is at the junction of such $\beta\alpha$ secondary structures, near the consensus sequences, that the guanine nucleotide binds. The same motif has been found in the crystal structures of other nucleotide binding proteins. Halliday regions are also found in a characteristic order and spacing in proteins that contain them. For example, they are sequentially arrayed along the primary protein structure as ACEGI from the amino toward the carboxyl terminus. The C, E, G, and I regions are separated from each other by about 20–50 amino acids. The distance of the A region from this CEGI group is more variable, ranging from 50 residues in ras to 270 residues in GP1α, but it is usually found within 10–40 residues of the amino terminus. Several discussions on the precise sequence homologies of ras and Ef-Tu to the α subunits have been published (Masters et al., 1986; references in Table I). Here only a brief updated review is provided.

The A-region consensus sequence is Gly-X-X-X-X-Gly-Lys. In Ef-Tu this region is near the α-phosphate of GDP. The lysine is thought to neutralize the negative charge on the phosphate. Mutations in the analogous region of ras reduce GTP binding and GTPase activity. Similar sequences are found in other purine nucleotide binding proteins.

The C-region consensus sequence is Asp-X-X-Gly. In the Ef-Tu structure, aspartic acid chelates a magnesium ion which is closest to the β-phosphate of GDP. A mutation of Asp-X-Ala-Gly to Asp-X-Thr-Gly in ras results in autophosphorylation of the threonine if GTP is used as a substrate. Mutation of other residues near this sequence in ras results in reduced GTPase activity (Der et al., 1986).

The E region is characterized as a very hydrophobic region between the C and G regions. It often contains an alanine that is located 26 amino acids distal to aspartic acid in the C-region consensus sequence. In Ef-Tu this alanine, along with other hydrophobic amino acids from the G and I regions, forms a hydrophobic pocket near the ribose and guanine rings. In ras, mutation of the analogous alanine to threonine results in a 30-fold reduction in GTP affinity (Feig et al., 1986).

The G region, which has the consensus sequence Asn-Lys-X-Asp, is appropriately named because this region determines the nucleotide binding specificity. In Ef-Tu asparagine is hydrogen bonded to the C-6 keto group of the guanine ring and aspartic acid is hydrogen bonded to the C-2 amino group. As might be expected, a mutation of aspartic acid to asparagine in Ef-Tu alters the nucleotide specificity of the protein to favor xanthine diphosphate over guanosine diphosphate (Hwang & Miller, 1987). Mutations in this region of ras reduce, alter, or abolish nucleotide binding (Feig et al., 1986; Sigal et al., 1986b; Walter et al., 1986; Clanton et al., 1986). The conserved lysine may hydrogen bond to backbone carbonyls or side chains of the two amino acids preceding Gly-Lys in the A region (le Cour et al., 1985). In ras mutation of this lysine to a glutamine results in a 75% reduction in nucleotide binding (Clanton et al., 1986). Such a change may distort the architecture of nucleotide binding but would conserve the hydrogen-bonding amino group.

A fifth region, the I region, may also be involved in nucleotide binding. Currently this region is not well defined. In Ef-Tu it may be near Gly-172, Ser-173, Ala-174 (la Cour et al., 1985), but the precise function of each amino acid awaits a more refined crystal structure. The analogous region of ras may be Thr-144, Ser-145, Ala-146. In a genetic screen designed to isolate GTP binding mutants of ras, one was obtained in which Thr-144 was changed to isoleucine (Feig et al., 1986). This change results in a 25-fold reduction in GTP affinity. The existence of an I region in α subunits has not been widely discussed. One possible I region with the sequence Thr-Cys-Ala is shown in Figure 1. It is found after the G region and, as with other Halliday regions, contains a cluster of invariant amino acids. The homology of this region to the Ef-Tu family of proteins is low, but a more striking and extended homology can be found when it is compared to the ras family, particularly the rab proteins (Touchot et al., 1987).

Mutations in the A, C, E, G, and I regions of ras have dominant biological effects in a variety of systems (references above) including yeast (Kataoka et al., 1985; Schmitt et al., 1986), *Xenopus* oocytes (Birchmeier et al., 1985), PC12 cells (Bar-Sagi & Feramisco, 1985), NIH3T3 cells (Seeburg et al., 1984), and transgenic mice (Quaife et al., 1987). Specific effects of recessive mutations have also been observed in yeast. The phenotypic and biochemical effects of analogous mutations made in G proteins is under investigation.

