The Many Roads That Lead to Ras

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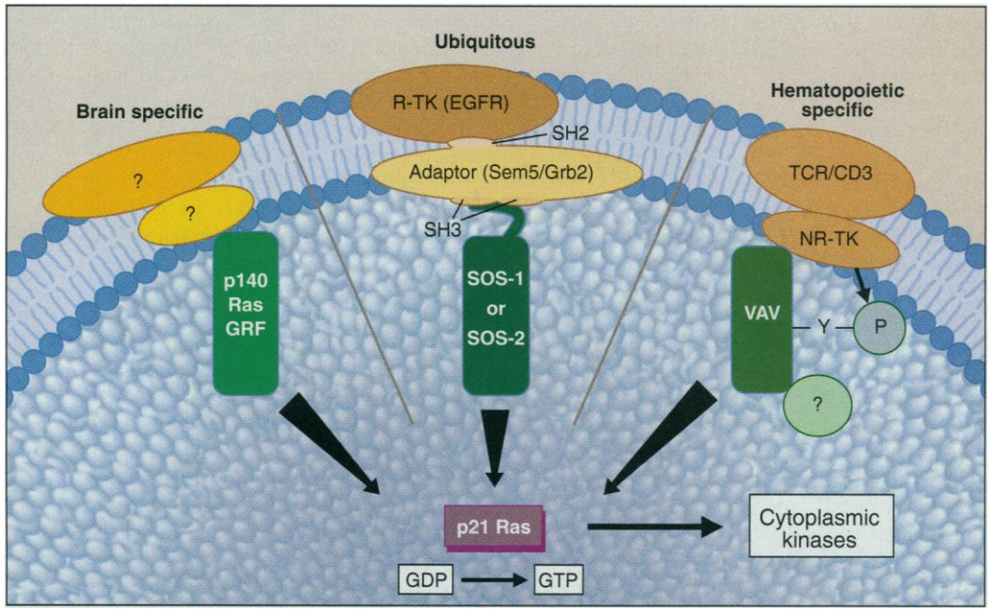
It has been a little over a decade since mutated *ras* genes with oncogenic potentia were first detected in human tumors. To understand how mutated Ras proteins contribute to oncogenesis, a major effort has been made to elucidate the normal function of Ras proteins in cells. During this time, it has become well established that the activation of Ras proteins is a key step in the biochemical pathways triggered by ligand-bound cell surface receptors that are themselves tyrosine kinases and those that are associated with tyrosine kinases (*1*). This class of receptors responds to a wide variety of cell modulators including growth and neurotrophic factors as well as factors involved in the immune response. Recently, a flurry of new information has begun to reveal how these stimulated receptors lead to Ras activation. These studies have identified both a family of guanine-nucleotide releasing factors (GRFs) [also called guanosine diphosphate (GDP) dissociation stimulators (GDSs)] that directly activate Ras in mammalian cells and an "adaptor" protein (Sem5/Grb2) that appears to mediate the interaction of some GRFs with activated receptor molecules. In this issue of *Science* (p. 822), a new GRF-containing pathway to Ras is outlined that is specific to the hematopoietic system; the protein Vav is reported to function as a GRF in the activation of Ras by the T cell receptor.

The three 21-kilodalton Ras proteins—H-Ras, N-Ras, and K-Ras—are members of a superfamily of proteins that can exist in two states: an active one that contains a bound guanosine triphosphate (GTP) molecule and an inactive one that contains GDP. Activation occurs by the replacement of bound GDP with GTP; the rate-limiting step in this exchange reaction, the release of bound GDP, is catalyzed by the GRFs. Deactivation occurs by the hydrolysis of GTP to GDP. GTPase activating proteins (GAPs) dramatically accelerate this hydrolysis. Thus, the relative activities of GRFs and GAPs acting on Ras at any particular time determine its activation state. Changes in both the rate of GDP release on Ras and the total cellular GAP activity have been observed (*2*) in various stimulated cells, suggesting that the regulation of Ras activation is quite complex.

