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The Ras superfamily of GTPases[1]

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ABSTRACT **The Ras superfamily of small GTPases comprises a group of molecular switches that regulate an astonishing diversity of cellular functions. A deep understanding of mitogenesis, cytoskeletal organization, vesicle traffic, and nuclear transport now requires the inclusion of the small GTPases as essential components of the molecular machines that drive these processes. The rich complexity of the control mechanisms involved is evidenced by the recent discoveries of GTPase cascades, multiple downstream effectors, and interconnected networks of GTPase-regulated protein kinase cascades. The 1995 FASEB Summer Conference at Snowmass Village, Colorado, on the Ras GTPase superfamily provided testimony to the broad impact that the study of these proteins continues to exert on cell biology.—Macara, I. G., Lounsbury, K. M., Richards, S. A., McKiernan, C., Bar-Sagi, D. The Ras superfamily of GTPases. *FASEB J.* 10, 625–630 (1996)**

Key Words: cytoskeleton · secretion · signal transduction · GTP binding proteins · nuclear transport

THE RAS GTPASES FUNCTION as molecular switches, the timing of which is modulated by a variety of factors. These factors include guanine nucleotide exchange factors (GEFs),[3] which catalyze the conversion of Ras to the GTP-bound "on" state, and GTPase activating proteins (GAPs), which accelerate the intrinsic rate of hydrolysis of bound GTP to GDP. The switch mechanism involves a guanine–nucleotide-dependent conformational change in two discrete regions of the Ras protein, called the Switch I and Switch II domains. This structural rearrangement allows GTP-bound Ras to interact with and activate specific downstream targets or effector proteins. Mutations in Ras that result in the constitutive accumulation of the GTP-bound form are oncogenic (1).

Within the last decade, a family of Ras-like GTPases has been discovered that governs an extraordinary diversity of cellular processes. Whereas the switching mechanism of these proteins is conserved, they are regulated by distinct GEFs and GAPs, and they couple to different effector proteins. The Ras-like GTPases can be clustered by structural relationships into five main groups whose members all possess related functions. At least 30 Rab GTPases, and another large group of GTP binding proteins called ARFs and ARL (ARF-like) proteins, play essential roles in the budding and fusion of vesicles that move between different membrane compartments in the cell. Members of the Rac/Rho group, which numbers about 10, control various types of cytoskeletal organization and indirectly regulate gene expression. The Ran GTPases are necessary for nuclear transport through the nuclear pore complex. There are also examples of other, less well-defined small GTPases that do not belong to any of these groups and possess as-yet-unknown functions. It is this diversity of roles, together with the accessibility of GTPase biochemistry and the high specificity of the protein-protein interactions that the GTPases undergo, that accounts for the impact the study of these molecular devices has had on cell biology over the last several years. In this review, we will attempt to provide a brief overview of the field and speculate on possible future developments.

RAS GTPASES

The Ras GTPases have been subjected to intensive study because activated mutants of these genes have been found in 10–20% of all human tumors. They sit at the heart of a signal transduction pathway that links cell-surface receptors through a protein kinase cascade to changes in gene expression and cell morphology. Two years ago, a series of exciting discoveries uncovered several missing links in the Ras-dependent signaling pathway. Grb2 and SHC were identified as adaptor proteins that couple phosphorylated tyrosine kinases at the plasma membrane to the mSOS guanine nucleotide exchange factor for Ras; the Raf1 protein kinase was identified as a direct downstream effector for Ras (2, 3). For a moment—if one ignored awkward questions regarding the mechanism of activation of SOS or of Raf1—it seemed as though all the major questions had been answered.

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[3] Abbreviations: PI 3-kinase, phosphatidylinositol 3-kinase; GEFs, guanine nucleotide exchange factors; GAPs, GTPase activating proteins; PLD, phospholipase D; PIP_2, phosphatidylinositol bisphosphate; PA, phosphatidic acid; JNK, Jun kinase; COPs, coatamer proteins; GDIs, guanine nucleotide dissociation inhibitors; RanBD, the Ran binding domain.

