The Cellular Functions of Small GTP-Binding Proteins

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A substantial number of novel guanine nucleotide binding regulatory proteins have been identified over the last few years but the function of many of them is largely unknown. This article will discuss a particular family of these proteins, structurally related to the Ras oncoprotein. Approximately 30 Ras-related small guanosine triphosphate (GTP)–binding proteins are known, and from yeast to man they appear to be involved in controlling a diverse set of essential cellular functions including growth, differentiation, cytoskeletal organization, and intracellular vesicle transport and secretion.

GUANINE NUCLEOTIDE BINDING PROTEINS FULFILL A wide range of regulatory functions in all organisms. However, they all share at least one mechanistic feature: these proteins can exist in two interconvertible conformational states—one inactive [guanosine diphosphate (GDP)–bound] and one active (GTP-bound) (Fig. 1). Superimposed on this central concept, evolution has modified and expanded the range of input and output signals to produce a diverse set of regulatory pathways.

The best understood of the guanine nucleotide binding proteins are those controlling peptide elongation in *Escherichia coli* (elongation factor, EF-Tu) and signal transduction across the plasma membrane in mammalian cells (G proteins), two very different tasks. EF-Tu is maintained in its active GTP form in growing cells by an exchange factor (input in Fig. 1) and, once activated, binds an aminoacylated transfer RNA (tRNA) and translocates to the ribosome. Stimulation by the ribosome of an otherwise slow intrinsic guanosine triphosphatase (GTPase) activity of EF-Tu rapidly generates the inactive GDP form and EF-Tu dissociates, leaving the aminoacylated tRNA behind (output). This simple regulatory protein, therefore, controls the unidirectional translocation of a single substrate (aminoacylated tRNA) to a specific location (ribosome) (*1*). The α subunit of the heterotrimeric (αβγ) G protein G_s, on the other hand, exchanges GDP for GTP only in response to a specific agonist-stimulated transmembrane receptor (input). The active GTP form of α_s then binds to and activates adenylate cyclase, leading to the generation of several thousand adenosine 3′,5′-monophosphate (cAMP) molecules (output) before the intrinsic GTPase activity of α_s inactivates itself. As a result, G proteins control the conversion of a signal transduced across the plasma membrane into an amplified intracellular messenger (*2*). This mechanism has been exploited in mammalian cells with many different G proteins responding to different input signals (receptors) and affecting a variety of intracellular signaling systems (*2*).

A large distinct group of guanine nucleotide binding proteins has emerged somewhat unexpectedly out of oncogene research. The *ras* genes were first highlighted in the early eighties as sites of somatic mutation in human cancers, and since then a great deal of effort has been put into understanding their function (*3, 4*). These genes encode a novel class of regulatory GTP-binding protein involved in the control of cell proliferation. A number of proteins have now been identified, either biochemically or through analysis of recombinant cDNA and genomic clones that have sequence homology to Ras; in fact, to date about 30 Ras-related proteins are known and the potential range of processes regulated by this family of proteins is just beginning to be appreciated (*5–7*). Although the well-characterized G proteins and EF-Tu have been used as paradigms for how Ras-related proteins might work, genetic and biochemical analyses suggest that the small GTP-binding proteins have many novel features. In this article, I will review our current understanding of their regulatory function.

Ras: A Ubiquitous GTP-Binding Protein

The three Ras proteins (H-Ras, Ki-Ras, and N-Ras) expressed in most, if not all, mammalian cell types are remarkably similar in sequence to the single Ras protein of *Schizosaccharomyces pombe* and the first 160 amino acids of the two somewhat larger Ras proteins of *Saccharomyces cerevisiae* (60% amino acid identity). This high degree of evolutionary conservation argues that Ras proteins fulfill basic essential cellular functions (*3, 6*). The structural properties of Ras indicate that they are regulatory GTP-binding proteins (*3*). For example, recombinant mammalian Ras protein obtained from *E. coli* expression systems contains 1 mol of tightly bound GDP, and the three-dimensional structure has many features in common with EF-Tu (*8*). Furthermore, the basic biochemical features of Ras are consistent with those in Fig. 1: bound nucleotide can slowly exchange spontaneously with added GTP or GDP (not other nucleotides), and the protein has a low but measurable intrinsic GTPase activity (*9, 10*). This GTPase activity is stimulated dramatically in vivo by a GTPase-activating protein (GAP) present in all mammalian cells and probably also in yeast (*11*).

