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G proteins – Critical control points in cellular signal transduction

Every living organism needs to communicate with its environment and adapt itself to changes in its surroundings. The signals for such changes could be nutrients, toxins, light and hormones, and sometimes all of them at the same time. Individual cells possess receptors for the various signals to which they should respond, and in the case of signals that cannot penetrate the cell membrane, specific receptors for the signals are present embedded within the membrane. Binding of ligands to these receptors must now result in biochemical changes within the cell; the process by which this extracellular message is transmitted is known as signal transduction. Studies carried out in the last twenty years by Drs Martin Rodbell and Arthur Gilman and, indeed, by a number of laboratories throughout the world, have shown that G proteins are essential components in a wide variety of signal-transducing mechanisms. The importance of G proteins in so many vital processes within a cell has been acknowledged in the joint award of the 1994 Nobel Prize for Medicine or Physiology to Rodbell and Gilman.

Discovery of G proteins

Rodbell, working in the early 1970s in the National Institute of Environmental Health Sciences, North Carolina, noticed that activation of adenylyl cyclase by various hormones showed a dependency on magnesium ion concentrations that could not solely be explained by the requirement of Mg–ATP as a substrate for the enzyme. The complex kinetics of ATP activation could be explained only later, by detecting a GTP contamination in the ATP preparation used for the assay![1]. On studying the glucagon-sensitive system in liver cells, Rodbell observed that GTP, in fact, reduced the affinity of the hormone for the glucagon receptor, and that the presence of GTP was essential for the stimulation of adenylyl cyclase by the hormone. GTP was hydrolysed during the course of the assay, and non-hydrolysable analogues of GTP could result in prolonged activation of adenylyl cyclase, strongly suggesting that a protein component was present in the membranes that bound GTP and could transduce the signal to adenylyl cyclase, leading to cAMP production[1]

In the late 1970s, Gilman working along with Eliot Ross at the Department of Pharmacology in the University of Texas, was able to restore adenylyl cyclase activity to membranes prepared from cells which were thought to be deficient in adenylyl cyclase activity, by the addition of extracts prepared from wild-type cells, and the hormone in a GTP-dependent manner. This assay allowed Gilman and co-workers to purify the first guanine nucleotide binding protein (G protein) responsible for stimulating adenylyl cyclase activity, Gs or α_s, and led to a general mechanism of G-protein-mediated hormone action[2].

Hormones that bring about their effects through G proteins bind to receptors on the cell surface. These G-protein-coupled receptors, a number of which have been cloned and sequenced, have a characteristic topology in which the receptor is predicted to span the membrane seven times. These 'seven-pass' receptors interact with the G proteins in well-defined regions of the receptor which project into the cytoplasm of the cell[2].

Mode of action of G proteins

The G proteins involved in signal transduction are heterotrimeric membrane-associated proteins composed of an α subunit, and β and γ subunits tightly associated with each other through non-covalent interactions[2]. The α subunit of the G protein binds the guanine nucleotide, either GTP or GDP, and in the GTP-bound form, dissociates from the $\beta\gamma$ subunits. The GTP-bound form of the α subunit is in the activated state and modulates the activity of a variety of molecules involved in the signal transduction process, such as adenylyl cyclases, phosphodiesterases, or ion channels The α subunit has an inherent ability to hydrolyse its bound GTP, and therefore returns to its basal GDP-bound, inactive state, allowing its interaction again with the $\beta\gamma$ subunits (Figure 1) This is known as the G protein cycle.

Activation of the G proteins is brought about by the binding of ligands with their specific receptors, and the conformational change in the receptor induced by ligand binding is sensed by the α subunit of the trimeric G protein present in the membrane. This leads to an association of the G protein with the activated receptor through the α subunit. This interaction reduces the affinity of the α subunit for its bound GDP, allowing GDP–GTP exchange via a transient 'empty' α subunit. The concentration of GTP is higher in the cell and therefore the vacant guanine nucleotide binding site in the α subunit is now occupied by GTP, leading to a dissociation of the $\beta\gamma$ subunits. The individual components of the G protein are now free to interact with their effectors (Figure 1 *a*).

It was initially believed that the GTP-bound α subunit was solely responsible for the activation of various effectors, but growing evidence in the last few years has indicated that the $\beta\gamma$ subunits are also able to activate a variety of ion channels, adenylyl cyclase and phospholipase C isoforms as well This implies that for a given hormone, a number of divergent signals are generated within a cell, the cumulative effect of which is reflected in the overall cellular response[3].

