

CHAPTER

21

G Proteins

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OUTLINE

Heterotrimeric G Proteins

The family of heterotrimeric G proteins is involved in transmembrane signaling in the nervous system, with certain exceptions

Multiple forms of heterotrimeric G protein exist in the nervous system

Each G protein is a heterotrimer composed of single α , β and γ subunits

The functional activity of G proteins involves their dissociation and reassociation in response to extracellular signals

G proteins couple some neurotransmitter receptors directly to ion channels

G proteins regulate intracellular concentrations of second messengers

G proteins have been implicated in membrane trafficking

G protein $\beta\gamma$ subunits subserve numerous functions in the cell

The functioning of heterotrimeric G proteins is modulated by other proteins

411	G proteins are modified covalently by the addition of long-chain fatty acids	418
411	The functioning of G proteins may be influenced by phosphorylation	418
412	Small G Proteins	418
412	The best-characterized small G protein is the Ras family, a series of related proteins of 21 kDa	418
412	Rab is a family of small G proteins involved in membrane vesicle trafficking	419
412	Other Features of G Proteins	419
412	G proteins can be modified by ADP-ribosylation catalyzed by certain bacterial toxins	419
415	G proteins are implicated in the pathophysiology and treatment of disease	420
	Box: Small G Proteins and Neurologic Disease	421
416	References	421

G proteins comprise several families of diverse cellular proteins that subserve an equally diverse array of cellular functions. These proteins derive their name from the fact that they bind the guanine nucleotides guanosine triphosphate (GTP) and guanosine diphosphate (GDP) and possess intrinsic GTPase activity. G proteins play a central role in signal transduction as well as in a myriad of cellular processes, including membrane vesicle transport, cytoskeletal assembly, cell growth and protein synthesis (see Chs. 7, 10 and 11). Mammalian G proteins can be divided into two major categories: heterotrimeric G proteins and small G proteins. This chapter reviews the types of G protein that exist in the nervous system and the ways in which they regulate signal transduction and other processes essential for brain function.

HETEROTRIMERIC G PROTEINS

The family of heterotrimeric G proteins is involved in transmembrane signaling in the nervous system, with certain exceptions

The family of heterotrimeric G proteins is involved in transmembrane signaling in the nervous system, with certain exceptions. The exceptions are instances of synaptic transmission mediated via receptors that contain intrinsic enzymatic activity, such as tyrosine kinase or guanylyl cyclase, or via receptors that form ion channels. Heterotrimeric G proteins were first identified, named and characterized by Alfred Gilman, Martin Rodbell and others close to 30 years ago. They consist of three

distinct subunits, α , β and γ . These proteins couple the activation of diverse types of plasmalemma receptor to a variety of intracellular processes. In fact, most types of neurotransmitter and peptide hormone receptor, as well as many cytokine and chemokine receptors, fall into a superfamily of structurally related molecules, termed G protein-coupled receptors. These receptors are named for the role of G proteins in mediating the varied biological effects of the receptors (see Ch. 12). Consequently, numerous effector proteins are influenced by these heterotrimeric G proteins: ion channels; adenylyl cyclase; phosphodiesterase (PDE); phosphoinositide-specific phospholipase C (PI-PLC), which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂); and phospholipase A₂ (PLA₂), which catalyzes the hydrolysis of membrane phospholipids to yield arachidonic acid. In addition, these G proteins have been implicated in several other intracellular processes, such as vesicular transport and cytoskeletal assembly.

Multiple forms of heterotrimeric G protein exist in the nervous system

Three types of heterotrimeric G protein were identified in early studies. G_i, termed transducin, was identified as the G protein that couples rhodopsin to regulation of photoreceptor cell function (see Ch. 51), and G_s and G_i were identified as the G proteins that couple plasma membrane receptors to the stimulation and inhibition, respectively, of adenylyl cyclase, the enzyme that catalyzes the synthesis of cAMP (see Ch. 22).

Since that time, over 35 heterotrimeric G protein subunits have been identified by a combination of biochemical and molecular cloning techniques (Neer, 1995; Wickman & Clapham, 1995). In addition to G_i, G_s and G_i, the other types of G protein in brain are designated G_o, G_{olf}, G_{gust}, G_z, G_q and G₁₁₋₁₆. Moreover, for most of these G proteins, multiple subtypes show unique distributions in the brain and peripheral tissues. G proteins are now divided into four main categories (Table 21-1): the G_s family stimulates adenylyl cyclase; the G_i family (which includes G_o, G_{gust}, G_z) can inhibit adenylyl cyclase, activate a certain type of K⁺ channel, inhibit voltage-gated Ca²⁺ channels, activate the MAP-kinase pathway or activate phosphodiesterase; the G_q family activates PI-PLC; and the G₁₂ family (composed of G₁₁₋₁₆) activates a group of proteins termed Rho-GEFs (guanine nucleotide exchange factors, which are described in greater detail below).

Each G protein is a heterotrimer composed of single α , β and γ subunits

The different types of G protein contain distinct α subunits, which confer part of the specificity of functional activity. G protein α subunits, listed in Table 21-1, are categorized on the basis of their structural and functional homologies. Current nomenclature identifies several subfamilies of G protein α subunit: G_{oα}, G_{oαi}, G_{oαq} and G_{oα12}. The M_r of these proteins varies between 38,000 and 52,000. As a first approximation, these distinct types of α subunits share common β and γ subunits. However, multiple subtypes of β and γ subunits are known: five β subunits of M_r 35,000–36,000 and 11 γ subunits of M_r 6,000–9,000. These proteins show distinct cellular distributions, and differences in

their functional properties are becoming increasingly apparent (Neer, 1995; Wickman & Clapham, 1995; Krumins & Gilman, 2006; Gibson & Gilman, 2006).

The functional activity of G proteins involves their dissociation and reassociation in response to extracellular signals

The functional activity of G proteins involves their dissociation and reassociation in response to extracellular signals. This G protein cycle is shown schematically in Figure 21-1. In the resting state, G proteins exist as heterotrimers that bind GDP and are associated with extracellular receptors (Fig. 21-1A). When a ligand binds to and activates the receptor, it produces a conformational change in the receptor, which in turn triggers a dramatic conformational change in the α subunit of the G protein (Fig. 21-1B). This conformational change leads to (a) a decrease in the affinity of the α subunit for GDP, which results in the dissociation of GDP from the α subunit and the subsequent binding of GTP because the cellular concentration of GTP is much higher than that of GDP; (b) dissociation of a $\beta\gamma$ subunit dimer from the α subunit; and (c) release of the receptor from the G protein (Fig. 21-1B, C). This process generates a free α subunit bound to GTP as well as a free $\beta\gamma$ subunit dimer, both of which are biologically active and regulate the functional activity of effector proteins within the cell. The GTP-bound α subunit is also capable of interacting with the receptor and reducing its affinity for ligand. The system returns to its resting state when the ligand is released from the receptor and the GTPase activity that resides in the α subunit hydrolyzes GTP to GDP (Fig. 21-1D). The latter action leads to reassociation of the free α subunit with the $\beta\gamma$ subunit complex to restore the original heterotrimers.