Post-translational modifications. Ras proteins require correct localization to the plasma membrane for functional activity. Mutations at the cysteine of the COOH-terminal CAAX box generate an inactive cytoplasmic protein by blocking a complex series of post-translational modifications that includes removal of the three terminal amino acids and polyisoprenylation of the new COOH-terminal cysteine (*12*). The CAAX box is a general signal for addition of the 15-carbon farnesyl lipid moiety, which increases the hydrophobicity of

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the protein, but a further signal is required for targeting to specific membranes (*13*). Localization of N-Ras and H-Ras to the plasma membrane requires addition of palmitic acid at another cysteine residue (181 or 184), whereas in cellular Ki-Ras, which does not contain an upstream cysteine and is not palmitoylated, a region rich in basic amino acids (residues 175–182) seems to provide this additional signal.

Mutational analysis of Ras. Researchers have subjected Ras to extensive mutational analysis since the discovery that amino acid substitutions (at codons 12, 13, or 61) are frequently found in human cancers (*3, 4*). These activating or oncogenic mutations block the ability of GAP to stimulate the GTPase activity of Ras (although they do not affect GAP binding to Ras), with the result that oncogenic Ras proteins are constitutively in the active GTP-bound conformation and give an uncontrolled output signal (*11*). Since this can result in abnormal proliferation of cells, it seems likely that the normal function of Ras, in some cells at least, is to control growth. Further mutational studies have localized the region of Ras that interacts with its downstream target to produce this effect (the effector domain) to between amino acids 32 and 40 and, consistent with this designation, the three-dimensional structure reveals an exposed stretch of residues between amino acids 25 and 45 (*8, 14*). A site of interaction of the GAP protein with Ras has also been mapped and coincides with the effector binding site (*15*). Although GAP can down-regulate Ras · GTP, an additional possibility is that GAP is also the downstream target for regulation by Ras.

Cellular Function of Yeast Ras

RAS in Saccharomyces cerevisiae. Genetic analysis has provided important insights into the function of the ras proteins in this organism. Deletion of both *RAS1* and *RAS2* genes, for example, is lethal, but cells can be rescued if the gene (*BCY1*) encoding the regulatory subunit of the cAMP-dependent protein kinase is disrupted (*16*). Furthermore, adenylate cyclase activity in membranes isolated from this RAS-deficient strain can be stimulated in vitro by addition of recombinant Ras protein (yeast or mammalian), and there is no doubt that *S. cerevisiae* RAS proteins regulate adenylate cyclase activity and cAMP concentrations (*17*). This in vitro assay of adenylate cyclase has confirmed that only the GTP form of RAS is active and that RAS can be constitutively activated by either guanosine-5′-*O*-(3-thiotriphosphate) (GTP-γ-S, a nonhydrolyzable GTP analog) or by mutation (for example Val^{19}, which is equivalent to an oncogenic Val^{12} mutation in mammalian Ras) (*17*).

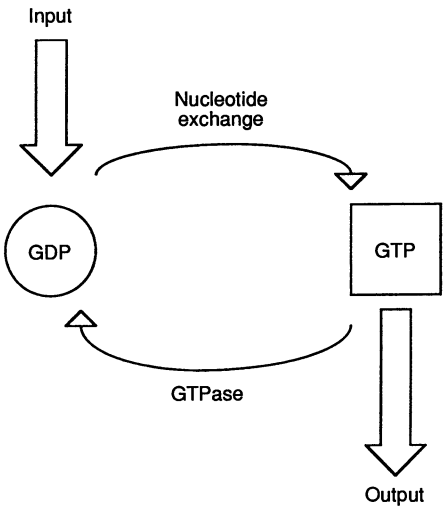

Fig. 1. The basic features of regulatory GTP-binding proteins. An active GTP-bound conformation is generated from an inactive GDP-bound form by nucleotide exchange and an intrinsic GTPase activity of the protein determines the half-life of the active state. A variety of upstream (input signal) and downstream (output signal) proteins interact with the system to affect the exchange rate and the GTP hydrolysis rate and to give the net downstream effect.