Structure of G proteins

With the advent of advanced molecular cloning techniques, it is now known that there are more than 20 different forms of the α subunit, four different β forms and at least seven different γ subunits in mammalian cells[3]. As a consequence, many combinations of the individual subunits are theoretically possible, allowing fine tuning of the cellular response. However, biochemical evidence suggests that only a few combinations actually do occur

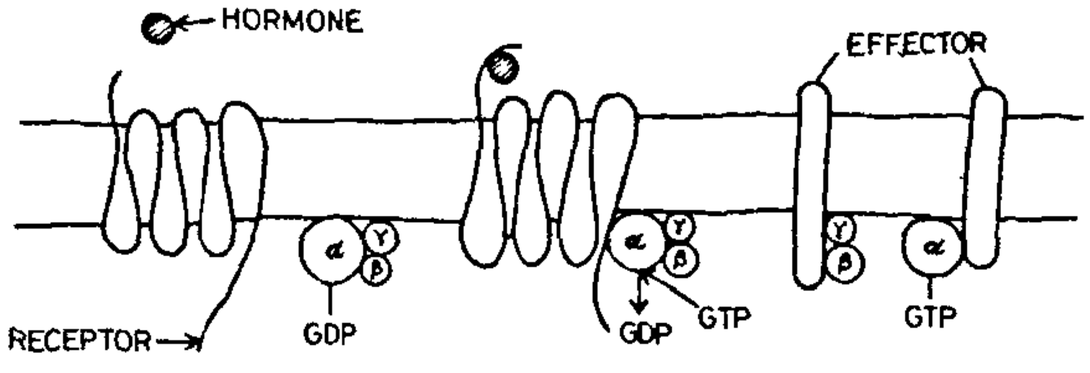

Figure 1. ***a***, The mechanism of activation of G proteins following ligand binding to its receptor; ***b***, the G protein cycle. Details are given in the text.

Table 1. Physiological effects mediated by G proteins

G protein	Stimulus/Cell type	Effects
Gs	Epinephrine, glucagon Liver cells	cAMP increase, glycogen breakdown
Gs	Luteinizing hormone Ovarian follicles	cAMP increase, estrogen and progesterone synthesis
Gs	Luteinizing hormone Sertoli cells/testes	cAMP increase, testosterone synthesis
Gi	Acetylcholine Heart muscle cells	Opens K^+ channels, slows heart rate
Gq	Angiotensin Smooth muscle *cells in blood vessels*	Phospholipase C Muscle contraction, *elevation of blood* pressure
Golf	Odorants Epithelial cells in nose	cAMP increase, detection of odorants
Gt (transducin)	Light Rod and cone cells of retina	cGMP increase, visual signal detection
βγ	Muscarinic agonists Cardiac atria	K^+ channel
βγ	*Nervous system*	*Type-1 adenylyl cyclase*

The α subunits are proteins which range in molecular weight from 39 to 52 kDa, and can be divided into four different classes based on similarities in their amino acid sequences. The α_s class is involved in the stimulation of adenylyl cyclase and the class includes α_{olf}, which is present in olfactory epithelium. The α_i class of proteins, which are able to inhibit adenylyl cyclase, includes transducin, the α subunit involved in visual signal transduction. The α_q class of proteins is involved in activating phospholipase C, and the fourth class includes α_{12} and α_{13}, whose functions are not yet known.

The α subunit of heterotrimeric G proteins belongs to a superfamily of proteins bearing resemblance to each other in the guanine nucleotide binding domain. Proteins in this superfamily include elongation factors EF–Tu, the oncogene *ras*, and other GTP-binding proteins related to *ras*. In these cases too, it is the GTP-bound form of the proteins which is active, though there are requirements for the interaction with other proteins to aid in the GDP–GTP exchange, as well as GTP hydrolysis[4].

There is greater identity in sequence amongst the different β and the γ subunits, which always remain tightly associated with each other, and with the cell membrane, through lipid modification of the γ subunit. In the last few years it is recognized that these two subunits are also able to activate a number of effectors, in what was considered a heretic concept when first suggested[3].

The crystal structures of *ras* and EF–Tu were reported a few years ago but it was in the last few months that the structures of transducin bound to GDP and a non-hydrolysable analogue of GTP, GTPγS, were available. An analysis of the structures reveals that observations made from studies on mutagenesis of various residues in the α subunit and the resultant effects on α subunit activity could be explained by the three-dimensional pictures that we now have of the α subunit[5]. It is also now possible to understand the chemical mechanism by which GTP hydrolysis is effected, allowing perhaps to regulate in future the rate of hydrolysis of GTP by the design of suitable analogues[6].