Genetic studies of a cell cycle mutant in the yeast *Saccharomyces cerevisiae* led to the identification of the first GRF for Ras, CDC25 (*3*). A mammalian counterpart, the brain-specific p140 Ras—GRF (also termed $CDC25^{Mm}$), was cloned from cDNA libraries by exploiting either its sequence (*4*, *5*) or functional similarity to CDC25 (*6*). The CDC25-like region of this protein promotes nucleotide exchange on Ras, but not on other members of the Ras superfamily, such as Rho and Ral (*4*). Genetic studies in *Drosophila* identified another putative Ras-GRF, son of sevenless (SOS) (*7*), that has a sequence similar to yeast CDC25. Three mammalian homologs to SOS have been cloned: Two from mouse—mSOS-1 and mSOS-2 (*8*)—and one from human—hSOS (*9*). The hSOS can also activate Ras (*9*). Unlike p140 Ras-GRF, which is found only in brain, mSOS-1 and -2 appear to be expressed ubiquitously. Another protein, GDP dissociation stimulator (GDS), that has the capacity to activate K-Ras has been cloned. It is unusual in that it can also activate Rho and Rap GTP-binding proteins, but not H-Ras and N-Ras, and that it requires its targets to be prenylated (*10*).

Studies of a Ras pathway in *Caenorhabditis elegans* again highlight the power of genetic analyses in relatively simple eukaryotes. These experiments identified a gene, Sem5, which has a product that functions upstream of Ras and downstream of tyrosine kinase receptors (*11*). The properties of the Sem5 protein are consistent with an adaptor-like role, because it contains no catalytic domain, only so-called SH2 and SH3 domains that are involved in protein-protein interactions. SH2 domains bind to certain tyrosine phosphorylated proteins. Counterparts to Sem5 in *Drosophila* (Drk) and mouse (Grb2) have now been detected, and their SH2 domains have been shown to bind to an activated *Drosophila* tyrosine kinase receptor and the epidermal growth factor (EGF) receptor, respectively (*12–15*).

SH3 domains bind proline-rich regions of specific proteins (*16*). Remarkably, SOS and mSOS-1 and -2 contain a 20-kilodalton stretch of proline-rich sequence at their carboxyl terminus that binds Drk and Sem5 in vitro (*12*) and in vivo (*14*, *15*). The two SH3 domains of Sem5/Grb2 bind to two cognate proline-rich peptides (*14*) that are similar in sequence to a consensus sequence established for the SH3 domain of the Abl proto-oncogene (*16*). In addition, the SH3 domains of hSOS are required for interaction with Grb2 (*9*). In fibroblasts, EGF stimulates the formation of a ternary complex containing SOS–Sem5/Grb2–EGF tyrosine kinase receptor (*14*, *15*). Thus, in this highly conserved



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Activation of Ras. Membrane-bound Ras can be activated by ligands that bind to cell surface receptors via a ubiquitous pathway consisting of the GRFs SOS1 or SOS2 and the adaptor molecule Sem5 (center) or via localized pathways that utilize tissue-specific GRFs such as p140 Ras—GRF in brain (left) and Vav in hematopoietic cells (right). R-TK, tyosine kinase receptor; NR-TK, nonreceptor tyrosine kinase; TCR, T cell receptor; EGFR, epidermal growth factor receptor; Y, tyrosine; P, phosphate.

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regulatory pathway, SOS is brought from the cytoplasm into the vicinity of its target, plasma membrane–associated Ras, by the adaptor Sem5/Grb2 (see figure). No change in the instrinsic GRF activity of SOS is observed upon cell activation, suggesting that regulation of nucleotide exchange on Ras is mainly due to the altered subcellular localization of SOS (*15*). The fact that SOS uses an adaptor molecule to bind to activated receptors, rather than encoding its own SH2 domain, suggests that we have detected only one of many ways SOS can interact wit cell surface receptors. In fact, another adaptor protein, SH2-containing transforming protein (Shc), has also been implicated in this signaling pathway by virtue of its association with Sem5/Grb2 in stimulated cells (*17*). The unique binding properties of the SH2 domain of Shc could ferry Sem5/Grb2-SOS complexes to additional membrane sites.

An additional complexity of Ras regulation is that, although Ras proteins are present in all cells, some GRFs are expressed in a tissue-specific manner. An example is the brain-specific p140 Ras—GRF. It is interesting that this GRF does not have the proline-rich, carboxyl-terminal tail that contains the Sem5/Grb2 binding site of SOS. This raises the possibility that p140 Ras—GRF allows Ras to be modulated by unique signals triggered at the cell surface of neurons (see figure).