The growth and cycling of yeast cells is controlled by the concentration of cAMP, which in turn appears to be regulated by nutritional factors (*18*). Starvation results in a decrease in cAMP, exit from the cell cycle, and, in the case of diploid cells, sporulation. Consistent with the idea that RAS links nutritional factors to cAMP concentrations, cells with an activating *RAS2* mutation (Val^{19}) maintain high amounts of cAMP when deprived of nutrients and consequently do not sporulate (*16*). A candidate nutritional-sensing gene, *CDC25*, identified genetically, is thought to encode a protein that regulates the amount of GTP on RAS, perhaps by controlling GTP ⇄ GDP exchange, but how this protein is itself controlled is not known (*18, 19*).

This apparently simple picture of nutritional regulation of *CDC25* leading to activation of RAS and to an increase in cAMP concentrations is actually far more complex. There is still some doubt, for example, whether RAS activates adenylate cyclase directly or through an intermediary cyclase-associated protein (CAP), and there is strong genetic evidence that RAS controls another additional signaling pathway distinct from adenylate cyclase but essential for cell viability (*20*). Other proteins affecting the RAS pathway have been identified and disruption of either the *IRA1* or *IRA2* genes, for example, gives a phenotype indistinguishable from a Val^{19} mutation in RAS (*21*). The proteins encoded by *IRA1* and *IRA2* both have homology to mammalian GAP and strains deleted in *IRA1* can be rescued by introducing the GAP gene (*21*). These findings suggest that *IRA1* and *IRA2* encode GAPs involved in the down-regulation of RAS, but they may have additional activities. Another gene product, *MST1*, can also down-regulate the RAS-cAMP pathway and, although there is no evidence for a direct interaction with RAS, *MST1* has some sequence homology to the β subunit of mammalian G proteins (*22*).

RAS in Schizosaccharomyces pombe. The role of RAS1 in *S. pombe* is less well understood. Deletion of the gene blocks conjugation and inhibits sporulation but has no effect on growth or cAMP concentrations, whereas an activating mutation in *RAS1* (Val^{17}) has no effect on growth or sporulation but blocks conjugation (*23*). Further genetic analysis has revealed a possible upstream regulator, STE6, which has some sequence homology to *CDC25* and *SCD25*,

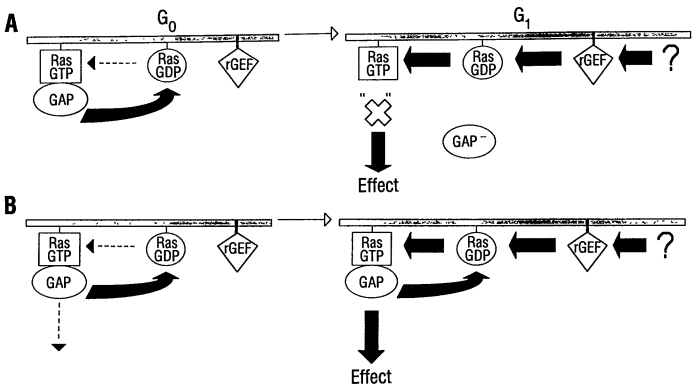

Fig. 2. Alternative models for the role of GAP in the mammalian Ras pathway. In both models the exchange factor (rGEF) can be regarded as a growth sensor, but its mechanism of control is not known. (**A**) GAP as a down-regulator. In quiescent cells (starved of growth factors or nutrients, or both) GAP down-regulates any Ras · GTP generated by spontaneous exchange. Stimulation of cells to enter G_1 occurs by inactivation of GAP allowing Ras · GTP to interact with its target (X). (**B**) GAP as the target. In quiescent cells the rate of formation of Ras · GTP is low and the rate of formation of a Ras · GTP-GAP complex is below a threshold. Stimulation of cells to enter G_1 results in catalyzed nucleotide exchange to give a dramatic increase in the rate of formation of Ras · GTP, and therefore of the Ras · GTP-GAP complex. It is the rapid flux through this cycle that allows Ras · GTP-GAP to produce a proliferative signal.

and a downstream target, BYRI (a protein kinase of unknown specificity) (*24*). *Schizosaccharomyces pombe* conjugates in response to a combination of mating factors and nutrient deprivation. It is thought that RAS might couple nutrient concentrations to a signal transduction pathway that synergizes with mating factor–induced signals to promote conjugation. The biochemical nature of these signals, however, is not known.