The structural basis of the interactions among the α , β and γ subunits of G proteins and between the subunits and the associated receptor has become increasingly understood as the three-dimensional structure of these proteins has been determined (Onrust et al., 1997; Oldham & Hamm, 2008). Each α subunit has two identifiable domains. One contains the GTPase activity and the GTP-binding site. This domain also appears to be most important in binding $\beta\gamma$ subunits as well as various effector proteins. The function of the other domain remains unknown, but it may be involved in the dramatic conformational shift that occurs in the protein upon exchanging GTP for GDP. The ability of the heterotrimeric G protein to bind to a receptor is thought to depend on sites located within all three G protein subunits. Thus, the different α subunits, as well as subtypes of β and γ subunits, seem to be responsible for targeting a particular type of G protein to a particular type of receptor.

G proteins couple some neurotransmitter receptors directly to ion channels

It is now clearly established that G protein subunits, when released from their G protein-receptor interactions, can directly gate (i.e., open or close) specific ion channels (Wickman & Clapham, 1995; Hatley et al., 2003; Riven et al., 2006; Dolphin, 2009). One of the best examples of this mechanism in brain is the coupling of many types of receptor to the activation of

TABLE 21-1 Heterotrimeric G Protein α -Subunits in Brain

Family	M_r^a	Toxin-mediated ADP-ribosylation	Effector protein(s) ^b
G_s			
$G_{\alpha s1}$	52,000	Cholera	Adenylyl cyclase (activation)
$G_{\alpha s2}$	52,000		
$G_{\alpha s3}$	45,000		
$G_{\alpha s4}$	45,000		
$G_{\alpha olf}$	45,000		
G_i			
$G_{\alpha i1}$	41,000	Pertussis	Adenylyl cyclase (inhibition)
$G_{\alpha i2}$	40,000		K^+ channel (activation)
$G_{\alpha i3}$	41,000		Ca^{2+} channel (inhibition) ?PI-Phospholipase C (activation) ?Phospholipase A ₂
$G_{\alpha o1}$	39,000	Pertussis	K^+ channel (activation)
$G_{\alpha o2}$	39,000		Ca^{2+} channel (inhibition)
$G_{\alpha t1}$	39,000	Cholera and pertussis	Phosphodiesterase (activation) in rods and cones
$G_{\alpha t2}$	40,000		
$G_{\alpha gust}$	41,000	Unknown	Phosphodiesterase (activation) in taste epithelium
$G_{\alpha z}$	41,000	None	?Adenylyl cyclase (inhibition)
G_q	41,000–43,000		
$G_{\alpha q}$		None	PI-Phospholipase C (activation)
$G_{\alpha 11}$		Unknown	
$G_{\alpha 14}$			
$G_{\alpha 15}$			
$G_{\alpha 16}$			
G_{12}	44,000	None	Rho-GEFs (activation)
$G_{\alpha 12}$			
$G_{\alpha 13}$			

Question marks indicate that the association between the particular G proteins and effector proteins shown in the table remains tentative.

^aValues shown reflect apparent M_r by gel electrophoresis in most cases. Values shown for $G_{\alpha gust}$, $G_{\alpha z}$ and $G_{\alpha 11-16}$ reflect calculated M_r based on amino acid sequence.

^bFor several of the effector proteins listed, the functional regulation of the effector is mediated, in part or in full, by the $\beta\gamma$ subunits associated with the indicated α subunit.

subtypes of inward-rectifying K^+ channel (GIRK) via pertussis toxin-sensitive G proteins, i.e., subtypes of $G_{i/o}$ in many types of neurons. The coupled receptors include opioid, α_2 -adrenergic, D₂-dopaminergic, muscarinic cholinergic, 5-HT_{1A}-serotonergic and GABA_B. In initial studies, it was controversial as to whether the free α subunit or the free $\beta\gamma$ dimer was responsible for this action. Based on the results of elegant studies in which cloned channel and G protein subunits have been expressed in a variety of cell types, the general consensus is now that the $\beta\gamma$ complex is the more important mechanism (Wickman & Clapham, 1995; Hatley et al., 2003; Riven et al., 2006). The region of the GIRK that binds to the $\beta\gamma$ complex

has been identified. Moreover, it seems that particular combinations of β and γ subtypes are more effective at opening this channel than others. In addition, some subtypes of α_i can open the channel, although not to the same extent as the $\beta\gamma$ subunits.

These same neurotransmitter receptors also are coupled via pertussis toxin-sensitive G proteins to voltage-gated Ca^{2+} channels. In this case the channels are inhibited by the interaction. Available evidence again supports a primary role for $\beta\gamma$ in mediating this effect, although there is some evidence that α_i and α_o subunits also can be involved. Binding of the G protein subunits to the Ca^{2+} channels reduces their probability of opening in response to membrane depolarization.

