Ras ubiquitination: Coupling spatial sorting and signal transmission

H-Ras, N-Ras, and K-Ras proteins have distinct biological properties, despite ubiquitous expression and similar affinities for regulators and effectors. C-terminal hypervariable regions that distinguish H-Ras, N-Ras, and K-Ras proteins direct them to distinct membrane compartments, where they may encounter regulators and effectors at different local concentrations. Jura and coworkers now report that these membrane-targeting domains direct differential ubiquitination of Ras proteins and so provide a molecular mechanism to explain the sorting process and, perhaps, some of the dramatic differences in biological potency among H-Ras, N-Ras, and K-Ras proteins.

Biological differences between members of the Ras family (K-Ras, N-Ras, and H-Ras) have been recognized for many years but have not been understood fully at the molecular level. Ras proteins play distinct roles in human cancer despite sharing the same regulators and effectors and despite similar patterns of gene expression. K-Ras is activated frequently in cancers of the lung, colon, pancreas, endometrium, and biliary tract, whereas activating mutations in N-Ras or H-Ras are rare in these diseases. On the other hand, H-Ras is frequently mutated in tumors derived from salivary glands, but N-Ras and K-Ras are not (http://www.sanger.ac.uk/genetics/CGP/). Activated alleles of N-Ras can lead to metastatic melanoma, whereas activated H-Ras is associated with Spitz nevi that do not progress to malignant disease (Bastian et al., 2000). Germline activating mutations in H-Ras cause Costello syndrome, a developmental disease that is associated with increased incidence of cancer (Aoki et al., 2005), whereas germline mutations in K-Ras can cause Noonan's syndrome (Schubbert et al., 2006). Analysis of Ras gene function in mice has revealed that K-Ras is an essential gene, but H-Ras and N-Ras are not, even when knocked out in combination (reviewed in Malumbres and Barbacid, 2002).

How can we account for these striking biological differences? All three Ras proteins are farnesylated, but they have different secondary membrane localization signals. N-Ras is acylated with one palmitoyl group and H-Ras with two. On the other hand, K-Ras4B, the most common splice variant, contains a polybasic stretch of amino acids (the K-Ras4A splice variant contains a single palmitoyl group like N-Ras). These different modifications are largely responsible for their localization in different plasma membrane microdomains, as well as different endomembrane compartments. For example, a constitutive depalmitoylation/repalmitoylation cycle is responsible for the continuous shuttling of H- and N-Ras proteins between the plasma membrane and Golgi complex (Rocks et al., 2005). K-Ras, previously thought to reside exclusively at the plasma membrane, has now been shown to relocalize to other endomembrane compartments, including endoplasmic reticulum, Golgi, and mitochondria, by a mechanism dependent on phosphorylation within the polybasic domain (Bivona et al., 2006). These dramatic spatial separations seem capable of explaining many of the biological differences noted above, since Ras regulators and effectors are themselves likely to be differentially localized. A new *Molecular Cell* paper by Jura et al. takes another major step toward explaining exactly how spatial sorting is regulated: they show that H-Ras and N-Ras, but not K-Ras4B, can be subject to ubiquitination (Jura et al., 2006), and that this differential modification plays a key role in compartmentalization and thus signal transduction.

Ubiquitination, the covalent attachment of ubiquitin to lysine residues of target proteins, has been implicated in the regulation of a wide variety of cellular functions. Ubiquitin, a highly conserved protein of 8 kDa, has several lysine residues itself, and ubiquitin molecules can form different types of chains by a process known as polyubiquitination. Lysine 48-linked polyubiquitin chains target substrates for proteosomal degradation. Other types of ubiquitin conjugates such as monoubiquitin or lysine 63-linked chains regulate other functions independently of proteolytic degradation (Haglund and Dikic, 2005). Among these, ubiquitination is well known to act as a regulatory signal directing internalization and sorting in the endocytic compartment of many membrane proteins (Hicke and Dunn, 2003).

Ubiquitination of H-Ras stabilizes its association with endosomes and modulates H-Ras' ability to activate the MAPK pathway. H-Ras ubiquitination was not affected by EGF treatment or by its activation state but still may be subject to regulatory mechanisms. The hypervariable region of H-Ras (the last 25 amino acids) is sufficient to dictate ubiquitination but does not seem to be the site of modification. A different lysine residue in the remaining portion of the H-Ras protein must be the acceptor site for ubiquitin. Both farnesylation and palmitoylation are necessary for ubiquitination. Jura et al. suggest that it is the subsequent localization at specific membrane microdomains that determines H- and N-Ras ubiquitination. It is also possible, however, that the hypervariable region may physically interact with a targeting component of the ubiquitin ligase complex.

An H-Ras mutant that can no longer be ubiquitinated is 4-fold more potent than wild-type at activating Erk, and this correlates with H-Ras' increased ability to recruit Raf-1 to the membrane fraction. Conversely, an H-Ras protein fused at its N terminus to an ubiquitin moiety preferentially localizes to early endosomes and is less efficient at recruiting Raf-1 and activating Erk. Thus, H-Ras ubiquitination and the resulting association with the endocytic compartment appears to impair H-Ras' ability to activate the MAPK pathway. This would be consistent with the known role of endocytosis in signal termination by removal of activated receptors from the plasma membrane. However, it is now clear that endocytic compartments are sites of active signaling and that endocytosis can play a crucial role in signal propagation and amplification and in the spatiotemporal regulation of signaling pathways (Miaczynska et al., 2004).

It will be interesting to determine the ability of ubiquitinated Ras to regulate other Ras effector pathways. It is plausible that, whereas endosomal-localized Ras may be impaired in its ability to interact with Raf, it could preferentially interact with other of Ras' many effectors and selectively activate other pathways. Even

results on the MAPK pathway should be interpreted with caution. This study does not rule out the possibility of localized Erk activation in the endosomal compartment having specific substrates and functions. It is intriguing that at least two MAPK pathway scaffolds, β-arrestin and MP1, are known to localize in endosomes (Kolch, 2005). SEF, a Golgi-localized scaffold, suggests a molecular mechanism for the differential spatial regulation of the MAPK pathway: SEF binding to Mek and Erk inhibits Erk translocation to the nucleus and allows signaling to cytosolic substrates but not nuclear substrates (Torii et al., 2004). It is possible that the various Ras isoforms will recruit Raf kinases (and other effectors) to different membrane subcompartments under different circumstances. This, in combination with the role of compartment-specific scaffolds, may result in Erk signaling output being channeled to different substrates and different functions.

A better understanding of the mechanisms regulating the segregation of the various Ras isoforms into different membrane compartments and how that is coupled to their signaling and biological properties in different cell types, may offer clues to their still unexplained mutational spectrum. Furthermore, as illustrated by the work of Bivona et al., it may also suggest new approaches for therapeutic intervention (Bivona et al., 2006). Despite the disappointing results of farnesyltransferase inhibitors as anti-Ras cancer drugs, modifications regulating other steps in their membrane localization process may offer new targets for isoform-specific, anti-Ras therapies.

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Selected reading

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