Cellular Function of Mammalian Ras

Genetic analysis of mammalian *ras* is not yet possible and biochemical approaches have been used to analyze its function. Although the *ras* genes cannot be deleted, the proteins can be neutralized and microinjection of antibodies to Ras into quiescent fibroblasts blocks stimulation by growth factors (*25*). Injection of antibodies at different times reveals that Ras is essential during the first 8 hours of G_1 of the cell cycle, but after this time cells are committed to complete G_1, enter S phase, and complete a cycle. Furthermore, antibodies block the action of a wide variety of growth factors, suggesting that Ras has a general role in growth control and is not linked to a specific receptor (*26*).

Downstream effects of Ras. Injection of recombinant oncogenic Ras protein into quiescent fibroblasts induces progression through G_1 and much effort has gone into defining the biochemical signals involved (*27*). No change in cAMP concentrations can be observed, and most research has been focused on the inositol phosphate signaling pathway (*28*). Activation of a phospholipase C by many growth factors can lead to the production of two second messengers, inositol triphosphate (IP_3) and 1,2 diacylglycerol (DAG), from a phosphoinositide lipid precursor. Furthermore, DAG is an activator of protein kinase C (PKC) and many groups have reported high PKC activity in *ras*-transformed cells (*29*). However, when phosphoinositide turnover is more closely examined, no convincing evidence for its direct stimulation by Ras can be found (*30*). Thus, although it has been shown that PKC activation is an essential step for the induction of DNA synthesis by oncogenic Ras, the mechanism of activation is unknown (*31, 32*). PKC is not required for induction of *myc* transcription or of morphological transformation by *ras*, suggesting that multiple pathways are activated as in *S. cerevisiae* (*31*).

Ras is also expressed in differentiated cells where it is likely to fulfill a more specialized role. Microinjection of Ras (Val^{12}) into Schwann cells, for example, stops growth and alters the differentiated phenotype (*33*). There is no reason to suppose that the biochemical nature of the Ras-induced signals is any different; instead mammalian cells may be programmed to interpret them differently.

So far, little progress in identifying the Ras signaling pathway has been made by analyzing changes in second messengers, and it is still an open question whether Ras controls the classical growth factor responses such as phospholipid turnover and protein kinase activation or whether it generates a novel signal. The discovery of GAP and the possibility that it might be a target for regulation has provided a more direct approach to examine Ras function (*11, 15*). GAP is a 120-kD cytoplasmic protein that has been cloned, although its sequence reveals few clues as to its function (*34*). GAP can associate with the platelet-derived growth factor (PDGF) receptor, and after stimulation of cells with a variety of growth factors or oncogene products GAP is phosphorylated on tyrosine (*35*). Although the function of GAP phosphorylation is unclear, this result provides a basis for the missing link between growth factors and Ras.

Two possible models for the role of GAP are presented in Fig. 2. In one model GAP serves only to down-regulate Ras · GTP and, for Ras to interact with its target (unknown), GAP must first be inactivated (Fig. 2A). It is possible that association with receptors or phosphorylation could inhibit GAP; alternatively, it has been suggested that phospholipid breakdown products can inactivate GAP (*36*). Although this model cannot be ruled out, in growing cells the steady-state concentrations of Ras in the GTP-bound state appear to be very low, and there is no evidence that GAP activity is significantly reduced compared to quiescent cells (*36, 37*). This is in contrast to oncogenically activated Ras, which is largely in the GTP-bound form in vivo. In the second model GAP is the target for regulation by Ras and translocates to the plasma membrane to interact with Ras · GTP and generate an effect (unknown) (Fig. 2B). The lifetime of the Ras · GTP-GAP complex would be expected to be short (explaining the low amounts of Ras · GTP in cells), but catalyzed nucleotide exchange on Ras would ensure its rapid and continuous formation. This second model has some similarities to EF-Tu function where interaction with its target (the ribosome) also stimulates GTPase activity. Unlike EF-Tu, however, which must continually cycle through a GDP form to perform its translocation function, Ras resembles G proteins in being constitutively activated by inhibition of GTPase activity. Until more biochemical details are available and some of the uncertainties resolved, models for GAP action will remain speculative.

