Molecular biology of signal-transducing G proteins

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Living cells possess the remarkable ability to discriminate among the enormous variety of chemical and physical stimuli that they receive. G proteins are a central component of the molecular machinery that allows cells to respond appropriately to each of these stimuli. G proteins are molecular switches whose properties of guanine nucleotide binding and hydrolysis are eminently suited to perform the transduction of external signals into intracellular effects. Molecular analysis shows that the components of signalling pathway-receptors and effectors linked by G proteins as well as the subunits of G proteins, are members of families of structurally diverse proteins. This diversity of molecules helps in processing the variety of signals a cell senses. But it is unclear how both specificity and cross-regulation of signalling pathways are achieved in the same cell with high fidelity.

CENTRAL to all mammalian physiological processes is the ability of a variety of receptors on cell surfaces to sense hormones, neurotransmitters and growth factors. Signals that are sensed by these receptors are transduced into intracellular effects through several different mechanisms to bring about transient or lasting changes in a cell's traits. The most widespread of such signalling pathways are those regulated by a family of guanine nucleotide binding proteins known as G proteins. G proteins are coupled to specific receptors on the plasma membrane. When the receptor senses a signal it triggers off the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) in the G protein. The activated G protein then modulates the function of an effector molecule (Figure 1). Diverse families of receptor molecules are coupled to G proteins. These include receptors for neuromodulators such as acetylcholine, norepinephrine, serotonin and neuropeptides as well as receptors for hormones such as adrenocorticotropic hormone (ACTH) and thyrotropin release hormone (TRH). Examples of G protein coupled receptors also include those in the sensory pathways such as the light-sensing opsins in the photoreceptors and odorant receptors. All these receptors share a putative tertiary structure that is made up of seven membrane spanning domains with the amino terminal of the protein projecting into extracellular space and the carboxyl terminal lying in the cytosol. There are also several effector molecules whose functions are modulated by G proteins. Examples are adenylate cyclases, phospholipases and ion-conducting channels. Families of related genes encode each of these effectors.

G protein-regulated signalling pathways show striking evolutionary conservation. The structure of G proteins and their receptors is similar in yeast, slime mould, nematode, insects and mammals. This review will focus on the molecular biology of G proteins in mammals. Present evidence indicates that in comparison to other eukaryotes, mammalian systems possess considerably more complex G protein-mediated signalling circuitry. Other reviews emphasizing various aspects of G protein biology are available[1–4].

To unravel the signalling circuitry regulated by G proteins, considerable effort in recent years has been devoted to the identification and characterization of their molecular components. Molecular cloning techniques

