GTPase switch: Ras then Rho and Rac

Anne Ridley

As a graduate student at Imperial Cancer Research Fund in London in the late 1980s, I worked on 'oncogene cooperation'. My graduate advisor, Hartmut Land, had shown that two different types of oncogenes, such as Ras and Myc, were needed to transform primary rodent fibroblasts into tumour-like fibroblasts. I made the intriguing observation that expression of oncogenic Ras by itself in primary Schwann cells inhibited proliferation, whereas Ras in combination with Myc or SV40 large T induced transformation. This finding led me to collaborate with Hugh Paterson from Chris Marshall's laboratory in the Institute for Cancer Research. Hugh had mastered the fine art of microinjecting purified proteins into cells, and when he injected my Schwann cells with oncogenic Ras protein, we found that Ras alone inhibited DNA synthesis. The power of microinjection as a tool to study immediate cellular responses to proteins struck me at the time, but after I completed my PhD I wanted to work in the US, and so moved to Boston armed with an EMBO postdoctoral fellowship. However, I soon realised that the research I was doing was not suited to me. When I discovered that Alan Hall, who worked with Chris Marshall on Ras, was advertising a postdoctoral position, I went for an interview and ended up taking the job and returning to London.

Alan Hall's and Chris Marshall's offices and labs were right next to each other, sharing equipment and Hugh's expertise, and providing a fantastic environment in which to work as a postdoctoral fellow. I considered initially working on neurofibromatosis 1, which had just been identified as a GTPase-activating protein for Ras. This seemed to be an excellent link to my graduate Ras and Schwann cell days, as neurofibromatosis is a Schwann cell cancer. I had even been to a neurofibromatosis workshop where I had presented my data on Ras in Schwann cells. My ideas changed, however, after a few trips to the library. Alan and Hugh had just published a paper showing that a recently identified relative of Ras called RhoA induced dramatic changes to cell shape. After reading papers such as Dafna Bar-Sagi's report that injection of oncogenic Ras stimulated membrane ruffling, and studies describing the effects of growth factors on the actin cytoskeleton, I became convinced that the link between the actin cytoskeleton and Ras and Rho proteins was the area I wanted to pursue, and so I asked Hugh to teach me how to microinject cells.

Anne Ridley is in the Randall Division of Cell and Molecular Biophysics, King's College London, New Hunt's House, Guy's Campus, London, SE1 1UL, UK.
e-mail: anne.ridley@kcl.ac.uk

This was a major turning point that shaped my scientific career. The combination of cell microinjection with Rho and Rac proteins together with stimulation with different types of growth factors, lipids and cytokines led us to identify the roles of Rho and Rac in regulating the actin cytoskeleton. With microinjection, a whole experiment could be done and analysed in a day: I would microinject proteins at multiple concentrations in the morning, and in some cases would stimulate the cells with growth factors or cytokines. After fixing and staining the cells, I would take reels of photos. Finally, I would develop the films by hand and make the prints myself in the dark room. This sounds very old-fashioned now that we have digital images, but it provided me with complete control over the whole process from culturing the cells to visualizing them on photographic paper.

Using these approaches, I first showed that several different extracellular stimuli, including serum, induced actin stress fibre assembly in serum-starved fibroblasts, similarly to the cellular response to microinjected Rho. Alan Hall, being a chemist by training, suggested I purify the factor in serum that induced stress fibres. I really benefited from the lab expertise in purifying proteins using fast protein liquid chromatography (FPLC) in the cold room — especially the tip to remember to wear a thick sweater. We found that the mystery 'factor' in serum was bound to serum albumin, and was heat-insensitive but sensitive to various phospholipases. This allowed us to identify the culprit as lysophosphatidic acid. The most exciting result came from experiments with C3 transferase, a clostridial enzyme that Klaus Aktories and Alan Hall had shown biochemically could modify RhoA, and was therefore a great tool to inhibit RhoA function. I showed that C3 transferase inhibited the increase in stress fibres induced by serum and other stimuli, uncovering a link between extracellular cues, RhoA activity and actin regulation. This detective work was great fun, and highlights the excitement of being a scientist that still keeps me going whenever experiments are not going well.

The combination of cell biology with biochemistry was very important for the success of this project, but the buzz in the Marshall and Hall labs as other important discoveries (such as the link from Ras to MAPK) were being made, and the freely shared expertise and reagents, also contributed a lot. In the twenty years since this work was performed, we have learnt much more about Rho GTPases, but many unanswered questions remain. Identifying the as-yet unknown actions of many of the twenty human Rho family members, as well as of their numerous relatives in other eukaryotes, will open exciting research paths in the coming years.

© 2013 Macmillan Publishers Limited. All rights reserved