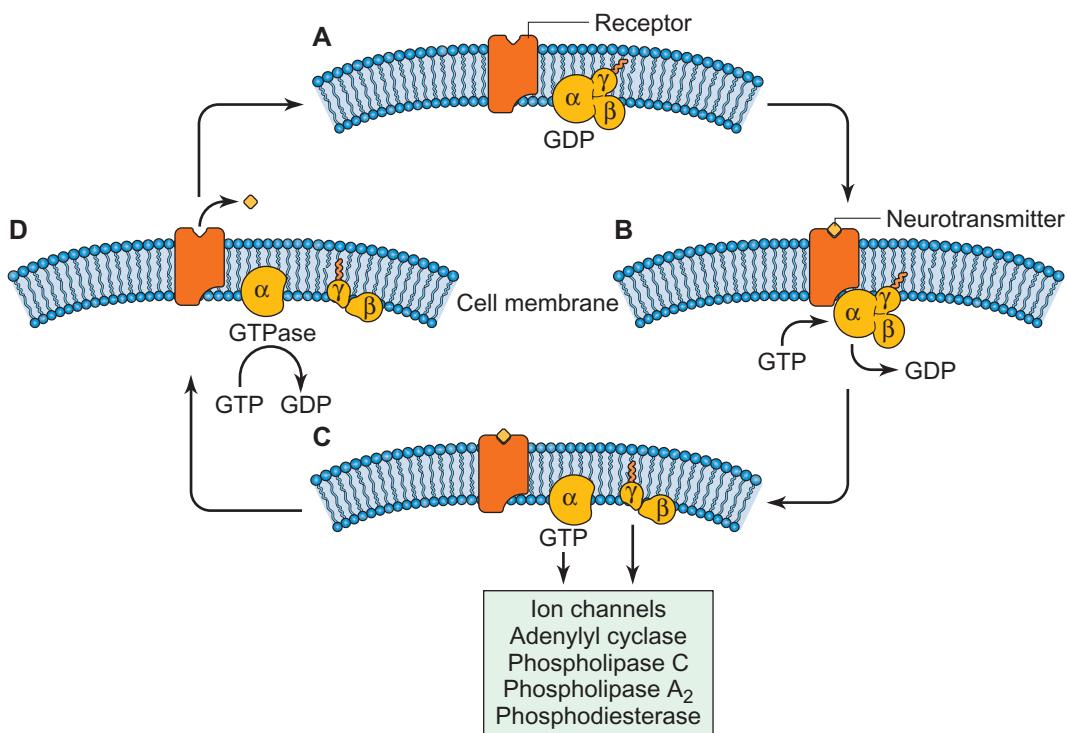


FIGURE 21-1 Functional cycle of heterotrimeric G proteins. (A) Under basal conditions, G proteins exist in cell membranes as heterotrimers composed of single α , β and γ subunits and are associated only loosely with neurotransmitter receptors. In this situation, GDP is bound to the α subunit. (B) Upon activation of the receptor by its ligand, such as a neurotransmitter, the receptor physically associates with the α subunit, which leads to the dissociation of GDP from the subunit and the binding of GTP instead. (C) GTP binding induces the generation of free α subunit by causing the dissociation of the α subunit from its β and γ subunits and the receptor. Free α subunits (bound to GTP) and free $\beta\gamma$ subunit dimers are functionally active and directly regulate a number of effector proteins, which, depending on the type of subunit and cell involved, can include ion channels, adenylyl cyclase, phospholipase C, phospholipase A₂ and phosphodiesterase. (D) GTPase activity intrinsic to the α subunit degrades GTP to GDP. This leads to the reassociation of the α and $\beta\gamma$ subunits, which, along with the dissociation of ligand from receptor, leads to restoration of the basal state.

This mechanism is best established for L-type Ca^{2+} channels, which are also inhibited by dihydropyridine antihypertensive drugs, such as verapamil, but may also operate for other types of voltage-gated Ca^{2+} channel (see also Chs. 4 and 24).

G proteins regulate intracellular concentrations of second messengers

G proteins control intracellular cAMP concentrations by mediating the ability of neurotransmitters to activate or inhibit adenylyl cyclase. The mechanism by which neurotransmitters stimulate adenylyl cyclase is well known. Activation of those neurotransmitter receptors that couple to G_s results in the generation of free G_{os} subunits, which bind to and thus directly activate adenylyl cyclase. In addition, free $\beta\gamma$ -subunit complexes activate certain subtypes of adenylyl cyclase (see Ch. 22). A similar mechanism appears to be the case for $G_{o\text{lf}}$, a type of G protein structurally related to G_{os} that is enriched in olfactory epithelium and striatum (Ch. 52).

The mechanism by which neurotransmitters inhibit adenylyl cyclase and decrease neuronal levels of cAMP has been more difficult to delineate. By analogy with the action of G_s , it was proposed originally that activation of neurotransmitter receptors that couple to G_i results in the generation of free G_{oi}

subunits, which bind to and, thereby, directly inhibit adenylyl cyclase. Initially, such direct inhibition of adenylyl cyclase by G_{oi} was difficult to demonstrate in some cell-free reconstitution experiments. Nevertheless, there is now strong evidence, including insight coming from the crystalline structures of these proteins, that certain subtypes of adenylyl cyclase are directly inhibited by G_{oi} . For other adenylyl cyclase isoforms, free $\beta\gamma$ -subunit complexes, generated by the release of G_{oi} , appear to mediate inhibition of the enzyme (Ch. 22).

The transducin family of G proteins mediates signal transduction in the visual system (Ch. 51) by regulating specific forms of phosphodiesterase that catalyze the metabolism of cyclic nucleotides (Ch. 22). G_{ot} activates PDE via direct binding to the enzyme. Gustducin (G_{ogust}) shares a high degree of homology with G_{ot} . It is enriched in taste epithelium and is believed to mediate signal transduction in this tissue via the activation of a distinct form of phosphodiesterase (Chapter 52).

The ability of many neurotransmitters and their G protein-coupled receptors to stimulate the phosphoinositide second-messenger pathway is mediated by the activation of PI-PLC, which, as mentioned above, catalyzes the hydrolysis of PIP_2 to form the second messengers inositol triphosphate (IP_3) and diacylglycerol (DAG) (Ch. 23). Such receptor-induced activation of PI-PLC is mediated via G proteins and involves the

regulation specifically of the β isoform of PI-PLC (Rhee, 2001; Ghosh & Smrcka, 2004). (By contrast, it is the γ isoform of PI-PLC that is regulated by growth factors (Ch. 29) and their tyrosine kinase receptors (Ch. 26)). In most cases, G_q mediates neurotransmitter regulation of PI-PLC β , and it is thought that $G_{\alpha q}$ and related α subunits bind to and directly activate the enzyme. In some cell types, $\beta\gamma$ subunits released from G_i proteins directly bind and activate PI-PLC β .

G proteins have been implicated in membrane trafficking

In addition to mediating signal transduction at the plasma membrane, evidence suggests that certain heterotrimeric G proteins are implicated in processes that involve the trafficking of cell membranes. For example, the $G_{\alpha i}$ subunit has been detected at relatively high concentrations in intracellular membranes, including the Golgi complex, *trans*-Golgi network and endoplasmic reticulum (Helms, 1995; Yamaguchi et al., 2000). Experiments that involve activation or inhibition of this subunit with various guanine nucleotides suggest that $G_{\alpha i}$ may regulate the budding of membrane vesicles through these organelles. It also has been suggested that $G_{\alpha i}$ could be involved in the process by which portions of the plasma membrane are vesicularized into the cytoplasm via endocytosis. Synaptic vesicle trafficking is discussed in detail in Chapter 7.

G protein $\beta\gamma$ subunits subserve numerous functions in the cell

In early studies, G protein $\beta\gamma$ subunits were thought to be inactive proteins that merely sequestered active α subunits or anchored them to the plasma membrane. However, it has become clear that $\beta\gamma$ subunits, acting as dimers, are highly active biological molecules that play important roles in several cellular functions. As mentioned above $\beta\gamma$ subunits directly bind to and activate a class of K channels called GIRKs and bind to and modulate the activity of PI-PLC β and certain classes of adenylyl cyclase. The $\beta\gamma$ subunits also bind to several other proteins, including certain protein kinases as well as phosphducin and Ras-GEFs [see below]. The ability of such diverse cellular proteins to bind $\beta\gamma$ subunits has led to a search for a common structural motif in these various target proteins that is responsible for binding to the $\beta\gamma$ subunits. One possibility is that the target proteins contain, within their primary structures, a specific amino acid sequence termed the pleckstrin homology (PH) domain, which binds $\beta\gamma$ with high affinity.

One class of protein kinase that binds $\beta\gamma$ subunits is called G-protein-receptor kinases (GRKs). These kinases phosphorylate G-protein-coupled receptors that are occupied by ligand and thereby mediate one form of receptor desensitization (Ch. 25). It now appears that $\beta\gamma$ subunits play a key role in this process (Tesmer et al., 2005). As shown in Figure 21-2, GRK is normally a cytoplasmic protein that does not come into appreciable contact with the plasma membrane receptor under basal conditions. Ligand binding to the receptor activates the associated G protein, which results in the generation of free α and $\beta\gamma$ subunits. The $\beta\gamma$ subunits, which remain membrane bound (as will be described below), are now free to bind to

