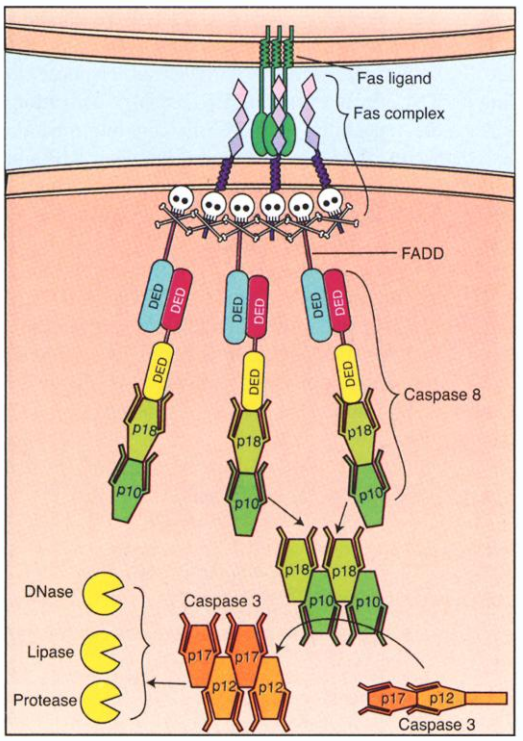

Fig. 1. Fas L-induced apoptosis occurs by autoproteolytical processing of caspase-8 (for details, see text).

in p53-induced apoptosis. Stimulation of Fas by membrane-bound FasL can be antagonized by the soluble decoy receptor DcR3, by various Fas isoforms lacking the transmembrane and/or death domains, and by soluble FasL generated by proteolytic processing or alternative splicing. The caspase-8–activating capacity of the Fas-DISC is mainly regulated by FADD-like interleukin-1β–converting enzyme (FLICE)–like inhibitory protein (FLIP) (*6*). FLIP exists in several isoforms that are structurally similar to caspase-8 although lacking in enzymatic activity (*6*). FLIP can be incorporated into the DISC of death receptors, thereby disabling DISC-mediated processing and release of active caspase-8 (*6*). In addition, Fas-mediated apoptosis is controlled by a plethora of regulators of the mitochondrial pathway of cell death, for example, by Bcl-2 family members, SMAC, or inhibitor of apoptosis proteins (*1*). Fas-mediated cell death occurs not only by apoptosis but also, depending on the cellular context, by necrosis. Fas-induced necrosis requires the adaptor protein FADD and the Fas-interacting serine/threonine kinase receptor-interacting protein (RIP), whereas caspase-8 seems to be dispensable (*7*). However, the molecular mechanisms linking Fas, FADD, and RIP to the execution processes of necrosis (for example, the production of reactive oxygen species) are not yet clear.

Although Fas is recognized predominantly as a death inducer, it also transduces proliferative signals in normal human diploid fibroblasts and T cells (*2*). The signaling pathways underlying Fas-induced proliferation might be partly related to the apoptotic pathway. In fact, stimulation of T cell growth by FasL can be blocked by caspase inhibitors. In addition, caspase-8, FADD, and FLIP are implicated in Fas-induced expression of the proto-oncogene c-*fos*, and mice deficient in these molecules have a defect in T cell proliferation. To what extent the Fas-mediated proliferation is related to activation of NF-κB, another non-apoptotic response elicited by Fas, remains to be clarified. A role of c-Jun NH_2-terminal kinase (JNK) activation in Fas-mediated proliferation seems rather unlikely, because FADD is normally not required for the activation of JNK. Rather, Fas promotes JNK activation through interaction with the Fas-binding protein DAXX and apoptosis signal–regulated kinase 1 (Ask1), a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family. Although JNK activation often correlates with Fas-mediated apoptosis, the pro-apoptotic effect of JNK activation (for example, up-regulation of FasL) normally does not directly contribute to Fas-dependent cell death. Some recent studies point to a role of JNK activation in Fas-mediated cardiac hypertrophy that occurs in response to the stress of pressure overload in the absence of apoptosis (*8*).