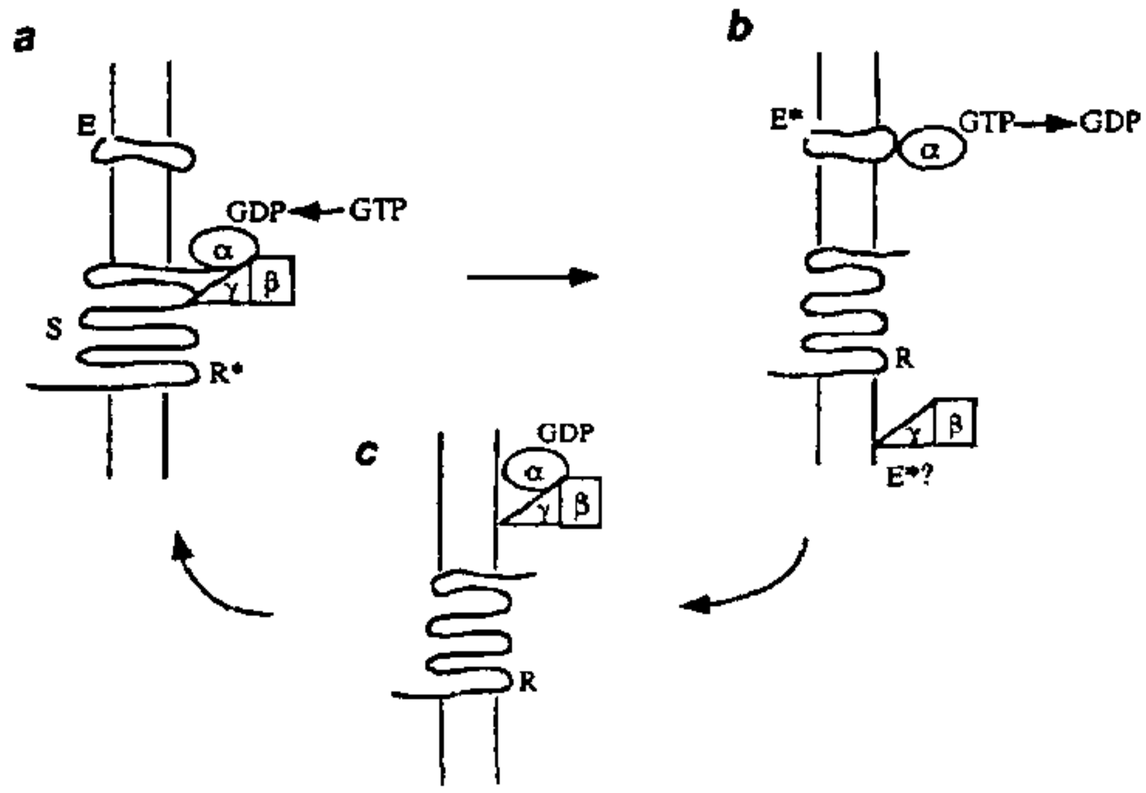

Figure 1. Transduction of a signal by a G protein *a*, The receptor (R) is activated by an external stimulus (S) The activated receptor (R*) induces the exchange of GDP for GTP in the G protein α subunit *b*, The α subunit with GTP bound to it dissociates from the βγ complex and activates or inhibits an effector (E) The βγ complex may also act on the same or another effector (E*?) *c*, The GTPase activity of the α subunit hydrolyses the GTP to GDP In the GDP bound form the affinity of the α subunit for the βγ complex increases and the heterotrimer forms again Thus the GTP bound α subunit is active and the GDP bound form is inactive The rate at which GTP is hydrolysed to GDP therefore controls the period during which the α subunit is capable of conveying a signal and bringing about a physiological change

have been used extensively to isolate and elucidate the primary structure of G proteins, receptors and effectors.

G protein subunit structure and function

G proteins are heterotrimers made up of α, β and γ subunits. Although the subunit stoichiometry has never been conclusively demonstrated it is generally assumed that each G protein comprises a single subunit of each kind. Molecular cloning of the complementary DNAs for different G protein subunits indicates that each subunit is part of a family of related proteins[3].

Although the primary structure of several of these proteins has been deduced from their cDNAs many subunit types are yet to be characterized at the protein level. About twenty α subunit types have been identified so far. This includes products of different genes as well as products resulting from the differential splicing of complex genes. Four β subunit types and at least eight γ subunit types have been identified so far. The potential exists for the occurrence of many more members of these families especially in the case of the β and γ subunits where the searches have not been carried out as intensively as in the case of the α subunit. Subtypes that occur either in small populations of specific cell types or subtypes that are expressed transiently during the differentiation of a cell type or during the development of an organism may not have been identified.

Of the three G protein subunits the primary structure of the α subunits is best understood in terms of function. They possess four structural domains that are involved in interactions with GTP. The amino-acid sequences in these domains are conserved in a variety of GTP-binding regulatory proteins including the ras family of oncogenic proteins[5]. In some well-characterized signalling systems there is convincing evidence that the α subunit modulates the function of the effector. αs stimulates the activity of all adenylate cyclases and αt activates cGMP phosphodiesterase in rod photoreceptors[6, 7]. αs is present in the G protein, Gs (stimulatory) and αt in Gt or transducin, a G protein present in photoreceptors. These two G proteins were among the first to be identified[1, 7]. G proteins have traditionally been defined by their α subunits.

The extent of primary structure homology among the α subunits of G proteins varies. Based on this homology they can be divided into subfamilies[3]. In comparison to the α subunits the four identified β subunits of G proteins, β1–β4, are highly homologous sharing more than 80% homology[3]. In the case of the G protein γ subunits, molecular characterization indicates the potential existence of subfamilies similar to the α subunits[3, 8].