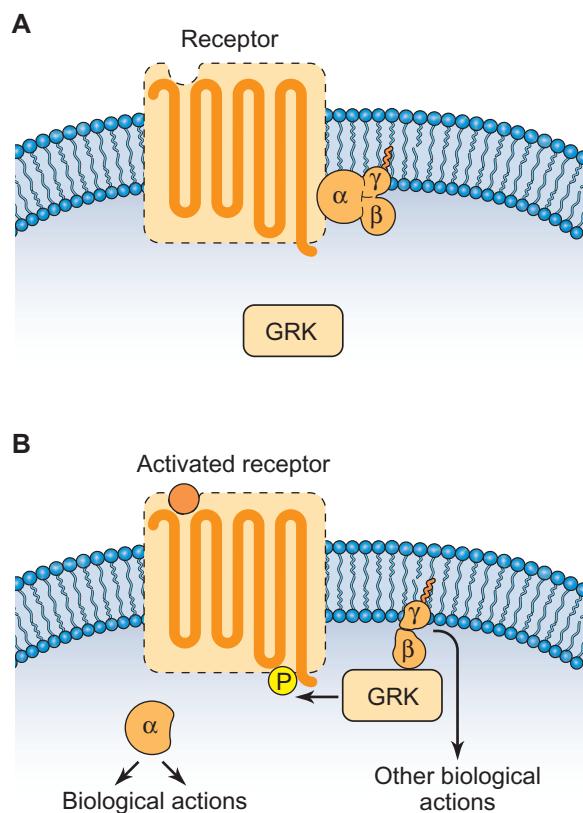


FIGURE 21-2 Schematic illustration of the role of G protein $\beta\gamma$ subunits in intracellular targeting of proteins. (A) Under resting conditions, the receptor is associated loosely with a heterotrimeric G protein and G protein-receptor kinases (GRK) are cytosolic and therefore unable to phosphorylate the receptor. (B) Upon activation of the receptor and G protein, free α subunit is generated, which can lead to a variety of physiological effects. In addition, a free $\beta\gamma$ subunit dimer is generated, which can bind to the GRK and draw it toward the membrane, where it can phosphorylate the ligand-occupied receptor. In this way, $\beta\gamma$ subunits can direct GRKs specifically to the targets, which are those receptor molecules occupied by ligand. Free $\beta\gamma$ also produces other physiological effects by interacting with other cellular proteins. The $\beta\gamma$ complex is tethered to the membrane by an isoprenyl group on the γ subunit, as depicted.

the C-terminal domain of the GRK. This draws the GRK into close physical proximity with the receptor and enables receptor phosphorylation. In this way, the $\beta\gamma$ subunits direct GRKs, which have constitutive catalytic activity, to those receptors that are ligand bound.

Another important role for $\beta\gamma$ subunits is regulation of the mitogen-activated protein kinase (MAP-kinase) pathway, including the MAP-kinase called ERK (extracellular-regulated kinase) (Schmitt & Stork, 2002). MAP-kinases are the major effector pathway for growth factor receptors (see Chs. 25, 26 and 29). However, signals that act through G protein-coupled receptors, particularly those coupled to G_i , can modulate growth factor activation of the MAP-kinase pathway. This is mediated via $\beta\gamma$ subunits. Activation of the receptors leads to the generation of free $\beta\gamma$ subunits, which then activate the MAP-kinase pathway at some early step in the cascade. Some

possibilities include direct action of the $\beta\gamma$ subunits on Ras (see below) or on one of several 'linker' proteins between the growth factor receptor itself and activation of Ras.

Recent research has implicated $G_{\beta\gamma}$ subunits in mediating G-protein signaling in the absence of activation of the G protein's associated receptor. This mechanism involves a newly discovered modulatory protein, called GoLoco, which triggers the release of free $\beta\gamma$ dimers from G protein-receptor complexes without receptor activation and leads to $\beta\gamma$ regulation of its several effector proteins. This GoLoco mechanism is described further in the next section of this chapter.

The activity of G protein $\beta\gamma$ subunits is modulated by another protein termed phosducin (Schulz, 2001). Phosducin is a cytosolic protein enriched in retina and pineal gland but also expressed in brain and other tissues. Phosducin binds to G protein $\beta\gamma$ subunits with high affinity. The result is prevention of $\beta\gamma$ subunit reassociation with the α subunit. In this way, phosducin may sequester $\beta\gamma$ subunits, which initially may prolong the biological activity of the α subunit. However, eventually this sequestration may inhibit G protein activity by preventing the direct biological effects of the $\beta\gamma$ subunits as well as preventing regeneration of the functional G protein heterotrimer. How phosducin functions in intact cells remains incompletely understood, although the ability of phosducin to bind to $\beta\gamma$ subunits is altered upon its phosphorylation by cAMP- or Ca^{2+} -dependent protein kinases (see Ch. 25). This raises the possibility that phosducin acts as an important physiological modulator of G protein function.

The molecular specificity of subtypes of β and γ subunits is an area of intense research (Gibson & Gilman, 2006; Smrcka et al., 2010). The five known forms of G protein β subunit, whose structures are highly similar, are divided into two families comprising $G_{\beta1-4}$ and $G_{\beta5}$. The 11 forms of γ subunit are more divergent structurally. Some show striking regional distributions in the brain: for example, $G_{\gamma7}$ is highly enriched in striatum (Schwindinger et al., 2003). Different forms of β and γ subunit interact with each other with widely varying abilities in *in vitro* expression systems. Identifying which forms of $\beta\gamma$ subunit complexes occur *in vivo* and the specificity of these complexes for various target proteins, such as adenylyl cyclases, K^+ channels, GRKs and others, remains an important area of investigation.

The functioning of heterotrimeric G proteins is modulated by other proteins

It has been known for years that the activity of small G proteins is modulated by proteins that bind to the G proteins and stimulate their intrinsic GTPase activity (see below). These are termed *GTPase-activating proteins* (GAPs; Fig. 21-3). Now, analogous proteins have been identified for heterotrimeric G proteins. These GAPs, first identified in yeast but subsequently found in mammalian tissues, are termed RGS proteins (Witherow & Slepak, 2003). RGS proteins bind to G protein α subunits and stimulate their GTPase activity. This action hastens the hydrolysis of GTP to GDP and more rapidly restores the inactive heterotrimer; thus, RGS proteins inhibit the biological activity of G proteins. In addition, RGS

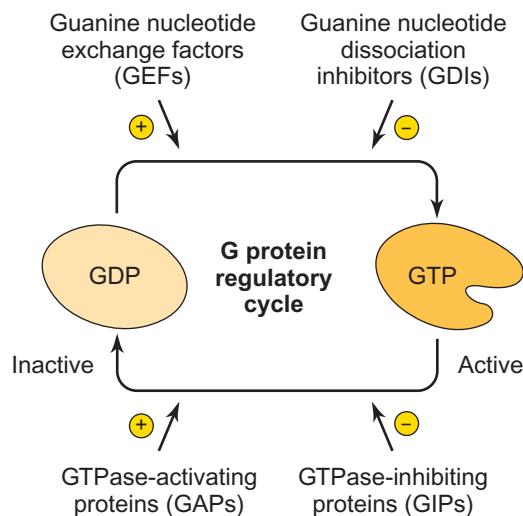


FIGURE 21-3 Schematic illustration of proteins that modulate the functioning of G proteins. The functional activity of G proteins is controlled by cycles of binding GDP versus GTP. This is associated with a major conformational change in the protein, as depicted. There are several proteins that regulate this cycle and thereby regulate the functional activity of G proteins. Analogous modulator proteins exist for heterotrimeric G protein α subunits and for small G proteins. There are proteins that facilitate the release of GDP from the G protein and thereby enhance G protein function. Examples of such guanine nucleotide exchange factors (GEFs) are receptors for heterotrimeric G proteins or a large number of GEFs specific for various small G proteins. There are proteins, GTPase-activating proteins (GAPs), that activate the GTPase activity intrinsic for the G proteins and thereby inhibit G protein function. Examples are the regulators of G protein-signaling (RGS) proteins for heterotrimeric G proteins and a series of GAPs specific for various small G proteins. There also may be GTPase-inhibitory proteins (GIPs) that exert the opposite effects. Heterotrimeric $\beta\gamma$ subunits can be viewed as such; analogous proteins have been proposed for the small G proteins. Phosducin, by binding to $\beta\gamma$ subunits, would represent yet another regulatory protein that modulates G protein function.

proteins impart important scaffolding properties that alter the function of multiprotein signaling complexes at the plasma membrane.