A new tissue-specific pathway to Ras is described by Gulbins and co-workers (p. 822). They have found that the protein Vav, which is expressed exclusively in hematopoietic cells, is involved in Ras activation by the T cell receptor. In fact, they present evidence supporting the surprising idea that Vav itself is a GRF specific for Ras. This finding is unexpected, because Vav has been predicted to be a GRF for the Ras-related Rho proteins. This prediction came from the fact that, instead of containing CDC25-like sequences that activate Ras, Vav has a domain similar to the Dbl protein, which is a GRF specific for CDC42 (*17*). CDC42 is a member of the Rho family of GTP-binding proteins and is involved in the regulation of the cytoskeleton. Thus, it may not be as easy as it originally seemed to predict the specificity of GRFs from their primary sequence.

The Vav gene was first detected by its transforming activity, which was apparent only after its 5' end was truncated during transfection assays (*18*). The oncogenic protein retained the "Dbl" domain as well as an SH2 and two SH3 domains. Gulbins and coworkers' work on Vav began with the finding that activation of the T cell receptor in the Jurkat cell line and in freshly isolated human T cells led to an increase in overall cellular GRF activity for Ras. This result was consistent with earlier work by Downward and his colleagues (*4*) showing an increase in the percentage of activated GTP-Ras in similar stimulated T cells, although the increase in the earlier study was shown to be due to a decrease in GAP activity, not a change in GRF activity.

Remarkably, all of the GRF activity on Ras observed by Gulbins and co-workers could be accounted for in immunoprecipitates of Vav protein from these activated T cells. The temporal appearance of activity after cell stimulation correlated with the tyrosine phosphorylation of Vav. Moreover, GRF activity in Vav immunoprecipitates from unstimulated cells could be activated by incubation with a potential mediator of T cell receptor action, the nonreceptor tyrosine kinase Lck ($p56^{lck}$ although it is not clear whether Lck is the kinase that phosphorylates Vav in vivo). Thus, activation of a T cell receptor—associated tyrosine kinase likely regulates the specific activity of a Vav-associated Ras-GRF activity. This is in contrast to the new studies in fibroblasts, which did not detect a difference in SOS-associated GRF activity in response to cell stimulation by the EGF receptor (*15*).

On the basis of an analogy to SOS regulation, one might have predicted that Vav was not a GRF but rather a tissue-specific adaptor molecule that binds to an SOS-like molecule via the former's SH3 domains. The SH2 domain of Vav could bring an unidentified SOS-like molecule to an autophosphorylated non-receptor tyrosine kinase such as Lck (see figure). SOS could then be activated by tyrosine phosphorylation. In fact, a tyrosine phosphorylated protein of about the size of SOS can be observed along with Vav in Lck-treated immunoprecipitates. Although this possibility has not been completely excluded, Gulbins and colleagues do show that a fragment of Vav containing the Dbl domain, translated in vitro in a wheat germ lysate, can promote GDP release from recombinant Ras. The authors postulate that this fragment of Vav must have lost its requirement for activation by tyrosine phosphorylation. Additional experiments will be required to confirm that Vav directly activates Ras in a tyrosine phosphorylation–dependent manner. In spite of these remaining questions, it is very likely that Vav is indeed a participant in a hematopoietic-specific pathway that leads to Ras from activated T cell receptors.

Although the many paths from receptors to Ras are becoming clearer, there is potentially another level of complexity. Some Ras-GRFs may be multifunctional! In addition to CDC25-like domains that specifically activate Ras (*4*), they contain Dbl-like domains (*4, 20*). Based on the discussion above, this may mean these proteins coordinately regulate more than one Ras superfamily member.

Finally, revealing how receptors lead to Ras activation only solves part of the puzzle. We still need to know why Ras activation is critical for the action of so many receptors and what role Ras plays in the generation of specific cellular responses to these individual receptors. By stimulating the activation of a set of cytoplasmic kinases (*1*) (see figure), Ras may be acting downstream of receptors as a general cell activator. This may be necessary in order for cells to respond to other specific signals generated by receptors.

Alternatively, as previously proposed (*21*), Ras may play a more active role in the proper functioning of tyrosine kinase receptors. In this view, for example, the binding of Sem5/Grb2 to the autophosphorylated receptor is important not only to bring SOS into the vicinity of its target, membrane-bound Ras, but also to bring membrane-bound Ras into a complex with tyrosine kinase receptors and other associated signaling molecules (*21*). Here, the presence of the active form of Ras may be necessary somehow for signals to be propagated effectively from activated tyrosine kinase receptors. This is consistent with GAP also being present in many receptor complexes. Again, specificity would have to be acheived by the presence of unique signaling molecules in each receptor signaling complex.

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