Since the cloning of Fas in 1991, tremendous progress has been made in the understanding of the molecular basis of apoptosis induction by this receptor. Nevertheless, many facets of Fas function are still poorly understood, in particular with respect to its non-apoptotic functions. Thus, it is easy to predict that, for now, the FasL-Fas system will remain as a death receptor paradigm; however, research in the years to come should shed more light onto other, non-apoptotic functions of these prominent members of the TNF/TNFR family.

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VIEWPOINT

G Protein Pathways

Susana R. Neves, Prahlad T. Ram, Ravi Iyengar*

The heterotrimeric guanine nucleotide–binding proteins (G proteins) are signal transducers that communicate signals from many hormones, neurotransmitters, chemokines, and autocrine and paracrine factors. The extracellular signals are received by members of a large superfamily of receptors with seven membrane-spanning regions that activate the G proteins, which route the signals to several distinct intracellular signaling pathways. These pathways interact with one another to form a network that regulates metabolic enzymes, ion channels, transporters, and other components of the cellular machinery controlling a broad range of cellular processes, including transcription, motility, contractility, and secretion. These cellular processes in turn regulate systemic functions such as embryonic development, gonadal development, learning and memory, and organismal homeostasis.

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are signal transducers, attached to the cell surface plasma membrane, that connect receptors to effectors and thus to intracellular signaling pathways (*1*). Receptors that couple to G proteins communicate signals from a large number of hormones, neurotransmitters, chemokines, and autocrine and paracrine factors. After the first four G proteins (G_s, G_t, G_i, and G_o) were identified by biochemical purification, a large number of G proteins and their subunits were identified by cDNA cloning (*2*). G proteins consist of three subunits, α, β, and γ. When signaling, they function in essence as dimers because the signal is communicated either by

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the Gα subunit or the Gβγ complex. In most cases, Gβγ subunits cannot be dissociated under nondenaturing conditions. Currently there are 20 known Gα, 6 Gβ, and 11 Gγ subunits.

On the basis of sequence similarity, the Gα subunits have been divided into four families and this classification has served to define both receptor and effector coupling, although there are always exceptions to the rule. In this Viewpoint and in the G protein Connections Maps in the Signal Transduction Knowledge Environment (STKE), we use the convention of naming the G proteins by the identity of their α-subunit [see $G\alpha_i$ Pathway, http://stke.sciencemag.org/cgi/cm/CMP_7430 (*3*); $G\alpha_s$ Pathway, http://stke.sciencemag.org/cgi/cm/CMP_6634 (*4*); $G\alpha_q$ Pathway, http://stke.sciencemag.org/cgi/cm/CMP_6680 (*5*); $G\alpha_{12}$ Pathway, http://stke.sciencemag.org/cgi/cm/CMP_8022 (*6*); and $G\alpha_{13}$, http://stke.sciencemag.org/cgi/cm/CMP_8809 (*7*)]. This approach defines both receptor specificity and, to a large extent, effector specificity, except when a signal is being transferred through the βγ subunits. The G_s and G_q families have very well defined effector pathways, the adenylyl cyclase and phospholipase C-β (PLC-β) pathways, respectively. The G_i and G_o families are more amorphous, and here the signal flows through both the Gα and Gβγ complexes. A number of new downstream effector pathways have been discovered for both the Gα ($G\alpha_o$, and $G\alpha_i$) and the Gβγ complexes. Perhaps the best understood of the G_i family pathways is the transducin pathway, which mediates detection of light in the eye.

Connectivity within the G_{12} and G_{13} pathways has been studied extensively. Although they do share some downstream signaling components, these pathways also exhibit selectivity. Although we present separate Connections Maps for $G\alpha_{12}$ (*6*) and $G\alpha_{13}$ (*7*), it is not entirely clear whether they always regulate distinct biological functions and are indeed distinct pathways.