Some 40 forms of mammalian RGS protein are now known, and several of them are expressed in brain with highly specific regional patterns (Gold et al., 1997). RGS proteins act on the G_α subunit of G proteins, with varying degrees of specificity. Figure 21-4 shows the categorization of mammalian RGS proteins based on their structural properties (Gold et al., 1997). All RGS proteins contain a core RGS domain, which is responsible for regulating G protein α subunit GTPase activity. However, several other domains contained within the RGS protein subtypes may control the protein's localization, stability and confer other diverse functions on these proteins, in addition to GAP. One example is the R7 subfamily of RGS proteins. This subfamily, which uniquely associates with $G_{\beta5}$, contains a GGL (G_γ -like) domain that may enable these RGS subtypes to substitute for G_γ subunits in regulation of G protein-coupled receptor function. Binding of $G_{\beta5}$ to the GGL domain of RGS proteins is essential for their stability and plays a key role in the process of

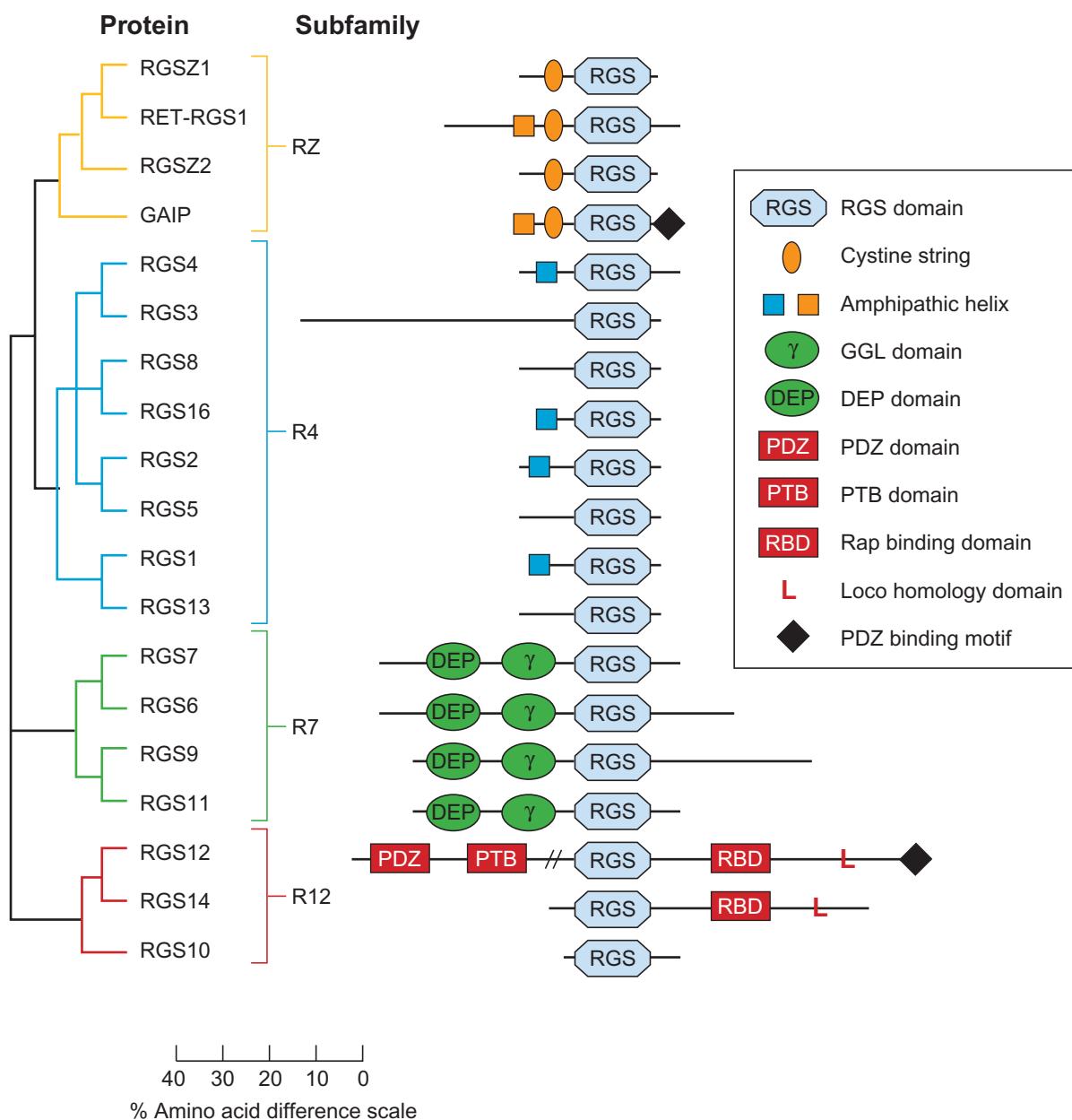


FIGURE 21-4 Schematic illustration of the structure and classification of mammalian RGS proteins. All the proteins contain a highly conserved RGS domain that has GAP activity. Most of the proteins contain additional domains that mediate other functions. The figure does not include several other types of homologous proteins, which lack the RGS domain but nevertheless are considered members of the RGS superfamily.

G protein-coupled receptor signaling and desensitization in the striatum (Neubig & Siderovsky, 2002; Traynor et al., 2009).

Another example is provided by the R12 subfamily of RGS proteins (Kimple et al., 2001). These subtypes contain the GoLoco motif described earlier. The name of the motif comes from the fact that it interacts with G proteins and the fact that the *Drosophila* homolog of RGS12 is called Loco. GoLoco binds directly to G_i and stabilizes it in its GDP-bound form. At the same time, it leads to the dissociation of G_{βγ} subunit dimers, which then activate numerous effectors. This occurs

independently of ligand activation of the associated G protein-coupled receptor. In this way, RGS12 proteins can stimulate receptor-independent G protein signaling. Several other types of protein contain Go-Loco motifs and similarly regulate G protein signaling.

