Thinking Outside the Box about Ras*

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The Ras oncoproteins are membrane-associated molecular switches that function to transduce extracellular signals to a panoply of intracellular response mechanisms. Activating mutations in ras genes are present in 15% of all cancers and perhaps as many as 30% of metastatic human cancers. Accordingly, considerable effort has been expended in understanding how Ras proteins work and, by extension, how their transforming activity can be blocked therapeutically (1–6).

The Ras oncproteins are encoded by three genes: Ki-ras, Ha-ras, and N-ras. Lesions in Ki-ras account for most of the Ras mutations found in human cancers. The three proteins are structurally and functionally quite similar. The three Ras oncproteins are part of a much larger family of genes, the Ras superfamily, encoding small monomeric GTPases (1–6).

As the term suggests, Ras proteins possess intrinsic GTPase activity. When bound to GTP, Ras proteins are active and capable of recruiting downstream effectors and influencing cell function. GTP hydrolysis results in a GDP-bound, inactive Ras protein. Both the intrinsic GTPase activity and GTP binding activity of Ras proteins are relatively slow and are greatly accelerated by accessory proteins (1–4). These accessory proteins serve to regulate Ras function. Thus, Ras GTPase activity is greatly accelerated by RasGAPs,2 which function to suppress signaling. Loss of RasGAP function by elevating basal GTP-bound Ras activity can contribute to oncogenesis. For example, neurofibromin is a RasGAP tumor suppressor protein. Inactivating mutations in neurofibromin occur in Von Recklinghausen disease (neurofibromatosis type 1). These inactivating mutations underlie much of the pathology of this disease, including the development of peripheral nerve sheath tumors (7, 8). RasGEFs promote the dissociation of GDP from inactive Ras. Inasmuch as GTP is in relative abundance in the cytosol, the dissociated GDP is rapidly replaced with GTP, thus promoting Ras activation (9, 10).

There are few prominent GEF oncogenes. Instead, oncogenic Ras mutations such as Val12-Ki-Ras function to inactivate Ras GTPase activity, leaving the mutant protein in a constitutively active, GTP-bound state (11).

In their GTP-bound state, Ras proteins physically interact with their downstream effector proteins, triggering activation of multiple signaling pathways with complex and divergent effects. Ras oncoprotein target proteins all contain conserved RBDs; these interact with an N-terminal effector loop on Ras (11). Given that Ras proteins are prenylated and, consequently, membrane-associated (12), Ras binding recruits Ras effectors to the plasma membrane. The best understood of the Ras effectors are the Raf Ser/Thr kinases, the class 1 catalytic subunit PI3Ks, and RafGEF (2, 13).

The Raf proteins (A-Raf, B-Raf, and Raf-1, also called c-Raf) are Ser/Thr protein kinases that function as MAPK kinase kinases (MAP3K). As such, they are specific direct activators of MAPK kinases (MKK) that lie upstream of the ERK group of MAPKs. MAPKs are highly conserved evolutionarily, and the ERK pathway represents a major mechanism by which mitogens stimulate cell proliferation (14).

The PI3Ks are lipid kinases that phosphorylate PI (typically PI-4,5-P) specifically on the inositol 3’-hydroxy group, thereby generating PI-3,4,5-P3. PI-3,4,5-P3 is an important second messenger that binds proteins containing pleckstrin homology domains, thereby fostering the assembly of signaling complexes. Typically, PI3Ks are recruited to the membrane by virtue of the binding of their regulatory subunit SH2 domains to phosphorytrosine residues present on activated Tyr kinases. However, active Ras (especially oncogenic Ras mutants) can also bind the RBDs present in class I PI3Ks. Both binding events serve to bring PI3Ks to the membrane, facilitating contact with substrate lipids. In the case of oncogenic Ras, this recruitment occurs in the absence of upstream stimuli. PI3K activity is absolutely required for the activation of Akt family Ser/Thr kinases, which, in turn, are pivotal in the inhibition of apoptosis and the promotion of cell survival. Other PI3K targets include the Akt-activating kinase PDK1 (PI3K-dependent kinase-1) and GEFs that target members of the Rho family of Ras superfamily GTPases (2, 13, 15–19).