Functions of G proteins

Various forms of the G proteins mediate a number of physiological actions of many hormones (Table 1). G proteins are involved in the perception of light, smell, taste, and the regulation of sex hormone secretion. Visual signal transduction is perhaps the most studied process involving G proteins, largely due to the high concentrations of the receptor rhodopsin, which couples with the G protein, and transducin, the α subunit involved in the stimulation of a cGMP-specific phosphodiesterase, in the membranes of the rod cells of the eye. Olfactory signalling is carried out by a large number of receptors – all bearing the characteristic seven transmembrane spanning domains – and G proteins that couple to adenylyl cyclase activation and phospholipase C activation. The luteinizing hormone (LH) and the follicle stimulating hormone both interact with distinct G-protein-coupled receptors and regulate the production of testosterone, progesterone and the development of follicles in the ovary[7].

A myriad of processes involve G proteins and their receptors, and so it is expected that any aberration in their function will lead to deleterious effects in the response of the cells. Cholera toxin, which is responsible for the acute, often lethal diarrhoea associated with cholera, covalently modifies α_s and thereby reduces the GTPase activity of the α subunit. The α subunit can then remain in an active state for a

prolonged period of time, leading to elevated levels of cAMP within the intestinal cell, resulting in a massive efflux of chloride and water, characteristic of the diarrhoea. Pertussis toxin, the toxin produced by the bacteria *Bordetella pertussis*, that causes whooping cough, modifies the Gi form of the α subunit, preventing its interaction with the receptor to which it couples, leading to elevated cAMP levels within the lung epithelia

A number of diseases are associated with mutations in the G proteins or in the receptors to which they couple[7]. Activated thyrotropin receptors, caused by somatic mutation of the receptor, which is G-protein-coupled, produces functioning thyroid adenomas with hyperthyroidism. Precocious puberty in males is due to an activating mutation in the LH receptor. Both these mutations map to regions in the receptors that interact with the G proteins, and presumably lead to constitutive activation of the α subunits that couple to adenylyl cyclase[8].

Mutations have also been identified in α subunits which either lead to the activation of the α subunit or loss of its expression. Activation is associated with a reduced GTPase activity of the α subunit, and many of the mutations are at residues which were earlier shown in *ras* to inhibit GTPase activity, and lead to cellular transformation. In fact, a majority of human tumours are associated with constitutively activated *ras* mutations. Certain growth-hormone-secreting pituitary tumours have identified mutations in the α_s subunit, as is the case in McCune–Albright syndrome, characterized by peripheral sexual precocity and other endocrinopathies. Albright's hereditary osteodystrophy is characterized by short stature and obesity, and is an inherited disease in which α_s levels are markedly reduced in cell membranes from affected tissues. This leads to resistance to a number of hormones, including thyroid stimulating hormone, gonadotropins and glucagon, all of which are coupled to the stimulation of adenylyl cyclase[7].

Conclusions

The fundamental nature of Rodbell and Gilman's discoveries, and the near ubiquitous role of G proteins in cellular signalling has laid the basis for extensive studies from a number of laboratories. G protein homologues have been identified in the yeast, *Saccharomyces cerevisiae*, where they are involved in the mating process, and in *Dictyostelium*, where they participate in developmental regulation; and G-protein-like genes have been detected in *Drosophila* and are essential for appropriate morphological changes[9].

Why has the guanine nucleotide been chosen to play such a critical role in the signalling process adopted by cells? The adenine nucleotide, ATP, is involved in all energy-dependent processes, and is used as a substrate for kinases within the cell. GTP, however, is used by guanylyl cyclases as a substrate for the production of cGMP. Interestingly, some membrane-bound forms of guanylyl cyclase, such as the receptor for atrial natriuretic factor, show an ATP-dependent, ligand-stimulated guanylyl cyclase activity[10]. In these receptors, there is a domain predicted to be lying towards the cytoplasmic side of the membrane, that bears homology to protein kinases. However, no protein kinase-like activity has been demonstrated by any of these receptor cyclases, suggesting that this ATP binding domain may influence the catalytic activity of the enzyme It is exciting to suggest that these kinase-like domains may function in a manner analogous to the α subunits of the G proteins, by activating the guanylyl cyclase activity when in the ATP-bound form[11].

With the critical role played by G proteins and their receptors in cellular signalling, it is no surprise to know that a number of drugs are in use that specifically interact with G protein-coupled receptors. These include drugs for hypertension, dopamine analogues, and the opiates. However, given the high degree of homology of the various subunits of the G proteins and their wide distribution within the cells, it appears unlikely that specific inhibitors for any α or $\beta\gamma$ subunits will be identified. Most therapeutics which interfere in the G-protein-coupled pathway are targeted at the effector enzymes, such as the methylxanthines, which inhibit phosphodiesterases and therefore can modulate cyclic nucleotide levels. In the coming years, clearer understanding of the structures of more α subunits, and perhaps the $\beta\gamma$ complex, will emerge. Further complexities in the intricate signalling pathways and cross-regulation of the different pathways will be identified. This will re-emphasize the fundamental discoveries made by Rodbell and Gilman, which will allow us in the future to control a variety of biological processes within the body.

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