These are just some examples of the diverse functions of RGS proteins in mammalian cells. Given the number and diversity of RGS proteins, and their region-specific expression in brain, there is considerable interest in targeting them in the development of new pharmacotherapeutic agents.

for neuropsychiatric disorders. There is also growing evidence that alterations in the activity of specific RGS proteins, for example via changes in their expression or association with interacting partners, modulate the activity of specific G proteins and, consequently, the sensitivity of specific G-protein-coupled receptors. Such mechanisms have been implicated, for example, in disorders as diverse as hypertension, drug addiction (Ch. 61), schizophrenia (see also Ch. 58) and Parkinson's disease, and over the last few years a large number of clinical studies link RGS proteins to many of these disorders (Zachariou et al., 2003; Levitt et al., 2006). Another family of proteins interacting with G protein signaling concerns the activators of G protein signaling (AGS). This group contains structurally diverse proteins that serve as binding partners of G protein components independent of receptor activation (Blumer et al., 2007). In neurons, AGS actions appear to mediate a series of conditions, from neurogenesis to drug addiction.

G proteins are modified covalently by the addition of long-chain fatty acids

All G protein α subunits are modified in their N-terminal domains by palmitoylation or myristylation. These modifications may regulate the affinity of the α subunit for its $\beta\gamma$ subunits and, thereby, the likelihood of dissociation or reassociation of the heterotrimer. The modifications also may help determine whether the α subunit, released upon ligand-receptor interaction, remains associated with the plasma membrane or diffuses into the cytoplasm. This could have important consequences on the types of effector protein regulated. It is also possible that palmitoylation, but not myristylation, is regulated dynamically. There is evidence that palmitoylation can be regulated by ligand binding, which makes this a potentially important control point. However, very little is known about palmitoyl transferases and depalmitoylases, the enzymes responsible for palmitoylation. In contrast, myristylation appears to be a one-time event in the life cycle of an α subunit.

G protein γ subunits are modified on their C-terminal cysteine residues by isoprenylation (Wedegaertner et al., 1995; Tu et al., 1997). There is now strong evidence that this modification plays a key role in anchoring the γ subunit and its associated β subunit to the plasmalemma. The importance of this anchoring is illustrated in Figure 21-2, which shows that the ability of $\beta\gamma$ subunits to direct GRKs to ligand-bound receptors depends on this membrane localization.

The functioning of G proteins may be influenced by phosphorylation

G proteins, as well as their associated receptors and RGS proteins, have been reported to undergo phosphorylation by a host of protein serine/threonine kinases and protein tyrosine kinases. While the ramifications of receptor phosphorylation are becoming increasingly well understood (see Chs. 25 and 26), the effect of phosphorylation of G proteins and RGS proteins, and its role in the regulation of physiological processes,

have been more difficult to establish with certainty. This remains an important area of future investigation.

SMALL G PROTEINS

In addition to the heterotrimeric G proteins, other forms of G protein play important roles in cell function. These proteins belong to a large superfamily often referred to as 'small G proteins' because of their low M_r (20,000–35,000) (Marshall, 1996). The small G proteins, like the heterotrimeric G proteins, bind guanine nucleotides, possess intrinsic GTPase activity, and cycle through GDP- and GTP-bound forms (as shown in Fig. 21-1). One unifying feature of the various classes of G protein is that the binding of GTP versus GDP dramatically alters the affinity of the protein for some target molecule, apparently by inducing a large conformational change. Small G proteins appear to function as molecular switches that control several cellular processes. Examples of small G proteins and their possible functional roles are given in Table 21-2.

The best-characterized small G protein is the Ras family, a series of related proteins of 21 kDa

Ras proteins were identified originally as the oncogene products of Harvey and Kirsten rat sarcoma viruses. Subsequently, normal cellular homologs, also known as proto-oncogenes, of viral Ras were identified. Mammalian Ras proteins are encoded by three homologous genes: the proto-oncogene for Harvey Ras virus (*H-ras*), the proto-oncogene for Kirsten Ras virus (*K-ras*) and neural Ras (*N-ras*), although all three forms of Ras are found in diverse mammalian tissues, including brain. All three forms of Ras are membrane-associated proteins (Marshall, 1996).

The activity of Ras is highly regulated by a variety of associated proteins, as shown in Figure 21-3. Guanine nucleotide

TABLE 21-2 Examples of Small G Proteins

Class	Proposed cellular function
Ras	Signal transduction (control of growth factor and MAP-kinase pathways)
Rac, CDC42	Signal transduction (control of cellular stress responses and MAP-kinase pathways)
Rab	Localized to synaptic vesicles, where it regulates vesicle trafficking and exocytosis
Rho	Assembly of cytoskeletal structures (e.g., actin microfilaments)
ARF	ADP-ribosylation of $G_{\alpha s}$; assembly and function of Golgi complex
EFTU	Associated with ribosomes, where it regulates protein synthesis
Ran	Nuclear-cytoplasmic trafficking of RNA and protein

ARF, ADP-ribosylation factor; EFTU, eukaryotic elongation factor.

exchange factors (GEFs) stimulate the release of GDP from inactive Ras, which facilitates the binding of GTP. Thus, GEFs increase the activity of Ras. Multiple forms of GEFs have been identified, some specific for Ras and others for different small G proteins. In contrast, GAPs bind to Ras and activate its intrinsic GTPase activity, thereby reducing the functional activity of Ras. There also may be GTPase-inhibitory proteins (GIPs) that bind Ras and inhibit this GTPase activity, although these remain poorly described. In addition, small G proteins are modified by guanine nucleotide dissociation inhibitors (GDIs), which reduce the rate of exchange of GDP for GTP.

The analogy between Ras and other small G proteins, on the one hand, and heterotrimeric G proteins and their related proteins, on the other, is striking (Fig. 21-3). G protein-coupled receptors, in their ligand-bound form, essentially function as GEFs for the heterotrimeric G proteins, whereas RGS proteins function as GTPase-activating proteins and $\beta\gamma$ subunits as GDIs. One major difference between the systems is that the intrinsic GTPase activity of Ras is far lower than that of heterotrimeric G protein α subunits. As a result, GAPs exert a much more profound effect on the functioning of Ras, essentially turning it on and off.

Upon binding GTP, a major conformational change occurs in Ras, which is thought to be responsible for its functional activation. Ras has long been known to be a major control point for cell growth and some of the effector molecules through which Ras produces its effects have been identified. Numerous types of cell signal, including many growth factors, converge on Ras to regulate MAP-kinase pathways and, thereby, to produce their diverse effects on cell function (see Chs. 26 and 29). Briefly, it appears that activation of growth factor receptors results in the activation of a GEF, termed Sos, which in turn activates Ras. Activated Ras then binds to the N-terminal domain of a protein kinase called Raf, the first protein kinase in the MAP-kinase pathway. Ras appears to activate Raf via an indirect mechanism. Ras, which is membrane bound, draws Raf to the plasmalemma, where it (Raf) is activated through other means that are not yet completely understood. Anchoring of Ras in the plasmalemma may be mediated by isoprenylation, another point of analogy between Ras and heterotrimeric G proteins (Fig. 21-2).

Although the major mechanism governing Ras activation is through growth factors, as outlined above, Ras function can also be modulated by heterotrimeric G protein and second-messenger pathways. The ability of free $\beta\gamma$ subunits, particularly those released from G_i , to activate Ras, was mentioned earlier. In addition, the cAMP cascade inhibits Ras activity in several systems, although it is unknown whether this occurs via direct phosphorylation by cAMP-dependent protein kinase of Ras or a closely associated protein. 'Cross-talk' such as this among these various intracellular cascades emphasizes that the multitude of intracellular messengers are highly integrated to coordinate the response of a cell to a myriad of extracellular signals.