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However, it soon became clear that a linear pathway from plasma membrane to nucleus was inadequate: G-protein-coupled receptors can activate Ras through a mechanism that involves βγ subunits; multiple MAP kinase pathways were identified; other small GTP binding proteins such as Rac and Rho were shown to mediate cytoskeletal changes induced by Ras; and other Ras binding proteins were discovered that were potential alternative effectors. An important advance was the discovery by White et al. (4) of complementing mutations in the effector domain of Ras that individually attenuate Ras-dependent transformation. Certain of these transformation-defective

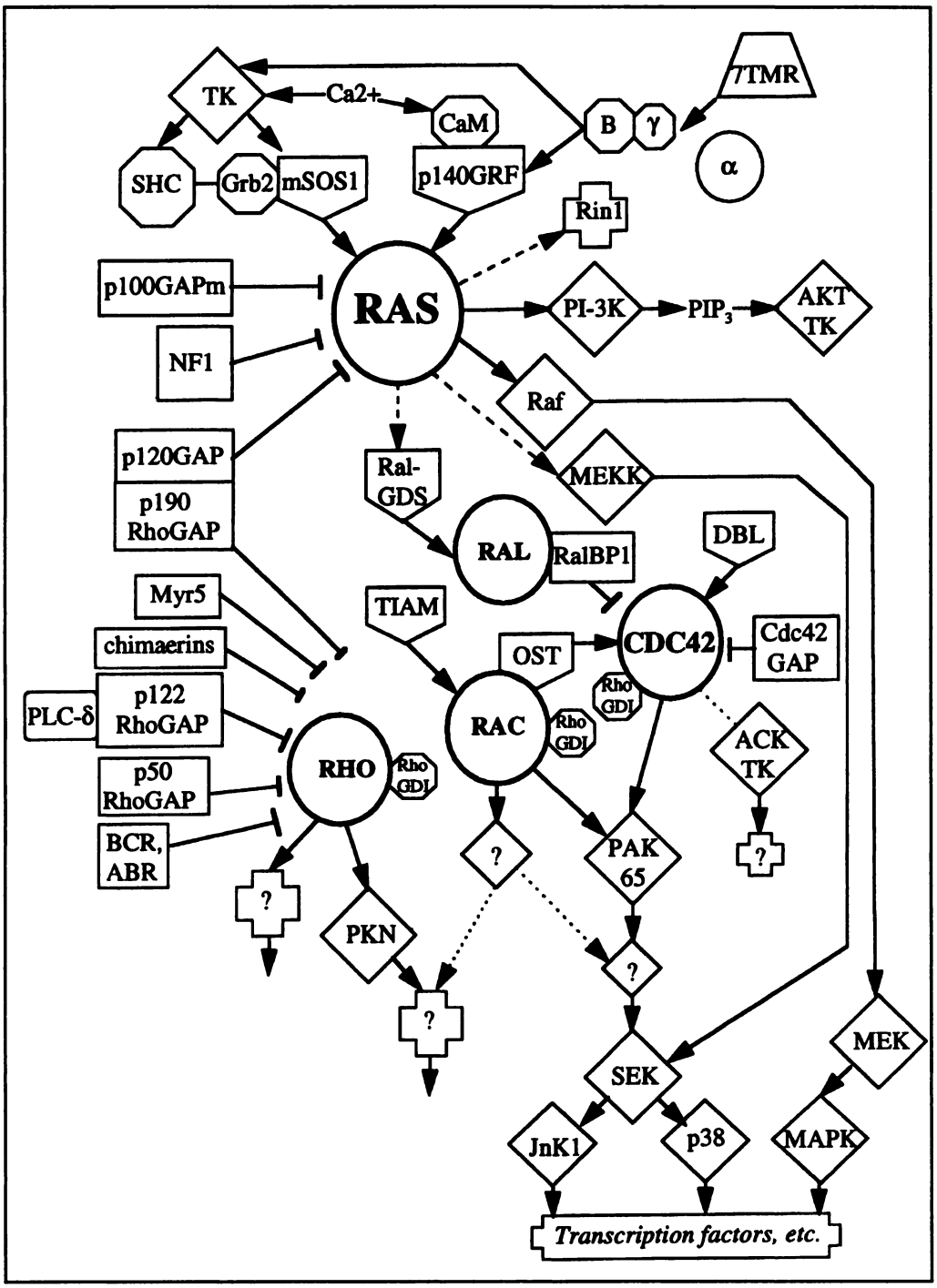

Figure 1. Overview of regulatory factors and downstream effectors for Ras and Rac/Rho GTPases. Kinases are shown as diamonds, GTPases as ovals, GAPs as rectangles, adaptor proteins as octagons, exchange factors as pentagons, other enzymes as round-cornered boxes, and proteins of unknown function are represented by crosses. Activating interactions are shown as arrows, inactivating interactions as bars. Abbreviations: 7TMR, seven transmembrane domain receptors; TK, tyrosine kinase; CaM, calmodulin. Because of space limitations not all the factors shown in this figure are discussed in the text. Further information could be accessed by using the names of these factors as key words in database searches.