In guanine nucleotide binding proteins that have Halliday regions the immediate area near each consensus sequence is similar within a family but is different from that of other families. Therefore, these regions form a "fingerprint" by which G proteins can be identified and distinguished from other guanine nucleotide binding proteins. The sequence of $G_z\alpha$ is interesting in this regard because it differs slightly from other α sequences in the A and E regions. Thus, $G_z\alpha$ may define a new subclass of G proteins. In fact, there may exist a wide continuum of α subunits, each slightly different from the other. Such a situation is emerging for the ras-like proteins, which now includes at least five related but distinct families.

Amino acid sequences outside of the Halliday regions are thought to be necessary for α-subunit specific functions such as receptor, effector, and $\beta\gamma$-subunit interactions. Structure/function studies on G proteins, particularly transducin, have provided a tentative functional assignment for some of those regions.

$\alpha/\beta\gamma$-Subunit Interactions. There is some evidence to suggest that the amino terminus of α is necessary for $\beta\gamma$ binding but the data does not indicate if it is also sufficient. Protease treatments that remove about the first 20 amino acids of $T_r\alpha$ abolish several reactions that depend on $T_r\beta\gamma$ such as ADP-ribosylation of α by pertussis toxin, rhodopsin binding, Gpp(NH)p binding, and immunoprecipitation of $T_r\beta\gamma$ by a monoclonal antibody against $T_r\alpha$ (Navon & Fung, 1987). The amino terminus is one of the more variable regions among α subunits. It is unusually hydrophilic and displays an amphipathic characteristic in which hydrophobic or uncharged amino acids are found at about every fourth position. It is not clear how these properties may contribute to $\beta\gamma$ binding. The γ subunits may provide most of the binding specificity since they are also known to be variable, but it has not been possible to separate the $\beta\gamma$ complex into pure subunits, without using denaturating conditions, to assess their individual binding properties.

α-Subunit/Receptor Interactions. Receptor/G protein interactions may be of two types. Conserved G protein sequences

may recognize conserved receptor sequences and function in processes basic to all receptor/G protein coupling systems such as the guanine nucleotide exchange reaction. Variable receptor and G protein sequences may interact to allow specific discriminations between closely related proteins.

There is evidence that receptor interactions are influenced by the extreme carboxyl terminus of the α subunit. First, pertussis toxin, which uncouples receptors from G proteins, ADP-ribosylates $T_r\alpha$ on Cys-347. Second, the UNC mutation, which uncouples $G_s\alpha$ from the β-adrenergic receptor, results in an arginine to proline change at amino acid 389 of $G_s\alpha$ (Rall & Harris, 1987; Sullivan et al., 1987).[2] Finally, there is a correlation between sequence homology to the carboxyl terminus of $T_r\alpha$ and the ability to interact with rhodopsin (Dohlman et al., 1987; Wistow et al., 1986).

However, a comparison of the interactions of rhodopsin and the α_2-adrenergic receptor with G_o, G_i, and T_r (Cerione et al., 1986) indicates another region may be important for providing receptor binding specificity. Rhodopsin can interact equally with G_o, G_i, and T_r whereas the α_2 receptor shows a distinct preference for G_o and G_i over T_r. Most of the positions where $G_o\alpha$ and $G_i\alpha$ sequences are identical but differ from $T_r\alpha$ are concentrated in the extreme amino terminus and in the carboxyl terminus on both sides of the putative I region. The variable amino terminus of α could indirectly be a determinant of receptor specificity since the amino terminus of α is required for binding to $\beta\gamma$ and $\beta\gamma$ is required for binding to receptor (Fung, 1983). A further indication that the extreme carboxyl terminus may not be a sole determinant of receptor interactions is the observation that the last 40 amino acids of $T_r\alpha$ and $T_c\alpha$, which are likely to bind to different receptors, are identical.

α-Subunit/Effector Interactions. Sequences involved in regulating effector proteins are thought to lie between the Halliday A and C regions where there is the most sequence variability among α subunits. The analogous region of Ef-Tu binds aminoacyl-tRNA in a GTP-dependent reaction, and mutations in ras in a limited region between A and C abolish its biological activity but do not interfere with nucleotide or membrane interactions (Sigal et al., 1986a; Stein et al., 1986). In fact a second site, allele specific suppressor of such a yeast ras mutation maps to adenylyl cyclase, an effector for ras in yeast (Marshall et al., 1988).

