Pathway discovery: The road to Ras and MAP kinase

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In 1980 I was recruited to the Institute of Cancer Research (ICR) by its new director, Robin Weiss. After seven years as a postdoctoral fellow, at last I was a tenure-track faculty member and anxious to do something really interesting. During my time as a postdoc at the Sidney Farber Cancer Institute (now Dana-Farber Cancer Institute) in Harvard Medical School, I had been very impressed by Robert Weinberg's work showing that oncogenes could be detected in the DNA of chemically transformed cells by transfection of this DNA into normal NIH3T3 cells (R. A. Weinberg, *Nat. Cell Biol.* **13,** 876; 2011). This functional assay seemed to be the first clear way to clone cellular genes that cause cancer and so, as many others, I also wanted to try it. Fortunately, Alan Hall was recruited to the ICR soon after I was, and we set up a rewarding collaboration that led to the cloning of NRAS, the third member of the Ras gene family.

Despite this success, searching for more human oncogenes was frustrating, so our thoughts turned to the Ras oncoprotein and if its function was related to that of normal Ras. Ras proteins had been shown to localize at the inner plasma membrane surface and to bind guanosine tri- and diphosphate (GTP and GDP), indicating that they might be involved in signalling pathways initiated at the plasma membrane, similarly to the heterotrimeric G proteins that bind GTP when seven-transmembrane-spanning receptors are activated. This idea received considerable support from two sources: first, Dennis Stacy and collaborators showed that growth factor signalling by receptor tyrosine kinases was inhibited if cells were micro-injected with an antibody that blocked the Ras protein; second, genetic analyses in *Drosophila melanogaster* and *Caenorhabditis elegans* showed that Ras function was downstream of receptor tyrosine kinases. Furthermore, normal Ras proteins were known to hydrolyse GTP, whereas the oncogenic mutants did not. This suggested that these mutants might be always activated, which was a very plausible mechanism for an oncogene.

Based on these observations, our working model was that in normal cells, Ras was activated to the GTP-bound state by growth factor receptors and switched on cytoplasmic signalling pathways, ultimately leading to gene expression in the nucleus. In contrast, oncogenic mutants that could not hydrolyse GTP were constitutively active in the absence of growth factors. The question was how to identify the cytoplasmic signalling events turned on by active Ras. Several lines of evidence indicated that cytoplasmic serine and threonine protein kinases might be involved, but comparing Ras-transformed and non-transformed cells at the steady state had not been fruitful. We needed a way to look at very early events following Ras activation. This was provided by Jonathan Morris, a postdoctoral fellow in Alan's lab. During his graduate studies, he had used 'scrape loading' to introduce proteins into cells by gently scraping the cells off the surface of a tissue culture dish in the presence of a protein solution. Jonathan showed that scrape-loading Ras oncoprotein worked because it led to DNA synthesis and signs of transformation, but we did not know whether cytoplasmic serine and threonine protein kinases were activated.

The turning point came when I heard a talk by Jim Ferrell from Steve Martin's lab at the 1989 Oncogene Meeting at Hood College in Frederick, Maryland USA, a great venue at that time to learn the latest scientific developments and, incidentally, to have a lot of fun. Jim described a very elegant approach to detect signalling-activated kinases, by renaturing western blots and performing a kinase assay *in situ* by adding radioactive adenosine triphosphate (ATP). This seemed better than the classical biochemical approach of fractionating cell extracts by chromatography. When Sally Leevers joined my lab as a graduate student in October 1989, I proposed scrape-loading Ras proteins into growth-factor-starved cells and using the renaturation approach to detect Ras-activated kinases. Soon Sally detected an inactive 42 K protein that was activated within minutes of scrape-loading Ras oncoprotein into cells. Importantly, the 42 K band did not appear in response to a Ras protein that was GTP-bound but not localized at the plasma membrane. Then disaster struck! For months Sally could not reproduce the result. After combing the literature for alternative approaches, she fortunately found a method in which the kinase assay was carried out after renaturing the polyacrylamide gel itself. This was more reproducible and allowed us to eventually show that Ras activated 42 K and 44 K bands that phosphorylated myelin basic protein. At this time, there was a lot of interest in growth-factor-activated cytoplasmic 42 K and 44 K kinases that phosphorylated myelin basic protein or microtubule-associated protein 2, so it made a lot of sense that oncogenic Ras could activate these mitogen-activated protein kinases (MAPKs) or extracellular-signal-regulated kinases (ERKs) in the absence of growth factors. Indeed, we were subsequently able to show that growth-factor signalling to MAPKs required normal Ras protein function. Following this, we collaborated with Philip Cohen to show that Ras activates MAPKs through a RAF–MAPK–kinase signalling module.

There have been a number of turning points in my career. Some, as the one described here, have been a talk at a conference, or even a chance meeting, but many others have been the result of ongoing dialogue with people in the lab.

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