Upstream regulation of Ras. Another important step towards understanding Ras function has been the identification of two apparently distinct proteins (rGEF and GRF) that can catalyze nucleotide exchange on Ras. rGEF is localized on the plasma membrane, whereas GRF is cytoplasmic, but it is not yet known how their activity may be controlled (*38*). A cytoplasmic protein (Rho GDI) that inhibits the dissociation of GDP from the Ras-related protein Rho has been reported, raising the possibility that more upstream proteins regulating Ras may emerge (*39*).

It has generally been assumed that growth factors somehow regulate nucleotide exchange on Ras. For example, growth factors may affect the activity of the Ras exchange factors. However, if the link between Ras and growth factors turns out to be through GAP phosphorylation, then alternative upstream activators of Ras will have to be examined. By analogy with *S. cerevisiae RAS*, nutritional factors might control nucleotide exchange on mammalian Ras and, indeed, it is known that in addition to growth factors, nutrients are required for progression through G_1 (*40*). Most adherent mammalian cells also have an absolute requirement for anchorage to a solid support for growth and this can be overcome, in established fibroblast lines at least, by oncogenic Ras proteins. Maybe Ras exchange factors are attachment sensors. Resolution of these issues will have to await further characterization of the proteins regulating GTP levels on Ras.

Ras-Related Proteins: Close Relatives

Seven proteins (to date) with relatively high (50 to 60%) amino acid homology to Ras have been described. Each of these proteins, Ral (A and B), Rap1 (A and B), Rap2, R-Ras, and TC21 in mammalian cells, and RSR1 in *S. cerevisiae* (Rap1A homolog) has a COOH-terminal CAAX box with either a potential palmitoylation site or a basic amino acid–rich region similar to Ki-Ras (*5, 6, 41, 42*). Rap1 is released from platelet membranes after phosphorylation by cAMP-dependent protein kinase (*43*). This putative regulatory event has not been observed for Ki-Ras (which also has a potential phosphorylation site close to the basic amino acid–rich region).

Cellular function. The function of these proteins is not clear. R-Ras, for example, interacts with the 120-kD Ras GAP protein, but transfection of cells with R-Ras containing an activating mutation

has no effect on cell proliferation (*44, 45*).

Rap1 also binds to Ras GAP, although the GTPase activity of Rap is not stimulated (*46*). This is not unexpected since normal Rap has a threonine at codon 61 (an activating mutation in Ras). Overexpression of Rap1A has been shown to revert *ras*-transformed cells and, on the basis of the model in Fig. 2B, this might suggest that Rap competes with Ras for GAP and blocks downstream signaling (*47*). However, this is probably an oversimplification because a Rap-specific GAP protein has been identified that can stimulate Rap GTPase activity (*48*). It is possible, therefore, that Rap controls a different signaling pathway; perhaps one antagonistic to Ras. Rap1 is also expressed in many differentiated cell types and, in neutrophils, for example, it is associated with a molecular complex at the plasma membrane responsible for superoxide generation (*49*). Whether Rap is involved in the assembly of this complex after stimulation of neutrophils is not known.

There is no evidence for a direct effect of the *S. cerevisiae* Rap1 homolog, RSR1, on growth signaling. However, growth of *S. cerevisiae* occurs by budding at a defined site on the plasma membrane and disruption of RSR1 results in random budding (*41*). This suggests that the protein is involved in organizing the growth process by orientating cell polarity and the exact function of RSR1 in budding yeast is now being analyzed genetically.

Ras-Related Proteins: Rho Family

The three mammalian Rho proteins (A, B, and C), two Rac proteins (1 and 2), and the protein TC10 are about 30% homologous to Ras (*5, 6, 42, 50*). *Saccharomyces cerevisiae* has at least two Rho-like proteins, RHO1 (closest to mammalian Rho) and RHO2, but CDC42 is also reported to have homology to Rho, although its sequence is not yet published (*41, 51*). These proteins each have a CAAX box and either a palmitoylation site or a basic amino acid–rich region, and it is likely, therefore, that they are active at the plasma membrane. Unlike Ras, however, significant amounts of Rho have been detected in the cytoplasm and in the Golgi (*52*).