mutants retain a functional interaction with the Raf1 kinase, whereas others do not. The data support a model in which multiple downstream effectors need to be activated by Ras to induce oncogenic transformation.

An illustration of Ras-dependent signaling pathways is provided in **Fig. 1**. The complexity of the network of interactions is immediately evident, and the pace of additions to the picture is accelerating. In addition to the tyrosine kinase receptor-mediated mSOS pathway, recent data suggest that seven-helix transmembrane receptors can induce the tyrosine phosphorylation of SHC via a mechanism that involves G-protein βγ subunits (5), and may be mediated by the Tsk and Btk tyrosine kinases. A new tyrosine kinase called PYK2 can also phosphorylate SHC, and is activated by Ca^{2+} and by protein kinase C (6). Phosphorylated SHC can then recruit the Grb2/SOS1 complex to the membrane, where SOS can activate Ras. This mechanism is complicated by the existence of a second Ras exchange factor, called p140 GRF or Cdc25Mm, which can also be activated both by Ca^{2+}—apparently through interaction with calmodulin(7)—and by βγ subunits through a mechanism involving phosphorylation (R. Mattingly and I. G. Macara, unpublished results). Multiple types of signal can therefore feed into Ras through at least two separate pathways.

The conversion of Ras to the GTP-bound state allows it to interact with other proteins that may function as downstream effectors. The "classical" effector is the Raf1 protein kinase (3). Other potential effectors include the phosphatidylinositol 3-kinase (PI 3-kinase) p110 subunit, a guanine nucleotide exchange factor for the Ral GTPase called RalGDS, a RalGDS-related protein, and Rin1, a protein of unknown function (8–10). In budding yeast the effector for Ras is adenylyl cyclase; in the fission yeast *Saccharomyces pombe*, a protein kinase, called byr2, and a putative GEF for the GTPase Cdc42p, called scd1, can both interact with Ras (11). Whether these proteins all function in a physiological context as true effectors for Ras is uncertain, however. For example, the phenotypic effects of a constitutively activated PI 3-kinase require functional Ras, which implies that this lipid kinase can act as an upstream regulator of Ras rather than as a downstream target (12).

It is likely that further Ras effectors remain to be discovered. Evidence that Rac and Rho are necessary for oncogenic transformation by Ras (13, 14) suggests that exchange factors for these small GTPases may operate downstream of Ras. Indeed, there is now evidence (C. J. Der, unpublished results) that at least three downstream effectors may be necessary for full oncogenic transformation by v-Ras.

The activation of the Raf1 kinase by Ras:GTP is believed to require recruitment of the kinase from the cytosol to the plasma membrane (15). The Ras effector loop provides a high-affinity binding site for Raf1, but the kinase also interacts with the Switch II region of Ras, and this interaction triggers a conformational change in Raf1 that leads to activation of the kinase. Autophosphorylation of the kinase then prevents its return to the inactive state, so that it is not switched off immediately after release of Ras from the complex. Nuclear magnetic resonance and crystal structures of Raf and the Ras:Raf complex should help determine the details of the switching mechanism (16).

Given the baroque complexity of the interactions described so far, it is not surprising to find that there are multiple GTPase activating proteins (GAPs) that function to shut the Ras switch off. The p120 GAP and NF1 (IRA1 and 2 in budding yeast) were the first to be discovered and cloned (1). Pawson (17) has used cell lines lacking p120 GAP and NF1, derived from transgenic mice, to show that whereas p120 controls the response of Ras to stimulation, NF1 appears to control basal Ras activity. Other GAPs have recently been discovered, one of which possesses a separate domain that can bind the inositol polyphosphate IP_4 (18). The mechanisms of regulation of these GAPs remain unclear.