A mutation in $G_s\alpha$ (H21a) has been isolated that prevents it from activating adenylyl cyclase. It is a single base pair change which results in a glycine to alanine change at position 228 of $G_s\alpha$ in the C-region consensus sequence. However, there is evidence that the inability of the H21a protein to activate adenylyl cyclase may be due to its inability to undergo the conformational change associated with GTP binding that is a prerequisite for effector interaction rather than a direct disruption of effector binding per se (R. T. Miller and H. R. Bourne, personal communication).

Recently a mechanism of cGMP phosphodiesterase (PDE) activation has been proposed in which $T_r\alpha$-GTP binds to PDE_γ, a known inhibitor of $PDE_{\alpha\beta}$, the catalytic subunits (Wensel & Stryer, 1986; Deterre et al., 1986). The amino acid sequence of PDE_γ has been determined (Ovchinnikov et al., 1986). In its 89 amino acid long sequence there is a region of 35 amino acids with 12 basic but no acidic residues. This probably explains why PDE can be activated by trypsin treatment. However, the molecular basis of the proposed interaction between PDE_γ and $T_r\alpha$ is not apparent from an examination of their amino acid sequences alone.

ADP-Ribosylation. The amino acid substrates of cholera and pertussis toxin in $T_r\alpha$ have been identified as Arg-174 (Van Dop et al., 1984) and Cys-347 (West et al., 1985), respectively. The cholera toxin substrate is conserved in all α-subunit sequences and the pertussis toxin substrate is found in $G_{i\text{-}1,2,3}\alpha$, $G_o\alpha$, $T_r\alpha$, and $T_c\alpha$. α subunits that have been purified can be ADP-ribosylated as predicted from this sequence similarity. However, since the precise toxin recognition elements have not been defined, it is not known if the presence of the substrate amino acid at a position analogous to that found in $T_r\alpha$ is necessary to allow ADP-ribosylation. Thus, for example, it remains to be determined if $G_z\alpha$ will be a pertussis toxin substrate since it has a cysteine offset by three amino acids from the other pertussis toxin substrates.

The amino acids that are ADP-ribosylated by cholera toxin and pertussis toxin are remarkably conserved. The cholera toxin substrate is invariant, and the region around it is highly conserved even in the yeast G proteins. This degree of conservation suggests these amino acids may perform an essential function. Since all G proteins contain similar β subunits and the region near the cholera toxin substrate is well conserved, it has been suggested to play a role in $\beta\gamma$ interactions (Stryer & Bourne, 1986). It has been reported that cholera toxin ADP-ribosylation promotes dissociation of the α and $\beta\gamma$ subunits (Kahn & Gilman, 1984).

Lipid Acylation. Most G proteins can be extracted from membranes only by using detergents, a property that is inconsistent with the hydrophilic nature of their amino acid sequences. One possible mechanism for membrane localization of G proteins that involves acylation with lipids can be formulated on the basis of subtle sequence homologies (Lochrie et al., 1985) to two other hydrophilic proteins, src and ras, that are also associated with the cytoplasmic surface of the plasma membrane.

The src oncogene product has been shown to be N-myristylated on a glycine at the second position (Gly-2) that is presumably exposed after removal of the initiating methionine by an aminopeptidase. Several observations indicate myristylation of src is involved in its membrane binding. Mutations that remove Gly-2 from src result in a protein that does not associate with the membrane, is not myristylated, and is incapable of neoplastic transformation (Kamps et al., 1985). Fusion of the first 15 amino acids of src to the amino terminus of globin results in membrane binding (Pellman et al., 1985). Numerous other proteins are known to be N-myristylated, in each case on a Gly-2 residue. In general, the function of fatty acid acylation is not well understood. Some myristylated proteins are soluble, indicating that myristylation can be, but is not always, a sole determinant of membrane association. The yeast and rat *N*-myristyltransferases (NMT) have been purified and their substrate specificities examined by using over 80 synthetic peptide substrates (Towler et al., 1988). On the basis of these studies and sequence comparisons to known N-myristylated proteins one consensus sequence that can be derived is Met-Gly-X-X-X-Ser. All G protein subunits listed in Table I except $G_s\alpha$, β35, β36, and $T_r\gamma$ fit this consensus. The β35, β36, and $T_r\gamma$ subunits do not have Gly-2, which is an indispensible substrate requirement. $G_s\alpha$ has a serine at position 7 but asparagine at 6. According to Towler et al. (1988) this difference could reduce the K_m of $G_s\alpha$ for NMT by a factor of 6000. Recently Buss et al. (1987) have published the first study to determine if G proteins are modified with lipids. It was found that $G_i\alpha$ and $G_o\alpha$ are myristylated but that $G_s\alpha$, β35, β36, and $T_r\gamma$ are not. It was also reported that