The capability of two different bacterial toxins to affect the function of some G protein α subunits has been of value in dissecting the role of G proteins in signalling pathways[9]. Both pertussis toxin and cholera toxin catalyse the addition of an adenosine diphosphate (ADP) ribose moiety to the α subunit. ADP ribosylation by pertussis toxin prevents interaction of the α subunit with the receptor. ADP ribosylation by cholera toxin inhibits GTPase activity and leads to constitutive activation of the α subunit. These toxins are specific to different G protein α subunit types. For instance αi and αo are modified by pertussis toxin while αs is modified by cholera toxin. Several α subunits such as αz and members of the αq family are insensitive to both toxins. Finally αt and αc fall in a distinct class of α subtypes that are modified by both toxins.

Different members of the α subunit family have been implicated in the modulation of a variety of signalling pathways. Apart from activating adenylate cyclase αs seems to activate Ca^{++} channels and inhibit Na^{+} channels[10, 11]. Members of the αq subfamily regulate phospholipase C isotypes[12]. Three properties of the α subtypes with regard to function are worthy of note. First, some α subtypes seem to modulate the activity of several effectors. Second, α subunits can show extreme specificity in terms of modulating the activity of effector molecules. For instance, members of the αq subfamily activate one type of phospholipase C, PLC-β1, but not PLC-β2 or other types such as PLC-γ or δ[12, 13]. Third, α subtypes that are closely related can still play distinctly different roles as in the case of the products of differential splicing from the αo gene—αo1 and αo2. αo1 couples the muscarinic receptor to inhibition of Ca^{++} channels in GH3 cells while αo2 couples the somatostatin receptor to inhibition of the same channels in these cells[14]. One explanation for the non-specific activity of some α subunits is that some of this activity is artifactual resulting from assaying in reconstituted systems. But if these functions are replicated *in vivo* it certainly raises questions about the mechanisms that prevent unwanted cross-talk between pathways mediated by the same G protein.

Comparison of the amino-acid sequences of the G protein β and γ subunits with other known proteins reveals little about their role in signalling. One of the γ subunits shows some homology to a ras oncogenic protein[15]. The β subunits of G proteins share a structural motif that is made up of eight repetitive domains of ~ 40 amino acids, each of which terminates with a tryptophan-aspartate sequence (WD). This motif is present in a number of other proteins that are all potentially involved in protein–protein interaction[16].

Reconstitution experiments with signalling components either *in vitro* or in cell lines have been more productive in identifying some of the roles of the βγ complex in signal transduction. Clues regarding the role of G protein βγ complexes have emerged from different lines of investigation. They point to a role for these subunits in receptor coupling of the holomeric G protein

and in the modulation of effector function. A number of reconstitution studies have indicated that the βγ complex is required for receptor activation of the α subunit. For instance, the βγ complex of Gt is required for αt to interact with rhodopsin[17]. Strong evidence now exists for the action of G protein βγ complexes on effector molecules. Purified βγ complexes from brain modulate the activity of adenylate cyclase, phospholipase C and K⁺ ion channels in the heart[6, 18, 19]. An unanticipated role for the βγ complex that does not fall into the functional classes above is in the mobilization of the β adrenergic receptor kinase to membrane bound receptors that are phosphorylated by the kinase[20]. It is possible that with the identification of more novel β and γ subunits other functions of these subunits will become apparent. Overall, it is already clear that a G protein when activated has the capability to act through both the α subunit and the βγ subunits. This introduces an additional level of control over the signalling pathways that receptors activate. Thus a single receptor could activate two different signalling paths. It is also possible that the action of an α subunit on an effector can be modified by a βγ complex activated by another receptor.

Specificity of G protein action

Knowledge that G proteins, receptors and effectors are families of molecules with varied structures provides a neat explanation for the complexity of signalling circuitry in mammalian cells. But the mechanisms that regulate their interactions are unclear. One mechanism that brings about specificity of action is cell type specific expression of G protein subunit types. For instance αtc and β3 are present in cone photoreceptors but not rods[21, 22]. γ3 is present predominantly in the ganglion cell layer of the retina[8]. αo is present in relative abundance in growth cones[23]. Although this has not been demonstrated, distinct functions of such specialized subunit types could confer unique signalling properties to these cells.

