Dimerization Opens New Avenues into Ras Signaling Research
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Several reports using recently developed physicochemical and imaging techniques have provided experimental evidence for the existence of dimers of Ras proteins, which was first postulated more than 25 years ago. These data have sparked renewed interest in the potential physiological and pathological importance of homooligomerization of Ras proteins. Understanding the structural and functional properties of Ras dimers should provide mechanistic explanations for unanswered questions regarding the involvement of Ras proteins in signaling pathways that contribute to both normal physiology and oncogenic processes. Furthermore, this knowledge should enable the development of new drugs targeting Ras proteins.

The Ras family of guanosine triphosphatase (GTPases) comprises three "canonical" isoforms (H-Ras, N-Ras, and K-Ras), which are associated with signal initiation at the plasma membrane. The different Ras isoforms appear to be functionally specific and are variably expressed in different cell lineages and tissues. Ras dimers first appeared in the scientific literature in a 1988 report describing the recovery of Ras proteins with a molecular weight indicative of homo-oligomers, possibly dimers, in radiation inactivation experiments performed on H-Ras–transformed cells or purified Ras proteins. That initial description of Ras dimers was followed by the characterization of the crystal structure of purified Ras proteins bound to guanosine diphosphate (GDP) or guanosine triphosphate (GTP). The initial descriptions of Ras crystals offered a structural basis for understanding the known biochemical and regulatory properties of normal and oncogenic Ras proteins without invoking the existence of dimers, thus contributing to the oversight or dismissal of the concept of functional Ras dimers at that time. More than a decade later, Ras dimers were mentioned again, this time in the context of the dimerization of kinases of the Raf family, which are activated by Ras proteins and are the first kinases in the three-tiered mitogen-activated protein kinase (MAPK) cascade. Crystal structures of B-Raf revealed amino acid residues conserved in all Raf proteins that mediate direct, side-to-side dimer formation. Furthermore, Raf dimers play a critical role in physiological, Ras-dependent activation of normal Raf proteins and in the pathological activity of various cancer-associated Raf mutants. Whereas dimerization has little impact on the transforming ability of B-Raf mutants with high kinase activity (B-Raf V600E), the ability to dimerize profoundly increases the transforming activity of other Raf mutants with intermediate or impaired kinase activity. Raf dimers are also important for the therapeutic response and mechanism of resistance to various inhibitors of the kinase activity of Raf. For example, ATP-competitive inhibitors of the kinase activity of B-Raf promote B-Raf homo- and heterodimerization, which provides a conceptual framework for understanding the paradoxical activation of downstream signaling in cells that exhibit resistance to these inhibitors and suggests that blocking Raf dimerization may be therapeutically beneficial. Given that Raf signaling is influenced by dimerization and that Ras proteins are the GTPases that activate these kinases, there is renewed interest in investigating the potential functional importance of Ras dimers. Artificially enforced homodimerization of H-Ras or KRas activates C-Raf (Raf-1), but whether Ras dimerization occurred natively or in vivo was not explored in this study. Several recent reports using sophisticated physicochemical and imaging techniques—including total reflectance Fourier transform infrared spectroscopy (AT-FTIR), Förster resonance energy transfer (FRET), fluorescence correlation spectroscopy (FCS), timeresolved fluorescence anisotropy (TRFA), and single-molecule tracking (SMT)—have produced experimental evidence for the existence of N-Ras, H-Ras, or K-Ras dimers under various experimental conditions. Güldenhaupt et al. noted that Ras forms dimers in at least 50 of the 71 published x-ray structural models and showed that lipidated N-Ras proteins form dimers in artificial membranes using AT-FTIR, biomolecular simulations, and FRET.
The authors propose that amino acid residues located in helices α4 and α5 and the loop between strands β2 and β3 of N-Ras form a stable dimerization interface, and these residues are conserved in H-Ras and K-Ras proteins. Lin et al. characterized various H-Ras mutants anchored to supported lipid bilayers using a combination of FCS and TRFA and confirmed the existence of HRas clusters. Photon-counting histogram analysis and SMT indicated that the H-Ras clusters were dimers, not higher-order oligomers. Interestingly, a Y64A point mutation in the loop between strand β3 and helix α2 abolished dimer formation, suggesting that this region is either part of, or allosterically coupled to, the dimer interface. Similar demonstrations of dimers of K-Ras, the Ras isoform most frequently activated in human tumors, have not been published but seem likely given the structural and sequence similarities of this protein with its related family members. Indeed, “blocking the formation of self-binding and other K-Ras complexes” is a specific goal listed in the National Cancer Institute’s Ras initiative to overcome the inability to successfully target Ras therapeutically. In addition to the three canonical Ras proteins, there are several families of Ras-related proteins. Although homo- and heterodimers of Ras-related GTPases drive specific cellular responses in yeast, such as bud-site selection and polarity establishment, experimental confirmation of the functional importance of dimers of the Ras family in mammals is still absent. If their functional relevance is confirmed, it will be important to ascertain whether such dimers are homo- or heterodimers, because the latter greatly increases the number of possible functional complexes and regulatory complexity of Ras-mediated signaling. Ras dimerization may also be important for the pathology of oncogenic Ras signals, which could offer molecular explanations for unresolved controversies, such as the contribution of homozygous or heterozygous Ras oncogenic mutations for cancer development and progression, and whether wild-type Ras contributes to oncogenic Ras–driven tumorigenicity. Dimerization might also catalyze nanoclustering of membrane-associated Ras. These nanoclusters are transient (<1 s lifetime), small (<20 nm in diameter), and estimated to contain ~6 to 7 Ras molecules (Fig. 1). The spatiotemporal dynamics of these Ras nanoclusters are essential for high-fidelity Ras-dependent signaling through the MAPK cascade. Small molecules that inhibit Ras by targeting an allosteric binding pocket and other drugs that bind the Ras-Sos complex disrupt downstream Ras signaling. Because allosteric interactions are clearly involved in establishing the drug-binding pockets targeted in these approaches, an interesting possibility is that Ras dimers, or even Ras nanoclusters, might become valid therapeutic targets for new drugs aimed at the interface between subunits in Ras dimers.
Fig. 1. Dynamic Ras clustering in the plasma membrane.

(A) Ras monomers and dimers viewed from the intracellular side of the plasma membrane. The inset shows a Ras dimer based on the crystal structure PDB ID 4LRW.

(B) Dimers may contribute to the formation of Ras nanoclusters, which are membrane nanodomains that transiently form and contain several membrane-bound Ras units. Whether these units are composed of complexes of dimers, complexes of dimers and monomers, or other high-order oligomers is unknown.