GEFs: master regulators of G-protein activation

What is a GEF?
GEF stands for guanine-nucleotide exchange factor, any of a large and diverse group of proteins that catalyze the release of GDP and the subsequent uptake of GTP by members of the Ras and heterotrimeric G-protein superfamilies (referred to here collectively as G proteins). When bound to GTP, G proteins are in the active conformation. Upon hydrolysis of this bound GTP, by virtue of their own intrinsic GTPase activities, G proteins are inactivated. GDP release is the rate-limiting step for the GTPase reaction in vitro; in vivo, this kinetic barrier is reduced by GEFs. Certain GEFs have also been called guanine-nucleotide release factors (GRFs) or guanine-nucleotide dissociation factors (GDFs). GEFs are not to be confused with guanine-nucleotide dissociation inhibitors (GDIs), which trap G proteins in an inactive GDP-bound state, nor with GTPase activating proteins (GAPs), which accelerate the rate of GTP hydrolysis (i.e. augment the intrinsic GTPase activity of the G protein) and thereby terminate G-protein signaling. GEFs constitute a highly diverse group of proteins in the cell. All GEFs contain one or more G-protein interaction domain that manifests exchange activity; most also contain other protein-interaction and regulatory domains.

Why are GEFs important?
By catalyzing the release of GDP from, and the subsequent uptake of GTP by, G proteins, GEFs enable G proteins to activate downstream effectors such as kinases, adenylate cyclases and ion channels. GEFs serve as the initial point of control for most G-protein-regulated pathways. The array of cellular activities regulated by GEFs reflects the functional diversity of G proteins themselves; hence, various GEFs are involved in transcriptional regulation (by modulating the activity of Ras, Rap and Ral G proteins), cytoskeletal rearrangement and motility (via Rho, Rac and Cdc42), vesicle trafficking (via Rab and ARF) and nuclear import (via Ran). A survey using the LocusLink utility of the NCBI (National Center for Biotechnology Information) server reveals the existence of nearly 80 genes encoding small protein-directed GEF homology domains in the human genome. Heptahelical G-protein-coupled receptors (GPCRs) constitute an enormous family of ligand-stimulated GEFs that activate heterotrimeric G proteins. In addition, exchange factors IEF1B and EFTu act upon IEF1A and EFTu, respectively, to ensure the fidelity of the initiation and translocation steps of ribosomal protein synthesis. GEFs that target signaling G proteins respond to extra- or intracellular signals, which can induce translocation of the GEFs to specific membrane compartments in the cell where they then activate G proteins. Certain GEFs also mediate crosstalk between different receptor-mediated signaling pathways, thereby acting as signal integrators.

Are there many varieties of GEF?
Members of each major subdivision of the Ras G-protein superfamily are recognized by one or more homologs of a distinct and corresponding family of GEFs. For example, the Rho–Cdc42–Rac-family G proteins are substrates of GEFs that contain tandem Dbl homology (DH) and pleckstrin homology (PH) domains. GEFs that act upon Ras, Ral and Rap contain domains homologous to that in the cdc25 protein of Saccharomyces cerevisiae. A separate class of sec7-type GEFs couple with the ARF-family of G proteins that are involved in vesicular transport. GEFs with similar guanine-nucleotide exchange domains can act on different G proteins of the same class, contain different sets of regulatory and localization domains, or exhibit different patterns of tissue-specific expression. For example, Vav and p115rhoGEF both contain DH and/or PH domains, but they also contain distinct regulatory and localization elements.

Have GEF domains diverged from a common ancestor?
Even though all regulatory G proteins appear to belong to a single, ancient family of proteins, GEF domains do not; the different classes of GEFs appear to have arisen independently. The structures of GEFs belonging to the sec7, DH, cdc25, RCC1 (acts on Ran) and EFTu families have been determined, and each has been shown to adopt a different three-dimensional fold (Fig. 1). The GPCR GEFs constitute a distinct family of integral membrane proteins.