Biochemically, mammalian Rho has many similarities with Ras despite only 30% amino acid homology. The nucleotide exchange and intrinsic GTPase rates are similar, for example, and a cytoplasmic, Rho-specific GAP protein has been identified (*44*). Furthermore, a codon 14 mutation in Rho (equivalent to codon 12 in Ras) acts as an activating mutation and prevents stimulation of the intrinsic GTPase activity by Rho GAP (*44*). Rho and Rac proteins are so far unique among the small GTP-binding proteins, however, in being substrates for adenosine diphosphate (ADP)–ribosylation. The exoenzyme C3, from *Clostridium botulinum*, ribosylates the proteins at an asparagine residue in the putative effector domain; this should provide a useful tool for examining their function (*53*).

Cellular function. There has been a question as to whether a neurotoxin (also from *C. botulinum*) that blocks exocytosis does so by ribosylating Rho. It is now evident that ribosylation of proteins by neurotoxin preparations is due to contaminating C3 transferase activity, and there is no evidence that Rho is involved in exocytosis (*54*).

Activated (Val^{14}) recombinant Rho protein has been introduced into mammalian cells by microinjection (*55*). The protein induces rapid and dramatic changes in cell shape; subconfluent fibroblast cells contract, leaving finger-like, polarized cytoplasmic extensions. Microinjection of cells with C3 transferase, on the other hand, causes cells to round up and induces dissolution of actin filaments through inactivation of endogenous Rho proteins (*55, 56*). These experiments suggest that Rho is involved in controlling some aspect of cytoskeletal organization. The role of Rho · GAP in this process is unclear, since ribosylation blocks the biological activity of Rho without affecting its interaction with GAP (*55*). There is some indirect evidence for a Rho GTP ⇄ GDP exchange factor, and a protein has been described that inhibits GDP dissociation from Rho (GDI-factor) (*39, 55*). Further characterization of these proteins and of the biochemical changes induced in cells by activated Rho should result in a greater understanding of the function of this family.

Genetic analysis of *RHO* genes in *S. cerevisiae* reveals that only *RHO1* deletions are lethal and, when an activating mutation is introduced into this gene, diploid strains can no longer sporulate (*51*). Although this appears to be similar to the effect of activating mutations in *RAS*, biochemically it is quite different; the cAMP pathway is unaffected by *RHO* mutations. The *CDC42* gene is also reported to have homology to *RHO*. Genetic analysis reveals that its function is similar to the *RSR1* gene described earlier and is involved in the establishment of cell polarity and the localization of budding (*41*). This role has some intriguing similarities to that proposed for mammalian Rho, since establishment of cell polarity in yeast appears to be mediated by the actin cytoskeleton (*57*).

Ras-Related Proteins: SEC4, YPT1, and Rab Family

YPT1 was first described in *S. cerevisiae* as a *ras*-related gene and since then its mammalian homologs Rab1A and Rab1B and about eight other Rab-like proteins (also called *smg25*) have been described, each with about 30% homology to Ras (*7*). *SEC4*, identified as a secretory mutant in *S. cerevisiae*, also belongs to this group, but no mammalian homolog of SEC4 has been found (*58*).

Although these proteins have only about 40% homology to each other, two aspects of their structure distinguish them from other Ras-related proteins. Foremost is the replacement of the COOH-terminal CAAX box with either CC or CXC motifs. The chemical modifications determined by these signals are unknown, but they are essential for membrane localization (*58*). The possibility that additional upstream sequences are required for localization has not been examined, although the proposed targeting of these proteins to different intracellular membranes would suggest this is likely. A second feature of the group is the absence of glycine at the equivalent position to codon 12 of Ras. Despite this, *YPT1* and Rab proteins have similar guanine nucleotide exchange rates and GTPase activities to Ras and Rho (*7*). So far there have been no published reports of GAP-like proteins specific for this group and there has been just one report of an exchange factor in brain cytosol active on Rab3 (*59*).

Cellular function of SEC4 *and* YPT1. There is strong genetic evidence that SEC4 and YPT1 are involved in secretion. Approximately 27 genes have been identified that control secretion in *S. cerevisiae*, and their characterization is revealing details of the biochemistry of this process (*60*). In *S. cerevisiae*, proteins destined for the plasma membrane or for secretion are first translocated from the endoplasmic reticulum (ER) to the Golgi apparatus. Although the Golgi in *S. cerevisiae* is much less well defined structurally than in mammalian cells, it is thought that proteins translocate between distinct compartments of the Golgi by vesicular transport. Finally, secretory vesicles carry the mature proteins from the Golgi to the budding site at the plasma membrane.