These four broad G protein families transduce signals from a very large number of extracellular agents. The agents listed in Fig. 1 constitute a very small subset of the extracellular signals that can couple to the various G protein pathways. The extracellular signal is routed to specific G proteins through distinct types of receptors. For example, epinephrine's signal is transmitted through the β-adrenergic receptor coupled to G_s, the α_2-adrenergic receptor to G_i, and the α_1-adrenergic receptor to G_q and G_{11}.

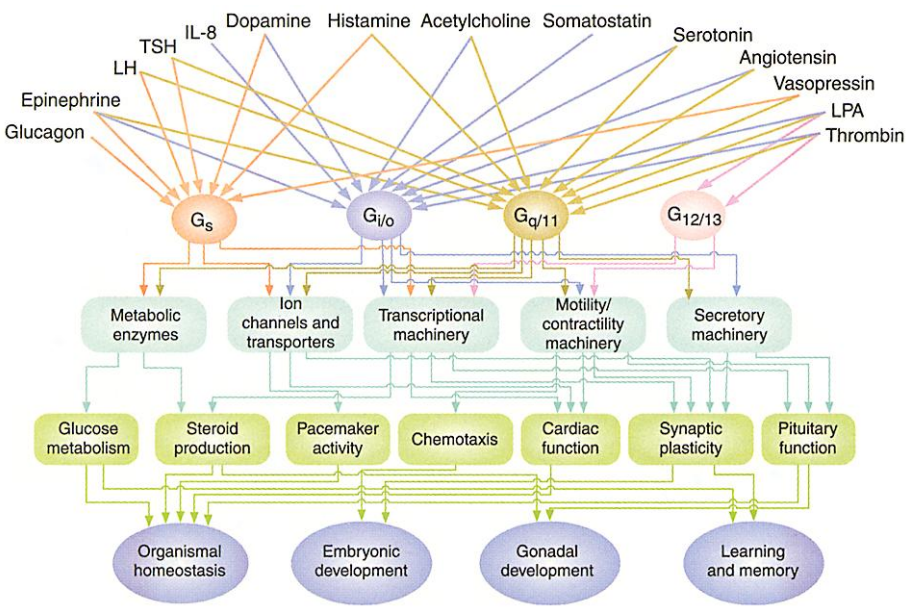

Fig. 1. Regulation of systemic functions by signaling through G protein pathways. A schematic representation of how signaling through G protein pathways can regulate systemic functions. Many extracellular agents, such as hormones (for example, glucagon, luteinizing hormone, and epinephrine), neurotransmitters (acetylcholine, dopamine, and seratonin), chemokines (IL-8), and local mediators (LPA), signal to the four main G protein families to regulate such cellular machinery as metabolic enzymes, ion channels, and transcriptional regulators. Modulation of the activities of the cellular machines in turn gives rise to altered cellular functions, such as changes in glucose metabolism in liver and muscle or altered activities of pacemaker cells in the heart. These cellular activities contribute to the regulation of large-scale systems such as organismal homeostasis and learning and memory. Thus, G protein pathways can propagate regulatory information through layers of increasing organizational complexity. At all levels, the examples shown here represent only a sample of extracellular agents that couple to the four G proteins, and the functions regulated by these pathways.

The G proteins, in turn, through signaling pathways described in more detail below, regulate important cellular components, such as metabolic enzymes, ion channels, and the transcriptional machinery. The resulting alterations in cellular behavior and function are manifested in many critical systemic functions, including embryonic development, learning and memory, and organismal homeostasis. This results in the propagation of regulated activities through increasingly complex layers of organization to serve as the basis of integration at the systemic level.

