MAMMALIAN CELL GENETICS

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During 1994, work in our group fell into two areas: signal transduction, the pathways that process the biochemical information that directs cellular activities, and the detection and characterization of genetic lesions in cancer cells. The first area stems from our long-term interest in the RAS genes, the genes that we first demonstrated were commonly activated in human cancers and that we subsequently discovered to be conserved in evolution. The second area stems from the use of representational difference analysis, or RDA, a method for comparative genomic analysis. These latter studies were conducted in collaboration with Dr. Nikolai Lisitsyn.

Signal Transduction

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We have been studying RAS pathways in yeasts and vertebrates. In the budding yeast, *Saccharomyces cerevisiae*, RAS regulates adenylyl cyclase and is required for the progression of the cell through the G_1 phase of the cell cycle (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). Our studies in this organism are nearly complete. Vincent Jung (Jung et al. 1995) has cloned the gene for a new component required for normal RAS function in *S. cerevisiae.* Called *SHR5* (the fifth suppressor of hyperactive RAS), it appears to be required for the efficient localization of RAS to the membrane. Studies of *SHR5* also confirm earlier studies demonstrating multiple functions of RAS in *S. cerevisiae.* Kathy O'Neill, a graduate student from Columbia University, continues to study the cellular role of CAP (the second suppressor of hyperactive RAS), a protein that we demonstrated to be associated with adenylyl cyclase and to be required in vivo for the efficient interaction of the latter with RAS. She has identified cytoskeletal elements that interact with CAP and that may direct its localization and consequently that of adenylyl cyclase to regions of the cell where it can encounter RAS.

The majority of our recent studies of RAS have been in the fission yeast *Schizosaccharomyces pombe.* In this organism, RAS is required for sexual differentiation. It regulates gene expression, as shown by Hao-Peng Xu and others (Xu et al. 1994), by way of a protein kinase cascade (Wang et al., *Mol. Cell. Biol. 11:* 3554 [1991]), and it also regulates cellular morphology by way of a completely distinct pathway, as shown by Eric Chang (Chang et al. 1994). These two distinct pathways are the clearest demonstration of multiple RAS functions in the same organism, a conclusion that was drawn first from studies in budding yeast (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). The morphogenic pathway has a homolog in budding yeast and may involve proteins that regulate another GTP-binding protein, a member of the RHO family (Chang et al., *Cell 79:* 131 [1994]). This in turn may regulate another protein kinase, SHK1, which is likewise conserved in evolution, as shown by Stevan Marcus and others (S. Marcus et al., in press). Further studies in the fission yeast by V. Jung have led to the discovery of a new mutation in RAS that dominantly interferes with wild-type RAS function (Jung et al. 1994). These studies suggest the existence of multiple independent regulators of RAS in *S. pombe.*

The kinase cascade regulated by RAS in *S. pombe* is conserved in evolution, and it is now called a MAP kinase module. We have extended our studies to this module. Working with the budding yeast pathway required for sexual differentiation, S. Marcus discovered that STE5 is a scaffolding protein that can interact with the STE11, STE7, and FUS3 protein kinases (Marcus et al. 1994). This led us to postulate that STE5 promotes the interaction of these protein kinases and also serves as an insulator, limiting cross talk between this MAP kinase module and others with distinct functions and regulators. In *S. pombe,* byr2 is the protein kinase required for sexual differentiation that interacts directly with RAS. Maureen Barr, a graduate student from Columbia University, has identified another upstream regulator of byr2. The existence of this upstream regulator may help explain the concerted action of RAS and G proteins in the sexual differentiation of *S. pombe* (Xu et al., *Mol. Cell. Biol. 14:* 1333 [1994]). Maureen Barr and Hua Tu, a graduate student from SUNY, Stony Brook, are identifying the domains of byr2 that interact with its upstream regulators.

The observation that a MAP kinase module was conserved in evolution and that this module was regulated by RAS in both fission yeast and vertebrates led to the demonstration that RAF was an immediate downstream target of RAS in vertebrates (Van Aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]). RAF is itself encoded by a proto-oncogene and is a protein kinase capable of activating the MAP kinase module. Current work by Javor Stolarov, a graduate student from Columbia University, is directed at defining the domains of RAF that are involved in its regulation. To ask further if RAF mediates RAS effects in vertebrates, Michael White created mutant RAS proteins that fail to interact with RAF and complementary RAF mutants that restored interaction (White et al., *Cell 80:* 533 [1995]). Experiments with these mutants led to the unambiguous conclusion that RAF can mediate transformation of mammalian cells and induction of gene expression by RAS. However, these same types of experiments also led to the clear conclusion that other RAF-independent pathways can also contribute to transformation by RAS (White et al., *Cell 80:* 533 [1995]).

As in fission yeast, RAS thus has multiple targets in vertebrate cells. To begin to define these, Linda Van Aelst has used the yeast two-hybrid system of Fields and Song to identify new genes encoding proteins that bind to RAS (Van Aelst et al. 1994).