[2] Since $G_s\alpha$ is longer than $T_r\alpha$, Arg-389 of $G_s\alpha$ and Cys-347 of $T_r\alpha$ are actually at adjacent positions when the homologous sequences in them are aligned.

$T_r\alpha$ is not myristylated. However, the analysis was done on transducin, which was purified without using detergent. Since there are indications that two forms of transducin exist, one that is easily solubilized and one that is more tightly membrane bound, it is possible that the difference between these forms is that one is acylated with lipid and one is not. It should be noted, though, that while NMT substrate consensus sequences may define amino acids that are critical for the myristylation reaction, other nearby residues can also have a substantial influence on the ability of a peptide to be an efficient NMT substrate.

Ras proteins are acylated with various lipids, primarily palmitate, on a cysteine that is the fourth amino acid from the carboxyl terminus (−4 Cys). Mutants that lack −4 Cys have normal nucleotide binding and hydrolysis activities but are not palmitylated and possess a greatly reduced biological activity. This deficiency can be compensated for by overexpression of the protein, indicating that the purpose of membrane association may be to increase the efficiency of interaction between ras and other membrane proteins. $G_o\alpha$, $G_{i\text{-}1,2,3}\alpha$, $T_c\alpha$, and $T_r\alpha$ have a −4 Cys. In fact, it is this amino acid that can be ADP-ribosylated by pertussis toxin, but there are no data to indicate whether it is posttranslationally modified by eukaryotic enzymes with ADP-ribose or any other adduct. Buss et al. (1987) found no evidence for palmitate or thioester-linked myristate in the G protein subunits they examined.

$T_r\gamma$ also has a −4 Cys. In this case, it is notable that two independent research groups failed to determine the amino acid sequence of $T_r\gamma$ past this cysteine as well as its presence by amino acid analysis (Ovchinnikov et al., 1985; McConnell et al., 1984), indicating that $T_r\gamma$ may be modified at this position. Pure $\beta\gamma$ will associate with phospholipid vesicles whereas α subunits do not unless $\beta\gamma$ is also present (Sternweis, 1986). Since the β and γ protein sequences are hydrophilic, a γ subunit modified with a hydrophobic group could mediate this interaction. Although Buss et al. found no myristate or palmitate linked to $T_r\gamma$, the carboxyl-terminal cysteines of other proteins have been found to be modified with other hydrophobic adducts besides fatty acids. A cysteine at the carboxyl terminus of the Thy-1 antigen is linked to phosphatidylinositol–glycan (Tse et al., 1985), and one in the yeast *Tremella* mating pheromone is linked to a farnesyl group (Sakagami et al., 1981). Perhaps the α and γ subunits are linked to one of these or another hydrophobic moiety. Of final note is that PDEα has a −4 Cys and a Gly-2 (Ovchinnikov et al., 1987a).