Although Fig. 1 depicts some of the rich knowledge of G protein regulation of important biological functions, it does not fully reveal the exquisite detail with which connectivity within the various G protein pathways is known. The remainder of this Viewpoint focuses on such connectivity. Over the years, approaches to the study of connectivity within the G protein pathways have changed. For the G_s and G_q pathways, connectivity was established by rigorous biochemical approaches. However, for many segments of the G_i, G_{12}, and G_{13} pathways, connectivity has been inferred from results of transfection experiments. In such cases, direct interactions must be established biochemically, and the presence of intermediate components cannot be ruled out. When considering these pathways, it might be assumed that reliable pairwise connectivity between components implies signal flow between the most distal parts of the pathway. This may not always be valid, and for many of the recently described connections, further experiments are needed to determine whether receptor activation does result in modulating the activity of the most distal effectors. The STKE Connections Maps contain both well-established and emerging connections and should be interpreted with this in mind. As new data are gathered, some of the newer connections may become well established, whereas others may have to be modified. Many excellent reviews and books have summarized G protein signaling (*8*–*14*). Here we focus on a few salient features of the pathways engaged by the four G protein families. Neither this review nor the Connections Maps are comprehensive. Connections that have not been widely verified or accepted are not shown. As more data are gathered, the Maps will be revised to reflect our new understanding.

G_s Pathway

The G_s pathway is the original cell signaling pathway to be described, and many key concepts, including that of second messengers (*15*), protein phosphorylation (*16*), and signal transducers (*17*, *18*), have come

from the study of this pathway. Most connections in this pathway have been established through biochemical experiments. Even after 40 years of study there are new details emerging for the G_s pathway. Recent discoveries include the identification of guanine-nucleotide exchange factors for the small guanosine triphosphatase (GTPase) Rap that are directly activated by the second messenger adenosine 3′,5′-monophosphate (cAMP) (*19*). This represents a mechanism by which G proteins regulate the activities of small GTPases.

Activation of Rap links G_s signals to activation of mitogen-activated protein kinase (MAPK) signaling modules. Other recent observations include the potential role of tyrosine kinase c-Src in the activation of Rap through cAMP-dependent protein kinase [protein kinase A (PKA)] (*20*) and a description of a putative GTPase-activating protein for $G\alpha_s$ (*21*) (Fig. 2).

G_i Pathway

This pathway was originally identified by the ability of $G\alpha_i$ to inhibit adenylyl cyclase. Many important hormones and neurotransmitters, including epinephrine, acetylcholine, dopamine, and serotonin, use the G_i and G_o pathway to evoke physiological responses. Signal flow through this pathway is inhibited by pertussis toxin, which adenosine diphosphate (ADP)–ribosylates the G protein α-subunit at its COOH-terminal region and thus prevents it from interacting with the receptor. In this pathway, both Gα and Gβγ subunits can communicate signals. Gβγ directly couples to at least four effector molecules, and indirectly to the small GTPase Ras, to activate MAPKs. The effectors directly regulated by Gβγ include PLC-β, K^+ channels, adenylyl cyclase, and phosphatidylinositol 3-kinase (PI3K). Although each of these effectors exists as multiple isoforms, only specific isoforms are regulated by Gβγ. Key physiological functions, such as muscarinic cholinergic regulation of pacemaker activity in the heart, occur through the coupling of M2-muscarinic receptors to G_i to release a Gβγ subunit that activates K^+ channels. $G\alpha_i$ and $G\alpha_o$ can regulate signals from c-Src to signal transducer and activator of transcription 3 (STAT3) and to the Rap pathways, as well as inhibit adenylyl cyclase. The well-studied inhibition of adenylyl cyclase may be physiologically relevant, especially in inhibiting the effects of cAMP to modulate secretion. However, the physiological consequences of $G\alpha_i$ and $G\alpha_o$ regulation of c-Src-STAT3 and Rap pathways remain to be established. Many connections in the $G\alpha_i$ and $G\alpha_o$ pathway have been established by biochemical experiments, although the newer pathways have been studied in transfected cells. It is currently not known how $G\alpha_i$ or $G\alpha_o$ activates c-Src, but some studies indicate possible direct interactions between Gα subunits and tyrosine kinases.