But many G protein subunit types are ubiquitously expressed and a single cell can possess many different receptors, G protein subunits and effectors. If all the different α subunits were equally capable of associating with all members of the β and γ subunit families, it would result in an enormous variety of G protein heterotrimers. This raises questions about the mechanisms that attenuate unwanted cross-talk between some pathways and enhance essential interaction between other pathways. One such mechanism could rely on the differential affinities that signalling components have for each other. There is evidence for selective association of G protein β and γ subunit types[24, 25]. Such selectivity in associations will allow only certain holomeric G proteins to form inside a cell even though many different types are present in it. As a result only certain signalling pathways can be active in this cell. There is also indirect and direct evidence for specific interaction of receptors with β and γ subtypes. In a cell line where the somatostatin and the muscarinic receptor are coupled to Ca^{++} channels, inhibition of expression of certain β or γ subtypes specifically affected either somatostatin or muscarinic receptor coupling to the ion channels[26, 27]. In our laboratory, we have examined the ability of Gt made up of the same α and β subunits but different recombinant γ subunits to interact with rhodopsin. The different heterotrimers show significant differences in affinity for rhodopsin indicating that the γ subunit has a strong influence on the affinity of the G protein for the receptor[28].

Another device that the cell could employ to increase specificity is to physically isolate the components of different signalling pathways. Even in a single cell that expresses more than one subunit type of a G protein these subtypes may be located in different parts of the cell. Some of the functions of G proteins indicate that such localization does take place. For instance, G protein involvement in endocytotic and exocytotic processes has been shown[29, 30]. Also, in polarized epithelial cells, α subunits similar to αs and αi regulate the sorting of proteins in the trans-Golgi network so that they are directed to different surfaces[31]. These actions of G proteins require them to be targeted to specific internal organelles where they may perform diverse functions. In support of this notion there is evidence for αi-3 presence on the Golgi apparatus and for the localization of G protein β and γ subunits to internal organelles[32-34]. It is possible that intracellular mechanisms exist that regulate both the assembly of G protein subunits into a heterotrimer and the appropriate localization of a holomeric G protein. This question has not been examined in detail so far.

Other mechanisms that determine specificity may depend on the post-translational modification of the G protein subunits. The α and γ subunits are modified by lipid addition. The role of the lipids is not fully clear. An obvious conjecture, since G proteins are associated with membranes is that they are necessary for membrane binding. Some of the α subunits which are myristoylated at the amino terminus, do not bind to membranes or the βγ complex when they are not myristoylated[35, 36]. Similarly, the γ subunits which are isoprenylated at a cysteine at the carboxyl terminus do not associate with membranes when not isoprenylated[37-39]. An exciting possibility is that these modifications of the α and the γ subunits are a mechanism for regulating the level of active G proteins. Two strands of evidence support this notion. The α subunit of transducin is modified at the amino terminus by a mixture of fatty acids with differing degrees of hydrophobicity and consequently different strengths of binding to the βγ complex[10]. The γ subunit of

transducin, γ1, is present in two forms[38]. One form that is isoprenylated and another that has the carboxyl cysteine removed. The form without the farnesylated cysteine interacts poorly with the αt subunit. If there were mechanisms that regulate the proportion of the different forms of αt or γ1, the kinetics of signalling processes mediated by these subunits can be significantly altered.

The properties of G proteins outlined above indicate their potential to play an important role in the three broadly related areas of mammalian biology discussed below. At present the evidence is limited for the role of G proteins in signalling in the nervous system and development as well as for G protein mutants being at the basis of many disease processes. In the future we can anticipate more evidence for G protein-regulated pathways playing a direct role in neuronal and developmental signalling. Also, it would be surprising if the intensive focus on the human genome does not implicate alterations in G protein subunits as a cause for disease states.