Phosphorylation. Since the membrane signal transduction system is the first step in a complicated pathway, it might be subject to feedback regulation. Regulation of receptors by phosphorylation has been well documented (Sibley et al., 1987). G proteins ($T_r\alpha$ and $G_i\alpha$ but possibly not $G_s\alpha$) have been reported to be substoichiometric substrates for phosphorylation by protein kinase C both in vitro and in vivo (Katada et al., 1985; Zick et al., 1987). The preferred protein substrate is α-GDP and not $\alpha\beta\gamma$ or α-GTP-γ-S. The amino acid substrate is a serine. In addition, $\beta\gamma$ inhibits phosphorylation of α-GDP. The precise substrate requirements for protein kinase C phosphorylation are not well understood, but for many kinase C substrates a basic amino acid is located two or three amino acids proximal to the substrate amino acid, which is a serine or threonine. In some cases the substrate is within about 10 amino acids of membrane attachment sites (Hunter et al., 1984; Gould et al., 1985; Ballester et al., 1987), consistent with the known membrane translocation properties of kinase C when it is activated. Given these guidelines Ser-12 (in $T_r\alpha$) is a good candidate for a kinase C substrate. This serine is conserved in all α subunits except $G_s\alpha$, 11 amino acids from putative membrane attachment site, and near several basic amino acids. Since it is located at the amino terminus, it is also in a region that could be shielded from phosphorylation in the $\alpha\beta\gamma$ trimer by $\beta\gamma$. Furthermore, since $\beta\gamma$ dissociates from α-GTP, this region may undergo a conformational change when α binds GTP such that Ser-12 would be unavailable for phosphorylation in the GTP-γ-S form of the α subunit. The functional consequences of transducin phosphorylation have not been reported, but it might regulate the interactions of α with $\beta\gamma$.

Table II: Percent Amino Acid Sequence Identity between G Protein α Subunits[a]

s	(100, M/H; 99.8, R/H; 99.8, B/M; 99.8, B/M; 99.8, M/R; 99.5, R/B; 99.2, B/H)							
o	46	(98.3, R/B)						
i-1	44	72	(100, H/B; 99.7, H/R; 99.7, R/B)					
i-2	43	69	88	(99.2, R/M; 98.6, H/R; 97.7, H/M)				
i-3	43	70	94	85	(98.6, R/H)			
c	44	62	70	69	69			
r	43	63	69	68	66	81		
z	41	60	67	67	67	57	55	
y	35	44	48	47	46	45	44	44
	s	o	i-1	i-2	i-3	c	r	z

[a] Abbreviations, sequences used, and computer program for calculating percent identity are as in Figure 1. For these calcualtions, the sequences of $G_s\alpha$ and GP1α were deleted in nonhomologous regions since they are significantly longer than the others. The percent identity between the same sequence from pairs of different species and the species compared (R, rat; B, bovine; H, human; M, mouse) is indicated on the diagonal in parentheses.

Evolution of G Protein Sequences. The interspecies divergence of any given mammalian α-subunit amino acid sequence ranges from 0 to 3% (Table II), but nucleotide sequences are typically about 10% different. G proteins from one organism can also have similar amino acid but different nucleotide sequences. For example, the amino acid sequences of $G_{i\text{-}1}\alpha$ and $G_{i\text{-}3}\alpha$ are 94% identical, yet their nucleotide sequences are only about 70% identical in the coding region. This suggests there is a very strong selective pressure to maintain protein structure. In fact, it may be that little if any of the amino acid sequence of an α subunit is nonfunctional. This may explain their high degree of evolutionary conservation. A similar situation is found for the two β proteins (Fong et al., 1987).

Differences in nucleotide sequence are also an indication that similar gene products are not encoded by one gene. In fact, the $G_{i\text{-}1}\alpha$ and $G_{i\text{-}3}\alpha$ genes do map to different human chromosomes (Table I). Other G protein genes also map to dispersed chromosomal positions in mice, humans, and yeast. Of those genes that have been mapped, only the $G_{i\text{-}2}\alpha$ and $T_r\alpha$ genes map near each other, possibly within 1 centimorgan (approximately one million base pairs), in a syntenic region that is found on both human and mouse chromosomes. Since the sequences, function, and tissue distribution of $G_{i\text{-}2}\alpha$ and $T_r\alpha$ are different, the reason for the proximity of their genes is not evident. It may be that the region as a whole has been conserved in organization for other reasons not related to G proteins. In any case, it would be interesting to determine if there is a cluster of G protein genes at this locus.

Tissue Distribution. By using gene specific probes, one can study the expression of mRNA encoding G proteins in different tissues by Northern blot analysis (Table I). Although G protein mRNA abundance does not always quantitatively agree with protein abundance (Brann et al., 1987), certain

trends emerge. First, very similar G proteins such as $G_{i\text{-}1}\alpha$ and $G_{i\text{-}3}\alpha$ can be expressed in different tissues. Second, there are "housekeeping" G proteins such as G_s that are expressed in almost every cell, and there are specialized G proteins such as the transducins that are expressed in one or a few cell types. Third, the sizes and relative expression levels for a G protein mRNA can vary between different tissues in the same organism and in the same tissue in different organisms. Finally, one tissue or clonal cell line can express multiple G proteins.