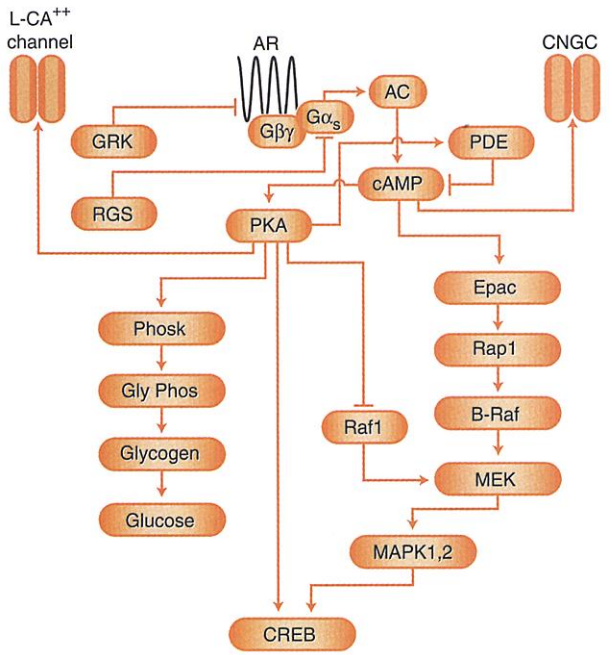

Fig. 2. The canonical Gs signaling pathway. This schematic diagram demonstrates how the cAMP pathway connects to multiple cellular machines, including ion channels, transcription factors, and metabolic enzymes. AC, adenylyl cyclase; PKA, protein kinase A; PDE, phosphodiesterase; L-Ca^{++} channel, L-type Ca^{2+} channel; CNGC, cyclic nucleotide–gated channel; PhosK, phosphorylase kinase; GlyPhos, glycogen phosphorylase; CREB, cAMP response element–binding protein; EPAC, the cAMP- and AMP-regulated exchange factor for Rap1; Rap1, a small GTPase; MAPK, mitogen-activated protein kinase; Raf1 and B-Raf, MAP kinase kinase kinases; MEK, MAPK/ERK kinase; MEKK, MAPK/ERK kinase kinase; GRK, G protein receptor kinase; RGS, regulators of G protein signaling; βAR, β-adrenergic receptor.

G_q Pathway

The G_q pathway is the classical pathway that is activated by calcium-mobilizing hormones and stimulates PLC-β to produce the intracellular messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 triggers the release of calcium from intracellular stores, and DAG recruits protein kinase C (PKC) to the membrane and activates it. These connections have been well established biochemically. In many cell types, the release of intracellular calcium activates the store-operated calcium channels at the cell surface to allow the inflow of extracellular calcium. $G\alpha_q$, working through PKC and possibly directly, also appears to regulate various isoforms of phospholipase D (*22*). $G\alpha_q$ is reported to activate the transcription factor NF-κB through PYK2 (*23*).

G_{12} and G_{13} Pathways

The $G\alpha_{12}$ and $G\alpha_{13}$ proteins were discovered through sequence similarity to known Gα proteins, and most of the experiments done to date have been in transfected cells. In many cases, direct interactions with effectors are not yet fully established. Which receptors endogenously couple through $G\alpha_{12}$ and $G\alpha_{13}$ pathways is still unclear. Although from sequence similarity it appears that $G\alpha_{12}$ and $G\alpha_{13}$ belong to the same family, they may produce different signaling outputs but generate a subset of overlapping effects.

$G\alpha_{12}$ has been reported to directly interact with a GTPase-activating protein for Ras, RasGAP, and Bruton's tyrosine kinase (Btk) (*24*). These observations require confirmation and extension to establish the cellular consequences in native systems of these direct interactions. $G\alpha_{12}$ is thought to stimulate phospholipase D, c-Src, and PKC by as-yet unidentified mechanisms. The endpoint physiological responses of these pathways are not yet fully understood. In many cases it appears that different members of the MAPK family, such as extracellular signal-regulated kinase 5 (ERK5) or c-Jun NH_2-terminal kinase (JNK), are activated. This activation should lead to regulation of gene expression. In fact, $G\alpha_{12}$ was identified as an oncogene in a functional screening assay (*25*) and hence effects on gene expression patterns are to be expected.