How do they work?
Although the specific molecular mechanisms differ, GEFs use a common approach to release nucleotide. G proteins share characteristic and well-conserved catalytic sites, composed of a P-loop that enforces the nucleotide phosphates, together with a P-loop that enforces the nucleotide phosphates, together with two mobile switch segments involved in catalysis and effector recognition. The very strong binding of guanine di- and trinucleotides to G proteins arises, in part,

Fig. 1. A gallery of GEFs, with Protein Data Bank (http://www.rcsb.org/pdb/) accession numbers in parentheses. (a) Elongation factor Ts from Thermus thermophilus (1IAP); (b) Ras-cdc25 domain of Human SOS1 (1BKD); (c) DH–PH domain from Human SOS1 (1DBH); (d) Human Ran-GEF RCC1 (1A12); (e) Bovine rhodopsin (1F88).

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from a network of interactions between the nucleotide phosphates and the P-loop, and between a magnesium cation and one of the switches. The four structures of GEF–G-protein complexes that have been determined are consistent with a scheme in which the GEF disrupts the magnesium ion binding site in the G protein, with a concomitant rearrangement of one or both switch segments. Some GEFs also disrupt the γ phosphate binding site and the P-loop (Fig. 2). By contrast, the guanine binding site is maintained, allowing the formation of a metastable ternary complex. GDP diffuses from the active site, possibly following an isomerization step, leaving an ‘open’ binary complex that is capable of accepting nucleotide. Because the intracellular concentration of GTP is approximately tenfold greater than that of GDP, an active, GTP-bound G protein is the usual product of an encounter between a G protein and its GEF. It is interesting to note that, for ARF1, interaction with the membrane is essential for the sec7-catalyzed nucleotide exchange mechanism.

How are GEFs regulated?

GEFs are the recipients of the regulatory signals that trigger G-protein-coupled pathways. Regulation of GEFs might involve their specific localization, as well as direct activation by protein–protein interactions. Information gleaned from studies of Ras-directed GEFs is instructive for our understanding of these mechanisms. There are several distinct families of Ras-GEF and, although all members of these families contain cdc25 domains, each is responsive to different sets of cellular inputs. SOS, for example, contains an SH3 domain, facilitating the localization of this GEF to the plasma membrane (where it activates Ras) upon receptor tyrosine kinase stimulation. Members of the CalDEG-GEF family of Ras GEFs possess EF-hand calcium binding domains as well as diacylglycerol-binding domains similar to those found in protein kinase C. These GEFs are, therefore, potentially responsive to phospholipase C activation. Members of the Rap-directed family of GEFs are regulated by cAMP and are thus subject to regulation through G-protein activation of adenylyl cyclase. Indeed, several examples of G-protein-regulated GEFs have emerged. For example, both Rap-GDF, which contains a cdc25 domain, and RIN1 (B. Horazdovsky, pers. commun.), a Vps9 domain-containing GEF for Rab5, are effectors of Ras. Such mechanisms allow a signal to diverge into multiple pathways. Signaling networks also involve indirect connections. For example, Rho-family GEFs Vav and Tiam have PDZ domains directly C-terminal to their GEF domains. These phosphoinositol-binding domains might not only function as membrane localization modules, but might also potentiate the GEF activity of the neighboring DH domain. In any case, PH domain-containing GEFs are potentially responsive to Ras-activated phosphoinositol 3-kinase. Novel members of the Rho family-directed GEFs have been identified that are regulated by heterotrimeric G proteins. The best characterized of these, p115rhoGEF, is activated by G13ct upon stimulation of lysophosphatidic acid receptors. Remarkably, p115rhoGEF contains an RGS (regulator of G-protein signaling) domain that stimulates the GTPase activity of, and thereby inactivates, its own stimulator, G13ct.

Where are the big challenges in GEF research?

Much remains to be understood about the molecular mechanisms of GEF action. For example, it is still a mystery how GPCRs work. G-protein α subunits do not, in general, require a magnesium ion as a cofactor for GDP binding. Therefore, we can expect fundamental differences in the way GPCRs promote nucleotide exchange compared with other GEFs. Nor is it understood how or, in some cases, to what extent, GEFs are stimulated by their own domains (e.g. cAMP-binding and PH) or other G proteins. Perhaps the most intriguing and far-reaching challenge is to understand the complex and highly-coupled network of signaling events in which GEF’s participate. The elaborate suites of regulatory modules with which many GEFs are adorned suggest that G-protein activation is localized, contingent and temporally defined.

References

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