G proteins in neuronal signalling and development

Signalling in the nervous system is immensely complex. The properties of G proteins in controlling information flow inside a cell are exquisitely well suited for their role in neuronal signalling. In some cases such as Gs, more than one signal can trigger off a single G protein. In other cases the same signal can activate more than one G protein[11]. G protein action on effector molecules is also similar to coupling with receptors. In some instances, one G protein can act on many effectors (e.g., Gs) and in others the same effector can be activated by many G proteins[12]. In addition, the potential for both the α subunit and the βγ complex of a G protein to act on effectors introduces the capacity to activate two pathways in response to one signal. If G proteins behave similarly *in vivo* they can both integrate signals and also disseminate information in different ways.

Electrical signalling resulting from the activity of ion channels is an ubiquitous property of neurons. G protein modulation of ion channels has an inbuilt malleability that is of considerable value in the regulation of membrane currents. The α subunit and the βγ complex are active at different concentrations in the modulation of K channels. The βγ complex is active at higher concentrations in comparison to the α subunit. But the α subunit activates fewer channels with a longer lag time[13]. In a cell, activation of a smaller number of receptors could result in the α subunit acting alone while the activation of more receptors and thus more G proteins would result in more rapid and fuller activation of channels. G protein action on an ion conducting channel can also be direct or indirect. Direct action would be more rapid but transient while indirect action, which resulted in covalent modification of the ion channel protein would be slower but longer lasting.

G proteins can be expected to play a role in development because of their ability to trigger off cellular changes. The inhibition of growth cones during the development of nerves has been shown to be mediated by G proteins. Receptor(s) that sense the target act through pertussis toxin sensitive G proteins[23]. Cell adhesion molecules, N-cadherin and NCAM that induce morphological differentiation of PC12 cells also act through pertussis toxin sensitive G proteins[43]. The nature of the receptors and also the G proteins involved in these pathways is of considerable interest. Are there classes of receptors and G proteins with distinct properties that are involved in developmental signalling? Not surprisingly G protein action has now been shown to be critical for the differentiation of one cell type into another[44]. In this case, teratocarcinoma cells differentiated into endoderm-like cells in the presence of retinoic acid when the expression of the αi2 subunit was reduced by antisense RNA.

G proteins in human disease

Given the widespread regulation of neurohormonal signalling pathways by G proteins, a variety of human disease states can be expected to result from alterations in these proteins. Cholera, the infectious disease that is life threatening in the developing countries results from a bacterial toxin entering intestinal cells and constitutively activating the G protein, Gs which affects water and salt retention. Albright hereditary osteodystrophy, a rare genetic disorder that is characterized by several abnormalities including skeletal defects is associated with αs mutations[15]. Human pituitary tumours have been shown to contain αs mutants whose GTPase activity is affected resulting in constitutive activation of the α subunit[16]. Similar mutant forms of αi are present in other endocrine tumours[17]. A similar mutant form of αq has been shown to transform a cell line indicating oncogenic potential[18]. These mutant α subunit types are reminiscent of the mutant forms of the ras proteins that cause oncogenic changes in cells[5]. Other evidence for diseases caused by aberrant G protein signalling is emerging. The amyloid precursor protein (APP) has been shown to be coupled to Go[19]. Mutant forms of APP have been implicated in Alzheimer's disease which is characterized by progressive dementia in older humans. Constitutive activation of Go is thus a potential cause for the disease.

An approach towards elucidating the role of G proteins in disease is mapping the genes for the various members of the α, β and γ subunit families on human chromosomes. Most of the genes for the α subunit types have been mapped on the mouse and human chromosomes[20].

If genes for G protein subunits show linkage to loci associated with human diseases it would indicate their potential role in causing the disease.

Remaining questions

How specific is the action of a G protein in a cell? Is it possible to design and target agents to a specific G protein that will accentuate or attenuate its activity? Such agents can be therapeutic in the case of known diseases that are due to aberrant neurohormonal regulation and in the case of disease processes induced by alterations in a G protein.

Inquiry into the basis of biological phenomena have been characterized by bursts of progress following the synthesis and application of novel techniques. It is therefore difficult to point out the best strategy to elucidate the basis of biological signalling. A personal predilection is for a molecular genetic approach where the effects of structurally altered proteins on native and reconstituted signalling systems are examined using biochemical as well as physical techniques.

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