RAC/RHO GTPASES

This group of GTPases has moved to center stage with recent discoveries of its involvement in morphogenesis and cytoskeletal organization, neutrophil activation, mitogenesis and transformation, protein kinase cascades, phospholipid metabolism, and transcriptional regulation. Elegant microinjection studies have defined an ordered GTPase cascade—at least for Swiss 3T3 cells—in which the Cdc42p GTPase acts upstream of Rac, which is upstream of Rho. Cdc42 induces filopodia formation, Rac stimulates formation of lamellipodia, and Rho induces production of stress fibers and adhesion plaques (19). Ral may act upstream of all of these GTPases in some circumstances, and Ras may act upstream of Ral. Feedback loops undoubtedly exist between these proteins, and one candidate factor in such a loop is a Ral binding protein that acts as a Cdc42p-GAP, described recently by two independent groups (20). The link between Rac and Rho appears to be indirect. Rac-dependent stimulation of phopholipase A_2 results in the formation and release of prostaglandins, which presumably interact with specific cell-surface receptors; these in turn activate Rho, perhaps via a heterotrimeric G-protein (21).

Of course each Rac/Rho GTPase can interact with a multitude of GEFs, GAPs, and effectors. Three exchange factors are known, each of which can have profound physiological effects when overexpressed in cells. Dbl and Ost can function as oncogenes, and Tiam is involved in metastasis (22). More exchange factors probably remain to be discovered. One large gap in current knowledge is the mechanism by which any of the Rac/Rho GEFs are controlled.

An array of Rac/Rho-GAPs have also been cloned, including Bcr, n- and β-chimerin, p50 RhoGAP, and p190 (23). Remarkably, many of these factors possess independent functional domains in addition to GAP activity

and they may act as links between different signaling pathways. The p190 can associate with p120 Ras-GAP and is itself a GTP binding protein. Another Rho-GAP, p122, can bind to and activate phospholipase C-δ (24), and still another is a member of the myosin family (25). Many of these GAPs and GEFs show no absolute specificity for any one member of the Rac/Rho class of GTPases, which confuses the assignment of physiological significance, but they do have clear preferences for different GTPases.

There have been important advances in identifying downstream effectors for these proteins. Two kinases, the ACK tyrosine kinase and the PAK65 serine/threonine kinase, have been cloned and shown to interact with Cdc42p and Rac (26). PAK65 kinase activity is stimulated by Cdc42p binding. It is only one of a family of similar protein kinases. In budding yeast, the Ste20 and CLA4 kinases, which possess sequence similarities to PAK, interact with the yeast Cdc42p. The Cdc42p GTPase functions in bud site selection, but may also act downstream of the βγ subunits of the pheromone receptor pathway (27). Two-hybrid screens and other approaches have identified a plethora of other potential targets for these GTPases, but further work is necessary to demonstrate physiological roles for them. Nonetheless, it has become clear that these GTPases sit at the head of protein kinase cascades that activate the Jun kinase (JNK) and p38 kinase rather than MAP kinases. It will be important to determine whether PAK65 or related kinases can activate MEKK1, which mediates the activation of JNK via SEK. In addition, exciting new evidence from two laboratories (28, 29) points to the protein kinase PKN as a downstream target for Rho. The Rac/Rho GTPases also appear to regulate phosphatidylinositol (PI) phosphate metabolism by binding to and/or activating PI kinases (30).

The emerging picture is one of a GTPase cascade that links to multiple protein and lipid kinase cascades, which ultimately link to different sets of target proteins, including transcription factor cascades. Tissue-specific expression of different regulatory factors and different kinases is likely to occur, providing a mechanism for cells to respond differently to an identical extracellular stimulus.

ARF AND RAB GTPASES

ARF and Rab proteins are responsible for the assembly, loading, and targeting of vesicles to appropriate cellular compartments. ARFs and the yeast GTPase Sar 1 act as structural components of vesicles that recruit coatamer proteins (COPs). They may also function as sensors to permit the transport only of cargo-loaded vesicles. Rab proteins may function by allowing docking and/or fusion of loaded vesicles to their target membrane only after mutual recognition has occurred (through interacting proteins called v- and t-SNARES). Much of our current knowledge of Rab GTPase function is based on elegant genetic studies in budding yeast on Sec4p and Ypt1 (31).