RafGEF is a GEF specific for the Raf family of Ras superfamily GTPases. RafGEF and Raf signaling is more poorly understood. Potential Raf targets include components of the exocyst complex, which may, in turn, regulate vesicular trafficking within the cell. In addition, elements of the exocyst complex have been implicated in the activation of the non-canonical IKK (inhibitor of κB kinase-ε). By this process, Ras, through Raf, may trigger NF-κB activation. Like Akt, NF-κB is anti-apoptotic; and together, engagement of these pathways may foster tumor cell survival (20).

Ras proteins are activated by the recruitment of RasGEFs. The best understood of these is mSOS (mammalian son of sevenless). mSOS contains an SH3 domain, which enables it to bind constitutively to the adapter protein Grb2. The SH2 domain of Grb2 binds to activated, Tyr-phosphorylated Tyr kinases (or their Tyr-phosphorylated substrate adapter proteins), thereby bringing the Grb2-mSOS complex to the mem-

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2 The abbreviations used are: GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; RBD, Ras-binding domain; PI3K, phosphatidylinositol 3-hydroxykinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI, phosphatidylinositol; SH, Src homology.
brane, where Ras resides. There, mSOS facilitates Ras GDP-GTP exchange (9, 10).

This initial notion of a pathway consisting of receptor Tyr kinase → Grb2-SOS (or a similar GEF) → Ras → Ras effector (Raf, PI3K, RalGEF) cell proliferation/survival was initially very satisfying insofar as it provided a link between mechanisms of cell-surface receptor engagement and activation of distal effectors, both of which had been the subject of intense work. A number of new therapeutic targets (especially for cancer) were also identified, and the sense of triumph was well justified. Indeed, allusions to Promontory Point and completion of the Transcontinental Railroad were bandied about.

Still, a number of frustrating observations remained that did not fit conveniently into this model. Among these was the observation that members of the Ras superfamily share numerous remarkably close structural similarities (1–4). Nowhere is this similarity greater than between the Ras proteins and the highly related Rap proteins. For example, Ras and Rap share nearly identical effector regions, and both can interact with Raf family kinases. Despite this similarity, Ras and Rap proteins perform quite different functions in vivo, with Ras controlling proliferative and survival signaling and Rap regulating cell adhesions, cell junctions, and polarity (21). How are two such similar proteins segregated to respond to such different sets of signals and produce such different responses? Moreover, for some time, it has been known that Ras can, in certain instances, promote apoptosis; and a poorly understood group of tumor suppressor proteins, the Ras association domain family (Rassf), had been identified as RBD-containing putative Ras effectors involved in Ras-induced apoptosis (22). Finally, the intensity and duration of Ras pathway activation (notably that of the ERK pathway) can exert profound effects on cell fate. How can the dynamic range of Ras pathway activation be regulated in situ?

It is these three important subjects that are discussed in the accompanying minireviews. In our first minireview, “Specifity in Ras and Rap Signaling,” Judith H. Raaijmakers and Johannes L. Bos provide a concise overview of the GEFs and GAPs that target Ras and Rap and how these regulatory proteins help define signaling specificity mediated by Ras and Rap. In our second minireview, “The Rassf Family of Tumor Suppressor Polypeptides,” Joseph Avruch et al. outline recent advances in our understanding of the Rassf Ras-associated tumor suppressors, notably how these proteins link in turn to a conserved pro-apoptotic family of protein kinases, Mst1/2 (mammalian sterile-20-like kinase-1/2). In the final minireview of this series, “Signaling Threshold Regulation by the Ras Effector IMP,” Sharon A. Matheny and Michael A. White discuss IMP (impedes mitogenetic signal propagation), an RBD-containing E3 ubiquitin ligase that functions as a rheostat controlling the intensity of activation of the Raf family kinases, a process that may affect cell fate.

It is becoming clear that Ras proteins are part of a complex network of signaling nodes rather than discrete components of linear pathways (2). The complexity of Ras protein regulation and the continuing identification and characterization of Ras targets with novel functions have cast aside old models of signal transduction. Together, these minireviews shed light on emerging areas of Ras research and reveal a new appreciation for the complexity of Ras signaling.

REFERENCES