Two receptors that couple to $G\alpha_{13}$ in the native setting are the lysophosphatidic acid (LPA) receptor and the thromboxane A2 receptor. $G\alpha_{13}$ directly interacts with and activates a guanine nucleotide exchange factor for the GTPase Rho, p115RhoGEF, and thus activates Rho, leading to a variety of effects that include regulation of the Na^+-H^+ exchanger. Through the activation of PYK2, $G\alpha_{13}$ may engage the PI3K pathway to activate the protein kinase Akt and regulate NF-κB (*23*). How $G\alpha_{13}$ activates PYK2 is currently not understood.

Perspectives

A map of the Gs pathway is shown in Figure 2 and a more comprehensive fam-

ily portrait of the heterotrimeric G protein pathways is shown on the STKE Web site (3). Although the composite map appears quite complex, this is a first-level representation where the multiple isoforms of the different components are not shown. Since these maps are canonical representations, not all of these pathways and connections would be present in every cell type. As cell type–specific Connections Maps are constructed, it will be interesting to compare those with the canonical maps to determine which pathways occur in which cell type. The Gs pathway in Fig. 2 illustrates several general patterns that emerge from this complex picture. First, all G proteins engage multiple signaling pathways and consequently different cellular machines. This often helps produce effects with distinct rates of activation and duration of response. In neurons, cAMP can act through PKA to produce short-term effects on channel functions, and through Rap and MAPK to regulate gene expression and produce long-term effects through regulation of the transcriptional machinery. Second, it appears that all G proteins regulate the activity of GTPases such as Rap and Rho. Third, all G protein pathways either stimulate or inhibit one or more of the MAPK signaling pathways. All of these interconnections result in a complex and likely robust network in which signals from G protein–coupled receptors can be fully integrated with signals from other receptors.

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VIEWPOINT

Control of T Cell Function by Positive and Negative Regulators

Andrew L. Singer and Gary A. Koretzky*

T cells are an essential element of the body's immune system. Engagement of the T cell receptor is responsible for initiating the signaling events that can activate, inactivate, or eliminate T cells, depending on the magnitude and duration of the signal. Control of T cell signaling occurs through both positive and negative regulation, as well as through the actions of molecular scaffolds that contribute to the formation of signaling complexes. The T Cell Signal Transduction Pathway at the STKE Connections Maps highlights the molecular components that are responsible for T cell activation. Understanding the mechanisms that regulate T cell responsiveness will aid in the development of therapeutic agents to treat infection, cancer, and autoimmune disease and immune deficiency.

T cells play critical roles in the body's defense against pathogenic challenges and its ability to recognize and eliminate cells that have undergone malignant transformation. These abilities require the T cell to discriminate between "self" and "nonself," whether in the form of foreign antigens or inappropriate expression of endogenous proteins bycancerous tissues. The T cell antigen receptor (TCR) is responsible for making this distinction. One component of the TCR is a disulfide-linked dimer (α/β) whose proteins arise from gene segment rearrangements that provide the opportunity for virtually unlimited diversity. The α and β chains associate on the cell surface with the invariant CD3 complex, which transduces signals into the cell after α/β engagement by antigen (*1*). As T cells mature in the thymus, cells expressing TCRs that fail to interact with major histocompatibility proteins and thus cannot respond to foreign antigen, and cells expressing TCRs prone to interact with normal self antigens, are eliminated through a selection process that interprets the magnitude and duration of TCR signaling. Those cells that pass selection emigrate from the thymus, where they again rely on their TCRs to detect ligand engagement and to provoke an effector response. Studies over the past few years have provided a wealth of new information regarding the molecular events that occur after ligation of the TCR and ultimately result in biological effects.

As with all complex biological systems, each step of the signaling pathway initiated by TCR engagement (*2*) is subject to both positive and negative regulation (Fig. 1). For example, one of the first biochemical consequences of TCR binding is activation of Lck (*3*), a Src-family protein tyrosine kinase (PTK). Lck itself is regulated positively and negatively by other enzymes. Positive regulation is accomplished through the cell-surface CD45 protein tyrosine phosphatase, which is required to dephosphorylate a COOH-terminal tyrosine that negatively regulates Lck function (*4*).

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