ARFs are predominantly cytosolic even though they are myristoylated. However, upon exchange of GDP for GTP, ARF:GTP translocates to membranes and recruits COPs to form coated vesicles. There is a concurrent stimulation of phospholipase D (PLD), which results in the hydrolysis of phosphatidyl choline to phosphatidic acid (PA) and choline (32). In turn, PA promotes phosphatidylinositol bisphosphate (PIP_2) synthesis, a cofactor for ARF-GAP (33). The resulting nucleotide hydrolysis is required for the uncoating of vesicles before their fusion with their target membranes. PIP_2 is also a required cofactor for PLD and is necessary for cytoskeletal rearrangements that accompany secretion. It is possible that these ARF-dependent interactions with phospholipids may provide a point of intersection with the Rac/Rho GTPases, which may regulate PI kinases.

Rabs are localized to specific membrane pools rather than the cytoplasm, and mutations that cause mislocalization result in loss of function (34). Rabs are delivered to their appropriate membrane by guanine nucleotide dissociation inhibitors (GDIs), which interact with geranylgeranylated Rab proteins in the GDP-bound state. Transfer of Rab from GDI to the membrane is obligatorily coupled with nucleotide exchange (35, 36). The presence of Rab subtype-specific GEFs on particular membranes could provide a targeting mechanism for the Rabs, and might explain the absence of detectable Rab receptors on the membranes. However, the picture is complicated by the existence of other soluble Rab-GEFs that are inhibited by GDIs.

Few downstream effectors for Rabs have yet been found. Rabphilin-3A is a likely target for Rab3A (37), and Zerial's group (38) has cloned an effector for Rab5, called Rabaptin-5. Undoubtedly many more are yet to be identified. The functions of these targets are unknown,

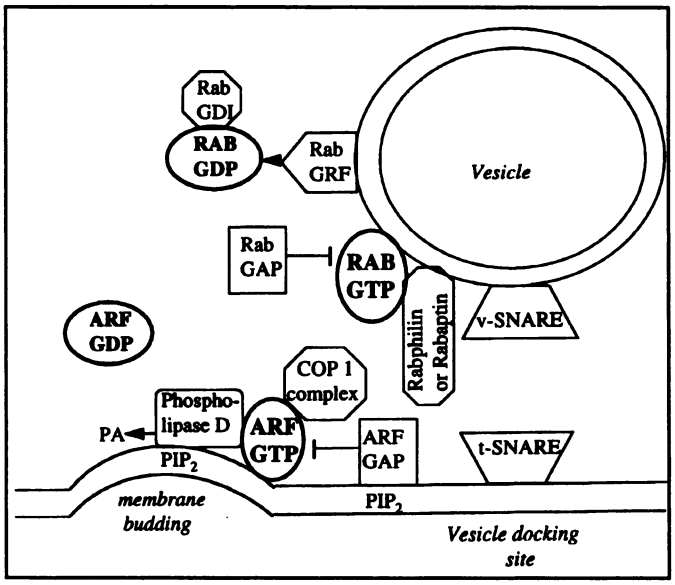

Figure 2. Regulatory factors and downstream effectors for the Rab and ARF GTPases. Shapes to indicate function follow the convention of Figs. 1 and 2.

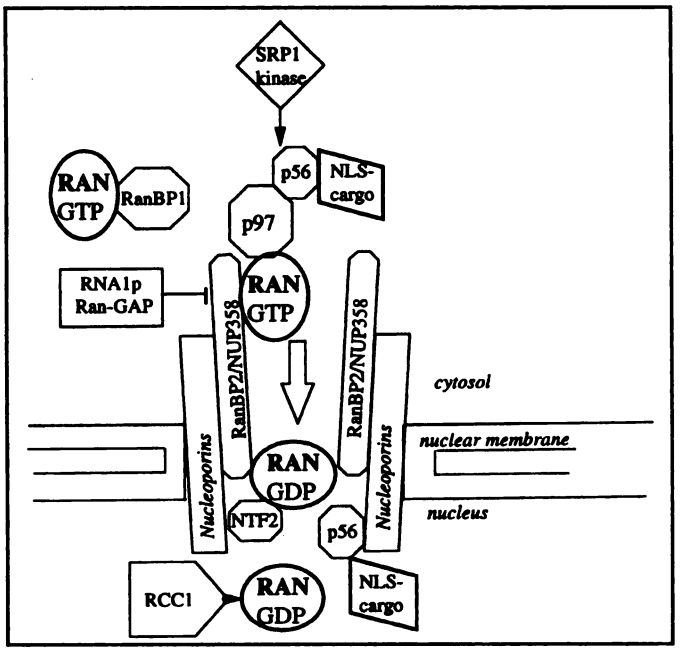

Figure 3. Regulatory factors and downstream effectors of the Ran/TC4 GTPase. Shapes to indicate function follow the convention of Fig. 1. p97 is β-karyopherin; p56 (also called importin and α-karyopherin) is the receptor for cargo possessing nuclear localization signals (NLS). NTF2 is nuclear transport factor 2 (40).