Disruption of the *SEC4* gene results in an accumulation of vesicles within the bud, suggesting that its product is essential for the final step of secretion, namely targeting or fusion of vesicles with the plasma membrane (*58*). In agreement with this, the protein is found predominantly on secretory vesicles and plasma membrane; only a small fraction (10%) is cytosolic (*61*). A model for *SEC4*

based on these observations has been proposed in which the protein is responsible for the unidirectional translocation of vesicles from a donor membrane (Golgi) to an acceptor membrane (plasma membrane) (*58, 62*). Such a scheme is shown in Fig. 3: vesicles leaving the Golgi contain *SEC4* in the GTP form and translocate to a target on the plasma membrane. Binding to the target is accompanied by fusion and stimulation of *SEC4* GTPase activity and SEC4 in the GDP form then leaves the plasma membrane to return to the Golgi via the cytoplasm. This model predicts that inhibition of *SEC4* GTPase activity would block vesicle transport, but this has not yet been tested directly.

Further genetic analysis, however, has revealed a more complex situation. At least ten genes are known to be essential for post-Golgi secretory events and one of these, *SEC15*, has been identified as a possible *SEC4* target (*63*). However, SEC15 is localized on secretory vesicles and not in the plasma membrane. This has led to the suggestion that SEC4 controls the formation of a complex between a docking protein, SEC15 (Fig. 3D), and a plasma membrane target that results in vesicle fusion. A GAP-like activity is assumed to be associated with this complex to generate SEC4 · GDP, which can then recycle to the Golgi. The model still does not account for why such a large proportion of SEC4 is found in the plasma membrane or how this integral membrane protein recycles back to the Golgi. In this respect, it is likely that the control of post-translational modification will be important. No information is available on how GTP concentrations on SEC4 are controlled and, in fact, since secretion in *S. cerevisiae* is constitutive, it is possible that a constitutively active exchange factor or even spontaneous nucleotide exchange could generate SEC4 · GTP.

Deletion of *YPT1* is lethal and, with the use of a temperature-sensitive mutation, it has been shown that its disruption results in an early block in secretion, with accumulation of small vesicles and unprocessed secretory proteins (*64*). One group has reported that these defects can be rescued by increasing extracellular Ca^{2+}, leading them to propose that the primary role of YPT1 is in Ca^{2+} regulation (*64*). Others have used an assay that allows reconstitution of protein transport through the Golgi complex in vitro and arrived at a different conclusion (*65*). Using molecular weight changes in a protein marker (pro α-factor) as it translocates through the Golgi, they showed that *ypt1* mutations block transport of the protein at an early step in the pathway and this could not be overcome by addition of Ca^{2+}. Furthermore, GTP-γ-*S* was found to block ER to Golgi transport. This has led to the idea that *YPT1* has a function similar to *SEC4* (Fig. 3) in controlling vesicle trafficking, but that its product acts at an early step in the secretory pathway between ER and cis-Golgi. In agreement with this, YPT1 appears to be localized in the Golgi (*66*). It is now known that both Ca^{2+} and GTP are essential for vesicular transport between the ER and the Golgi and that they are required at distinct steps (*67*). With in vitro assays, it has been shown that antibodies to YPT1 block transport from ER to Golgi and that Ca^{2+} is required at a later stage, perhaps for fusion of vesicles with the cis-Golgi membrane. Genetic and biochemical identification of proteins interacting with YPT1 will be required to clarify its exact function.