Rab is a family of small G proteins involved in membrane vesicle trafficking

Mammalian tissues contain around 70 forms of Rab, which specifically associate with the various types of membrane

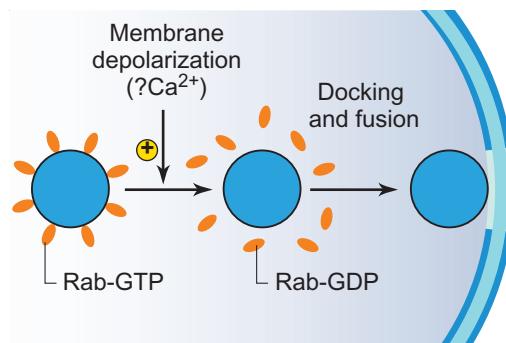


FIGURE 21-5 Schematic illustration of the proposed role of Rab in neurotransmitter release. Under basal conditions, GTP is bound to Rab3, which allows Rab3 to associate with other proteins on synaptic vesicles. This creates conditions unfavorable for the docking and fusion of vesicles at the nerve terminal plasma membrane. Membrane depolarization results in hydrolysis of the GTP bound to Rab, possibly via a Ca^{2+} -induced activation of Rab-GTPase activity. Rab then dissociates from the vesicle, creating a condition more favorable for docking and fusion. A large number of other synaptic vesicle proteins contribute to this process.

vesicles and organelles that exist in cells (Stenmark, 2009; Vanlandingham & Ceresa, 2008). Rab proteins, named originally as *ras*-related proteins in brain, are isoprenylated and associate with membranes, as do isoprenylated Ras and G protein γ subunits. However, unlike these other G proteins, the GTP and GDP binding to Rab appear to regulate its association with membrane compartments.

Subtypes of Rab, particularly Rab3, have been implicated in the regulation of exocytosis and neurotransmitter release at nerve terminals (see also Ch. 8 and 12) (Stenmark, 2009; Vanlandingham & Ceresa, 2008). One possible scheme by which this might occur is shown in Figure 21-5. In its GTP-bound form, Rab associates with synaptic vesicles and interacts with other membrane proteins to create a complex unfavorable for vesicle docking and perhaps fusion. Upon depolarization of the nerve terminal, a Rab GAP is activated, which results in dissociation of the GDP form of Rab from the vesicle membrane. This enables the synaptic vesicle to proceed with docking and fusion. The mechanism by which depolarization leads to activation of a Rab GAP and subsequently to release of Rab from the vesicle remains unknown, but Ca^{2+} is believed to be involved. To complicate matters further, several other proteins are involved in this Rab-synaptic vesicle cycle, including a Rab GDI, which regulates the ability of Rab to bind to synaptic vesicles, and rabphilins and RIMs (Rab-interacting molecules), among several synaptic vesicle proteins that can bind Rab.

OTHER FEATURES OF G PROTEINS

G proteins can be modified by ADP-ribosylation catalyzed by certain bacterial toxins

Among the tools that facilitated the discovery and characterization of G proteins were the bacterial toxins cholera and

pertussis, which were known to influence adenylyl cyclase activity. Subsequently, it was shown that the actions of these toxins are achieved by their ability to catalyze the addition of an ADP-ribose group donated from nicotinamide adenine dinucleotide (NAD) to specific amino acid residues in certain heterotrimeric G protein α subunits (Neer, 1995).

Cholera toxin catalyzes the ADP-ribosylation of a specific arginine residue in $G_{\alpha s}$ and $G_{\alpha t}$. This covalent modification inhibits the intrinsic GTPase activity of these α subunits and thereby ‘freezes’ them in their activated, or free, state (Fig. 21-1C). By this mechanism, cholera toxin stimulates adenylyl cyclase activity and photoreceptor transduction mechanisms. The ability of cholera toxin to ADP-ribosylate $G_{\alpha s}$ may require the presence of a distinct protein, ADP-ribosylation factor (ARF). ARF, which is itself a small G protein (Table 21-2), also is ADP-ribosylated by cholera toxin. ARF is implicated in controlling membrane vesicle trafficking (see Ch. 3).

In contrast, pertussis toxin catalyzes the ADP-ribosylation of a specific cysteine residue in $G_{\alpha i}$, $G_{\alpha o}$ and $G_{\alpha t}$ (Neer, 1995). Only α subunits bound to their $\beta\gamma$ subunits can undergo this modification. Pertussis toxin–mediated ADP-ribosylation inactivates these α subunits such that they cannot exchange GTP for GDP in response to receptor activation (Fig. 21-1B). By this mechanism, pertussis toxin blocks the ability of neurotransmitters to inhibit adenylyl cyclase or to influence the gating of K^+ and Ca^{2+} channels in target neurons. However, since $G_{\alpha z}$ is not a substrate for pertussis toxin, the toxin may not be able to block neurotransmitter–mediated inhibition of adenylyl cyclase in all cases. The G_q and G_{11-16} types of G protein α subunit are not known to undergo ADP-ribosylation.

A third type of bacterial toxin, diphtheria toxin, catalyzes the ADP-ribosylation of eukaryotic elongation factor (EFTU), a type of small G protein involved in protein synthesis (Table 21-2). The functional activity of the elongation factor is inhibited by this reaction. Finally, a botulinum toxin ADP-ribosylates and disrupts the function of the small G protein Rho, which appears to be involved in assembly and rearrangement of the actin cytoskeleton (Table 21-2). These toxins may be involved in neuropathy (see Ch. 38) and membrane trafficking (see Ch. 6).

G proteins are implicated in the pathophysiology and treatment of disease

Neurofibromatosis type 1 (NF1), a familial disorder characterized by multiple benign tumors of certain glial cells, is due to a mutation in the gene that codes for one form of GAP that regulates Ras (Dasgupta & Gutmann, 2003). The mutation in the NF1 GAP that leads to neurofibromatosis renders GAP unable to activate GTPase activity of Ras. This means that the GTP-bound form of Ras remains active for abnormally long periods of time, which leads to abnormal cellular growth.

Of course, the critical importance of Ras and most other small G proteins in cell growth and differentiation is highlighted by the consideration, stated above, that several forms of these proteins are proto-oncogenes (see also Ch. 27). This means that mutations in these proteins that result in

alterations in their regulatory properties can lead to oncogenesis. Ras in particular has been implicated in several human cancers. It has been estimated that as many as 30% of all human cancers contain mutations in one of the three Ras genes. While the frequency of Ras mutations in some types of human cancer is very low, its frequency in certain cancers, such as squamous cell carcinoma, lymphatic cancers and colorectal adenocarcinoma, is very high.