Implications of G Protein Multiplicity. The realization that multiple G proteins exist should alter experimental procedures and may provoke a reexamination of previous data. For example, the old view that pertussis toxin affects one protein, G_i, was derived from the observation that after ADP-ribosylation of membrane proteins using radioactive NAD^+ as a substrate a single band could be seen after analysis of the labeled proteins by sodium dodceyl sulfate gel electrophoresis. This is clearly not valid since multiple pertussis toxin substrates with nearly identical molecular weights can exist in one cell type. In many other types of studies such as those of in vitro biochemical reconstitution and in situ localization it now becomes important to use highly specific probes to characterize the preparations being used. Synthetic oligonucleotides and antibodies against synthetic peptides that correspond in sequence to the most divergent regions found in G proteins are being used successfully to uniquely identify the numerous related G protein subunits.

Another aspect of G protein diversity is the isolation of cDNA clones encoding G proteins that have not been purified and thus have an unknown function. Several approaches can be taken to identify the function of such G proteins. Hopefully, as new G proteins of known function are purified and sequenced, they will be found to correspond to a previously isolated cDNA. In cases where a predicted G protein is rare or has not yet been purified one could express cDNAs in a heterologous system such as bacteria and assay for a particular function such as potassium channel activation. The expression of G proteins for such purposes is just beginning. For example, Graziano et al. (1987) have shown that the long and the short forms of $G_s\alpha$ expressed in *Escherichia coli* are capable of activating adenylyl cyclase. Such in vitro approaches may not be sufficient to determine the true in vivo function of a new G protein given the moderate promiscuity observed in receptor/G protein interactions, but effector/G protein interactions may be more monogamous. An examination of the precise cellular expression pattern of a novel G protein by in situ hybridization may aid in providing a clue to its function since a G protein and the receptors and effectors it interacts with should be expressed in the same cells. Such studies are beginning [cf. Worley et al. (1986)].

The discovery of G proteins in lower eukaryotes should facilitate a genetic analysis of G proteins and their transduction pathways. In yeast the ability to isolate, express, mutate, delete, and replace genes will provide powerful tools that are currently unavailable in other systems. By isolating suppressor mutations, it should be possible to identify components of the G protein signaling systems of yeast as has been elegantly done for the yeast ras/adenylyl cyclase pathway. In fact, one yeast α-subunit gene was isolated fortuitously as a suppressor of a mating hormone supersensitive mutant (Dietzel & Kurjan, 1987). Similar techniques can be used in *Drosophila* and *Dictyostelium*, which have the added advantage of being multicellular organisms with more observable phenotypes. It may be possible to analyze the genetics of G proteins in mammals by introducing genes with dominant mutations into transgenic mice or cultured cells. Ultimately, recessive phenotypes could be generated in such systems by methods, such as the use of antisense RNA, which are currently under development.

Future Goals. One topic of interest that will continue to be of importance is that of structure/function relationships. How do G proteins interact with other proteins at a molecular level? What is the basis for changes in molecular recognition that are regulated by the presence or absence of a single phosphate group? How might posttranslational modifications alter function? Ultimately, it will be desirable to obtain X-ray crystal structures. As more information becomes available on receptor/effector interactions with G proteins, it may be possible to predict the type of signal transduction system a new G protein operates in, design G proteins with novel interaction specificities, and engineer drugs which affect the function of specific coupling systems.

Another interesting subject that has been largely unexplored is the temporal nature of G protein expression and how it changes. Are there G proteins that are only expressed during embryogenesis? What signals regulate the expression of the signaling proteins themselves? Why are G proteins that are almost identical in structure expressed in different cells? Such questions can be approached by the isolation of G protein genes and the examination of promoter function.

Given the number of receptors reported to interact with G proteins, the variable responses of different cells to the same signal, and the number of highly specialized cell types, it is not surprising that multiple species of G proteins with diverse structures and functions exist. It is possible that there are still many more than those mentioned here. Therefore, a continuing goal will be to determine how many G protein coupled transduction systems exist and to understand the significance of their diversity.

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