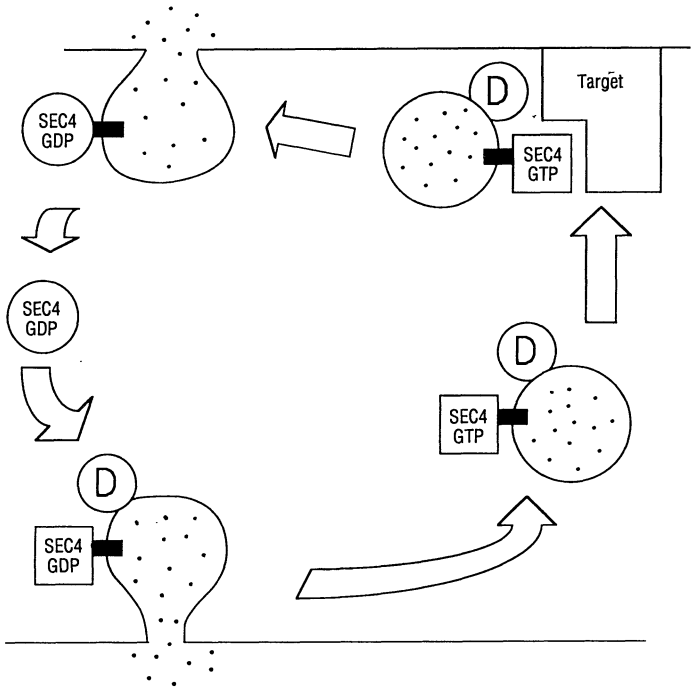

Fig. 3. Model for the role of SEC4 in vesicle trafficking. SEC4 · GTP leaves the Golgi on secretory vesicles that recognize a target on the plasma membrane. SEC4 promotes binding of a docking protein (D) to the target resulting in vesicle fusion and stimulation of SEC4 GTPase activity. SEC4 GDP then recycles back to Golgi via a soluble cytoplasmic form.

More recently, two other small GTP-binding proteins, ARF and SAR1, have been localized to the Golgi and ER (*68*). These proteins are more distantly related to the Ras-related family, although an ARF-specific GTPase-activating protein has been detected in yeast cell extracts (*68*). ARF and SAR1 are involved in transport, but their contribution to the process is unclear.

Function of mammalian Rab proteins. With the exception of Rab3, which is expressed only in neural tissues, the mammalian Rab proteins are ubiquitous. Rab3 is found in synaptic vesicles and in the soluble fraction of bovine brain (*69*). Rab1 is localized to the Golgi and the *rab1* gene can complement *YPT1* deletions in *S. cervisiae* (*66, 70*). On the basis of these observations and with SEC4 and YPT1 as models, a role for Rab proteins in intracellular trafficking of vesicles has been proposed.

As with yeast, proteins in mammalian cells destined for organelles or for secretion undergo a complex series of enzymatic processing steps in the ER and in different compartments of the Golgi (*71*). Movement between these compartments occurs by vesicle transport and a mechanism for routing vesicles to exact locations is essential. Some of these processes have been analyzed in vitro, and it has been shown that transport is dependent on GTP and is blocked by nonhydrolyzable GTP analogs (GTP-γ-*S*) (*71, 72*). This suggests that trafficking is controlled by guanine nucleotide binding proteins and in a way similar to that described for SEC4 and YPT1. In addition to constitutive transport pathways, mammalian cells also show more differentiated functions such as regulated secretion in secretory cells, axonal vesicle transport in neuronal cells, and polarized trafficking in epithelial cells. There is evidence that small GTP-binding proteins are also involved in these specialized processes, although they remain uncharacterized (*73*). The large number of Rab proteins could readily account for the diversity of targeting molecules required for vesicle trafficking in mammalian cells. At the moment, however, this remains speculation.

Conclusions

The Ras-related small GTP-binding proteins are involved in the regulation of a wide variety of biological processes, but there is still much uncertainty as to their exact function. The SEC4-like proteins constitute a distinct subgroup of the family and seem to have evolved to control the complex intracellular trafficking that occurs in all eukaryotic cells. The more Ras-like, CAAX box containing proteins, however, act at a single location and appear to regulate the assembly of plasma membrane complexes with unknown catalytic

activities. Genetic analysis has allowed identification of gene products that interact with RAS and RAS-related proteins in *S. cerevisiae*, although their biochemical analysis has proved much more difficult. It has still not been shown biochemically, for example, whether CDC25 catalyzes nucleotide exchange on RAS or if RAS activates adenylate cyclase directly. In mammalian cells on the other hand, a number of proteins that interact with the Ras-related proteins have been identified biochemically and the analysis of nucleotide exchange factors and GAPs will help to identify upstream and downstream signals, respectively. A combination of yeast genetics and mammalian cell biology and biochemistry should elucidate the function of the small guanine nucleotide binding proteins in the near future.

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