Mental retardation is a disorder that affects about 3% of the population. It is attributed to dysfunctions of more than 100 different genes with roles in synaptic function, intracellular trafficking or chromatin remodeling. Among them is the small GTPase Rab39B, a neuronal-specific, Golgi-localized protein, which is an essential element for neurite outgrowth and vesicular trafficking. Loss-of-function mutations of Rab39B were identified in X-linked mental retardation cases, whereas family members carrying Rab39 mutations were associated with intellectual disorders such as autism spectrum disorder, epileptic seizures, and macrocephaly. Further evidence of a role of small GTPases in cognition come from studies in *Drosophila* that indicate that Rac proteins are involved in memory processes (Shuai et al., 2010). Ablation of Rac does not affect acquisition of information but contributes to forgetting newly acquired memories. Another small G protein, Rho GTPase, is necessary for the initiation of sequential steps in long-term potentiation.

The RGS protein RGS4 is very abundant in the prefrontal cortex and other areas associated with cognitive function, including the hippocampus and the striatum. A large body of clinical and preclinical studies implicates RGS4 in schizophrenia. Microarray analysis of human cortical tissue reveals a decrease in RGS4 transcript in schizophrenic patients. Following this report, several studies point to RGS4 as a schizophrenia vulnerability factor (Levitt et al., 2006), whereas they show no role of this protein in antipsychotic drug actions (Chapter 58).

In addition to their involvement in specific disease states, concentrations of heterotrimeric G protein subunits, as well as of particular RGS protein subtypes, are altered in specific regions of the central nervous system in response to chronic exposure to many types of psychoactive drugs. Evidence has been presented to suggest that drug-induced alterations in complexes containing G proteins and RGS proteins may influence the signal transduction events that follow receptor activation and thereby they may affect the therapeutic or side effects of drugs currently used for the treatment of neuropsychiatric disorders (Traynor et al., 2009). Genetic animal models in which G proteins or RGS proteins are deleted or overexpressed reveal that mutations in some of these proteins lead to severe behavioral abnormalities and, importantly, may influence specific actions of psychotropic drugs. Examples include the opiate analgesic morphine, which potently modulates RGS9-2 levels in the brain. In fact, genetic ablation of RGS9-2 induces a 10-fold increase in sensitivity to morphine, suggesting that interventions in regulation of G protein-coupled receptor signaling by RGS proteins may improve treatment efficiency (Zachariou et al., 2003) (see also Ch. 61). These data further highlight the importance of G protein signaling in the brain in health and disease and open new paths for investigation in the years ahead.

SMALL G PROTEINS AND NEUROLOGIC DISEASE

George J. Siegel

Tuberous sclerosis and Rheb

This is a fascinating story of research that unfolds a biochemical process in regulating growth. Tuberous sclerosis is a multi-systemic tumor syndrome inherited as an autosomal dominant trait manifested by numerous benign tumors (hamartomas) that develop most often in the brain, kidneys, skin, heart and lungs. The most critical morbidity results from these tumors in the brain resulting in epilepsy, autism and mental retardation; angiomyolipomas in kidney; and lymphangioleiomyomatosis in lung. The cause is a mutation in either of the tuberous sclerosis complex genes, *TSC1* (OMIM #605284) or *TSC2* (OMIM #191092). These were first identified as tumor suppressor genes.

The gene products, hamartin from *TSC1* and tuberin from *TSC2*, associate to form what is thought to be a heterodimeric complex, TSC1-TSC2. TSC1 is required to stabilize TSC2 and prevent its ubiquitin-mediated degradation while TSC2 acts as a GTPase-activating protein (GAP). Missense mutation in the TSC2 GAP domain affects its GAP function and mutations in either TSC1 or TSC2 commonly destabilize the complex, thereby leading to TSC2 ubiquitination and degradation. The main function of the TSC1-TSC2 complex is the negative regulation of the mTORC1 (mammalian target of rapamycin complex 1), which itself promotes cell growth via several processes including activation of S6K (ribosomal S6 kinase) and inhibition of 4E-BP (a protein translation inhibitor) (Huang & Manning, 2008). TSC1-TSC2 complex overexpression inhibits mTORC1-mediated phosphorylation of S6K1 and 4E-BP1 while this phosphorylation is increased by the lack of TSC1-TSC2 complex.

How does TSC1-TSC2 control mTORC1 activation? Through the biochemistry and genetic studies of several groups, the molecular switch in this case was identified as the small G protein Rheb (Ras Homologue Enriched in Brain). The GAP domain of TSC2 in the TSC1-TSC2 complex stimulates the GTPase activity of Rheb converting Rheb-GTP to Rheb-GDP. Rheb-GTP leads to activation of mTORC1 so that the conversion to Rheb-GDP decreases the activation. Thus, in the absence of functional TSC1-TSC2 complex, both the ratios of Rheb-GTP to Rheb-GDP and of active to inactive mTORC1 remain high. However, the molecular mechanism by which Rheb-GTP controls mTORC1 activity is not yet clear.

Since Rheb-GTP-mTORC1 appears to be the main switching locus for stimulating tumor growth, potential therapies for tuberous sclerosis, and possibly other tumors, may ensue from discovery of inhibitors of mTORC1, such as rapamycin, or inhibitors of the Rheb-GTP activating effect on mTORC1. For the interested reader, there is an excellent, detailed review of the

biochemical and genetic work elucidating the biochemical pathways discussed above (Huang & Manning, 2008).

Heredity sensory-motor neuropathy (HMSN) and Rab

HMSN is a heterogeneous group of inherited peripheral neuropathies with length-dependent sensory, motor and autonomic nerve dysfunction in various combinations caused by mutations in a number of different genes (see Ch. 38). One such syndrome, HMSN2 (Charcot-Marie-Tooth type 2B), is characterized by dominantly inherited peripheral nerve axon degeneration without demyelination and with normal conduction velocities, in contrast to HMSN1 in which there is demyelination and slowed conduction.

HMSN2 produces sensory and autonomic neuropathy, which leads to ulcerations and mutilation. It is caused by mutations in the gene for Rab7 [OMIM #602298], a member of the Rab family of about 70 proteins in the Ras super family. Rab proteins regulate vesicular transport, maturation and fusion of vesicles with membranes. Activation and deactivation of the downstream effectors ensue from the cycling of Rab through its active GTP-bound form, hydrolysis of GTP by its GTPase function and to its inactive GDP-bound form. This Rab cycling is tightly regulated by other regulatory proteins so that its signaling is orchestrated with other signaling pathways. Rab regulation is involved in ensuring cargo unloading at correct locations and vesicle recycling at specialized target membranes, such as in presynaptic nerve terminals and in retrograde transport of neurotrophins (types of nerve growth factors). Rab7 is located in the late endosome and controls vesicular transport to late endosomes and lysosomes in the endocytic pathway (Vanlandingham & Ceresa, 2008; Zweifel et al., 2005).

Disease-associated mutations in Rab7 result in increased activation of the downstream signaling pathway, increased GTP binding to Rab, and dysregulation of GDP-GTP exchange on the protein with increased proportions of GTP- relative to GDP-bound Rab which is the probable cause of excessive and dysregulated activation. There is hydrolysis-independent inactivation, abnormal retention of Rab7 on target membranes, and dysregulated, hydrolysis-independent membrane cycling. The abnormal Rab7 function leads to spatiotemporal dysregulation of vesicle traffic and cycling (Cogli et al., 2009; McCray et al., 2010).

These genetic disturbances help elucidate the specific molecular reactions critical to axonal function and which may be dysfunctional in acquired as well as inherited neuropathies. These biochemicals may be targeted for pharmacologic therapy.

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