but it seems possible that the Rab proteins target the effector to the correct membrane, just as Ras triggers the translocation of Raf1 to the plasma membrane. The Rab:effector complex may then interact with other proteins, allowing hydrolysis of the Rab:GTP and release of the Rab protein (**Fig. 2**). Recent studies of Rabphilin-3 suggest that it may form an inhibitory complex that prevents docking and/or exocytotic vesicle fusion until a secretory signal is received by the cell (39).

RAN GTPASES

The study of the nuclear GTPase Ran/TC4 recently gained momentum with the discovery that Ran is an essential component of the nuclear protein import machinery (40). Moreover, RCC1, a protein essential for entry into and exit from mitosis, associates with and functions as a nucleotide exchange factor for Ran.

Mutations that prevent GTP hydrolysis of Ran or inhibit the exchange of GTP for GDP on Ran alter both nuclear import pathways and the proper regulation of the entry into mitosis, possibly through different mechanisms. Several proteins that bind to Ran:GTP have been described. These include a 29 kDa cytosolic protein, RanBP1/HTF9A (41), a 360 kDa nuclear pore protein, RanBP2/NUP358 (42, 43), and a 96 kDa *Caenorhabditus elegans* protein, Ranup96. All these binding proteins possess one or more copies of a highly conserved motif, the Ran binding domain (RanBD) (44). RanBP2 and Ranup96 also possess sequence similarities to nuclear porins, and RanBP2 is associated with the nuclear pore complex. The RanBD acts as a potent inhibitor of guanyl nucleotide release from Ran and is a coactivator of the RanGAP, RNA1p. The Ran:RanBD dimer can also associate with other proteins to form ternary complexes. One of these proteins is β-karyopherin, which is a component of a docking complex for nuclear-targeted cargo (45). Ran and Ran binding proteins may act to couple the docking step at the nuclear pore complex with translocation of the cargo through the pore (**Fig. 3**). Rapid advances in the cloning of many essential factors required for nuclear transport and the development of in vitro assays (40) are together opening a new era in understanding of the molecular mechanisms of nucleocytoplasmic transport.

CONCLUSIONS

A general model appears to be emerging from the diverse studies of Ras-like GTPases in which these proteins function to induce the translocation of proteins to specific cellular sites and/or to induce the assembly of multicomponent complexes. In both situations, the GTPase must be in the GTP-bound state, but can be released from the site or complex after GTP hydrolysis without immediately causing dissociation of the complex. In this view, the Ras-like GTPases act as assembly catalysts rather than as simple switches or structural components.

A second emerging theme is that different GTPases interact with one another. This has been most clearly demonstrated for the Rac/Rho signaling pathway in which CDC42p can activate Rac, which can activate Rho. The links between the GTPases that form this chain are not yet known, but will undoubtedly be identified within the next few years. It will be interesting if the Rab and ARF GTPases turn out to be linked to one another in a similar way.

A third new concept is that the Ras-like GTPases have multiple downstream effectors. Evidence is strongest for Ras itself, but large numbers of potential effectors for the Rac/Rho family are being identified, and several effectors for Ran have been cloned, with more on the way. It would not be too surprising to find that each Rab and ARF protein also interacts with multiple targets.

Finally, it is instructive to consider how many different gene products are likely to be involved in regulating and interacting with Ras-like GTPases. At least a dozen proteins are known to bind to Ras and a similar number to Rac. About eight have already been found to bind to Ran. Mammalian cells probably contain more than 50 Ras-like GTPases. It seems reasonable, therefore, to speculate that there may well be 500 or so genes in higher eukaryotes that encode proteins that bind directly to members of this GTPase family. There will certainly be no dearth of topics for many future FASEB conferences on the Ras GTPase superfamily. FJ

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