MAMMALIAN CELL GENETICS

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Work in our group is divided into three parts. The first is the study of the signal transduction pathways that relate to the Ras proteins, small GTP/GDP-binding regulatory molecules. Ras proteins are frequently activated by mutation in human cancers. The second is the application of representational difference analysis (RDA) to the definition of the acquired genetic lesions of breast and gastrointestinal cancers. RDA is a method, developed in collaboration with Nikolai Lisitsyn, for the comparison of similar genomes (Lisitsyn et al., *Science 259:* 946 [1993]). The third is the development of combinatiorial techniques for the discovery of small molecules capable of binding to and altering the biological activity of signal transduction proteins. These studies, emerging out of earlier work with chemists at Columbia University, are conducted in collaboration with Peter Nestler (Ohlmeyer et al., *Proc. Natl. Acad. Sci. 90:* 10922 [1993]).

Ras Signaling Pathways

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Previous work from our group defined the effector targets of the Ras proteins in the budding yeast *Saccharomyces cerevisiae* (Toda et al., *Cell 40:* 27 [1985]), the fission yeast *Schizosaccharomyces pombe* (Wang et al., *Mol. Cell. Biol. 11:* 3554 [1991]; Van Aelst et al., *Proc. Natl. Acad. Sci 90:* 6213 [1993]; Chang et al., *Cell 79:* 131 [1994]), and mammalian cells (Van aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]). Most recently, we provided the most rigorous genetic evidence that one target for Ras in mammalian cells is the Raf protein (White et al. 1995). Our studies also provide clear evidence for other targets important to the ability of Ras to induce malignant transformation, morphological change,

mitosis, and gene expression (White et al. 1995; Joneson et al. 1996). We have therefore directed considerable effort to the discovery of, and analysis of, other potential targets for Ras (Van Aelst et al. 1995). The candidate targets are found by two hybrid interactions (Fields et al., *Nature 340:* 245 [1989]). The threshing of candidates proceeds by mutational analysis, coupled with gene transfer and tests for genetic interactions.

In the paper by White et al. (1995), we demonstrate a general genetic approach to testing the importance of particular protein/protein interactions. Using the two-hybrid system, we generate allele-specific mutations between genes encoding interacting proteins. We are applying this approach to a number of signal transduction problems, including an analysis of the interactions between Raf and 14.3.3, a highly conserved protein with which Raf interacts (Freed et al., *Science 265:* 1713 [1994]; Irie et al., *Science 265:* 1716 [1994]) and an analysis of other candidate Ras targets.

Concomitant with these mammalian studies, we have continued our studies of the Ras pathway in yeasts. In particular, we have identified a new gene product that appears to facilitate the arrival of Ras at its proper site within *S. cerevisiae* (Jung et al. 1995). We continue to explore the function of Cap, an adenylyl-cyclase-associated *S. cerevisiae* protein that is required, in vivo, for Ras activation of adenylyl cyclase (Field et al., *Cell 61:* 319 [1990]). Two-hybrid and biochemical studies indicate that Cap can bridge adenylyl cyclase to Sla2, a yeast homolog of mammalian talin. Mutational studies indicate that this bridging function is required for the sensitivity of adenylyl cyclase to Ras in vivo.

Our earlier studies in *S. pombe* revealed two distinct Ras-dependent pathways, one leading to activation of a MAP kinase module, through the direct activation of the Byr2 protein kinase (Wang et al., *Mol. Cell. Biol. 11:* 3554 [1991]; Van Aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]), and one leading to the activation of a Rho-like protein, Cdc42, through a protein complex involving the Scd1 and Scd2 proteins (Chang et al., *Cell 79:* 131 [1994]). Both pathways are required for sexual conjugation. We have identified a second component that interacts with Byr2, namely Ste4, a leucine zipper protein, and have shown that Ste4 and Ras1 both bind the regulatory domain of Byr2, but at separable sites. The Ste4 and Ras1 proteins evidently act together, although they can act partially independently to activate Byr2. Ste4 shows weak homology with Ste50, an *S. cerevisiae* protein involved in sexual differentiation in that organism. Ste50 binds Ste11, the *S. cerevisiae* homolog of Byr2, and Ste50, like Ste4, is capable of homotypic interaction. We have hypothesized that Ste4 (and perhaps Ste50) acts by causing the dimerization of its target protein kinase.

A potential target for *S. pombe* Cdc42 has been identified, the Shk1 protein kinase (Marcus et al. 1995). It is the homolog of mammalian $p65^{PAK}$ and *S. cerevisiae* Ste20, and genetic evidence suggests it may mediate some (if not all) of the effects of Cdc42 on cell shape and conjugation. Thus, this kinase may also be indirectly under the control of Ras1 in *S. pombe*, through the latter's effects on Scd1 and Scd2.

Genomic Difference Analysis of Human Cancer

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In 1993, we published a powerful method for genomic difference analysis called RDA (Lisitsyn et al., *Science 259:* 946 [1993]). In collaboration with N. Lisitsyn, we have utilized RDA to identify probes for loci that undergo amplification, homozygous loss, or loss of heterozygosity in tumor cell lines and in tumor cells (Lisitsyn et al. 1995a,b). Several of the loci we have identified are frequently the sites of genetic alteration in cancers of gastrointestinal origin. We are searching for transcripts from these loci. Using RDA, we have also discovered several loci in humans that appear to contain deletions (or insertions) within the normal population.

Most of our effort now focuses on breast cancer, using material sent to us from collaborators at Sloan-Kettering Memorial Cancer Center (Dr. Larry Norton) and North Shore University Medical Center (Dr. Margaret Kemeny). From these sources, we have identified greater than a half dozen loci, including several previously unidentified, that become amplified in breast cancer, and greater than a half dozen loci that appear to suffer repeated homozygous loss in breast cancers. Together, these probes detect lesions in nearly 90% of aneuploid tumors. Our future work will include continuing to identify loci that are common sites of genetic alteration in breast cancer, correlating these alterations with disease outcome and response to therapy, and isolating the genes and gene products that are the targets of these alterations.

Combinatorial Chemistry

D. Dong

In 1993, together with the Still lab at Columbia University, we published a method for the efficient generation of libraries of organic molecules indexed with molecular tags (Ohlmeyer et al., *Proc. Natl. Acad. Sci. 90:* 10922 [1993]). Recently, Peter Nestler from the Still lab was appointed to the staff at Cold Spring Harbor Laboratory. In collaboration with him, we have planned to generate libraries of branched peptide-like molecules in search of small molecules that can behave locally like antibodies, i.e., that can bind to directed protein targets with high specificity. We are especially interested in targeting domains of the Ras protein, in the hope of interfering with its function. In preparation for this, we have generated reagents and designed systems to detect the binding between Ras and members of our libraries.

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In Press

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