# From RAS to RHO: The making of the great cell biologist Alan Hall (1952–2015)

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**The fields of cell biology and cancer research are deeply saddened by the untimely death of Alan Hall at the young age of 62. There will be many obituaries, so in this remembrance I would like to describe some events in Alan's career, and give a personal view on what I think made him an outstanding scientist and led to him having such a major impact on our understanding of cell biology.**

Alan and I worked together for 12 years. We became close colleagues and a deep friendship developed. Robin Weiss recruited both of us to the Institute of Cancer Research (ICR) in London after he became director in 1980. Robin was very keen to introduce modern methods of cancer research into ICR and was on the lookout to recruit promising molecular biologists and cell biologists. Robin also brought Hugh Paterson with him from his laboratory at the Imperial Cancer Research Fund—Hugh and his cell biology and microscopy expertise were to play a central role in Alan's work.

Alan started at ICR early in 1981. He had gained an undergraduate degree in chemistry at Oxford University and then a PhD with Jeremy Knowles in the enzymology of β-lactamase at Oxford and Harvard University, from which he published his first *Nature* paper (Hall and Knowles, 1976). He then made a decisive change, switching to working with recombinant DNA technology by becoming a postdoc in the laboratories of Ken Murray (University of Edinburgh) and then Charles Weissmann (University of Zurich). Murray and Weismann were major influences on the development and exploitation of recombinant DNA technology, and Alan played a key role in the cloning of α-interferon in Weismann's laboratory.

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I joined ICR about six months before Alan and embarked on a project to identify human oncogenes using genomic DNA transfection into NIH3T3 cells, a methodology pioneered by Bob Weinberg at MIT. Alan and I met soon after he joined ICR and discussed what we were planning to do. A couple of days later, Alan came to see me and said he really wasn't interested in what Robin had proposed as a project; he was much more interested in what I was trying to do and suggested we work together. This suggestion was probably one of the most significant events in both our careers. Our skill sets and, as others observed, our rather different personalities complemented each other beautifully. From the outset I think we both sensed that we could work together effectively.

Although we had our methodologies firmly established, the search for novel transforming activities from human tumor DNA was frustrating. I presented the results of screening about 60 tumor DNAs at a laboratory meeting, and Robin was so critical that Alan and I decided we needed to have a meeting to discuss what to do. He suggested I bring my family out to his home on the next Sunday, and so we took our children to the park in the drizzling rain while we talked. In the end, we decided we had a robust assay and would look at another 20 tumor DNAs. If nothing came out, we would have to think about alternatives for our careers. Fortunately, in those 20 DNAs we identified novel transforming activities, and we were able to clone the third member of the RAS family NRAS (Hall et al., 1983).

The next thing to do was to study how RAS proteins actually worked. The known GDP and GTP binding activities

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**Alan Hall**

of RAS proteins suggested that they were regulatory GTP proteins that might be involved in signal transduction. As we were interested in the biochemical proteins of NRAS proteins and wanted to use recombinant proteins as a probe for biological function, Alan and our student Robin Brown produced cDNAs encoding normal NRAS (Gly12) and an oncogenic mutant NRAS-Asp12 derived from an acute myeloid leukemia NRAS oncogene that Alan had isolated in collaboration with Christoph Moroni. We then worked with Frank McCormick to express NRAS proteins in *Escherichia coli*. An interesting finding from studies with the protein was that microinjecting oncogenic RAS protein rapidly stimulated cell motility, an observation





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that got us thinking about GTPases and cell motility (Trahey et al., 1987). Frank used the NRAS proteins to great effect to identify RAS GTPase-activating protein (GAP), a protein that increased the rate of GTP hydrolysis by normal RAS but did not stimulate oncogenic RAS. These early studies on cell motility and GAP piqued Alan's interest, and were to reemerge when he started to study the RAS-related RHO protein family.

To study signaling by RAS, we had three principles. These principles were very much influenced by the rigor of Alan's thinking, perhaps derived from his training in enzymology. First, we wanted to use recombinant proteins to study early events stimulated by active GTP-bound RAS. We believed that this would be more informative than comparing RAS-transformed cells with their nontransformed counterparts, which seemed to be a rather indirect and potentially messy approach. To study early events, we needed a way of introducing recombinant RAS into cells. Here, Hugh Paterson was to prove invaluable because he had become adept at cell microinjection and could even microinject fragile quiescent Swiss 3T3 cells, at that time one of the best substrates for studying cell signaling. Later on, Alan's laboratory used the technique of "scrape-loading" recombinant RAS proteins into cells that allowed us to carry out biochemical assays (Morris et al., 1989). Second, because it was thought that oncogenic RAS would activate intracellular signaling in the absence of growth factors, active RAS proteins should be able to do this in growth factor–free conditions. Third, blocking normal RAS signaling with dominant-negative mutants should stop a RAS-dependent signaling response to growth factors. These principles were crucial to dissecting RAS signaling, and Alan then applied them to great effect to study RHO family GTPase signaling.

In the mid-1980s, RHO was identified as a protein highly related to RAS. Since the function of RHO was unclear, Alan saw this as great opportunity to study something new and decided to use similar approaches for RHO that were proving to be powerful tools to study RAS. He produced recombinant RHO proteins that could be studied biochemically in vitro and introduced into cells by microinjection. Because activated mutant versions of RHO did not occur naturally, he made the glycine-to-valine mutant at codon 14 equivalent to Val12 in RAS. One of the first fruits of producing recombinant RHO protein was the demonstration with Klaus Aktories that the C3 toxin of *Clostridium botulinum* ADP-ribosylated Rho and that ribosylation of Rho accounted for the cytoskeletal effects of C3 (Aktories et al., 1989). These studies with C3 provided a powerful tool for studying RHO and provided an insight into the function of RHO. The next key step was RHO protein microinjection experiments performed by Hugh Paterson that further demonstrated that RHO was acting on the actin cytoskeleton (Paterson et al., 1990). However, the crucial steps were taken when Anne Ridley joined Alan's laboratory as a postdoctoral fellow.

Anne took on two projects, one to study RHO itself and the other a recently identified closely related protein RAC. Her work beautifully exemplified the principles that Alan was using to analyze signaling: the responses to introducing active proteins were rapid, they occurred in the absence of growth factors, and when RHO or RAC were inhibited, the ability of external stimuli to evoke the responses, such as LPA stimulation of actin stress fiber formation, was blocked. Strikingly, although they are quite similar proteins, RHO and RAC elicited very different responses on the actin cytoskeleton: RHO was involved in forming stress fibers and focal adhesions whereas RAC drove membrane ruffling. These studies, published in back-to-back papers in *Cell* in 1992 (Ridley and Hall, 1992; Ridley et al., 1992), revolutionized the study of actin in cellular responses by linking activation of signaling pathways of RHO family proteins to specific dynamic actin responses. This work truly revolutionized the field of cell biology and spawned a new field investigating how RHO family GTPases transduce stimuli into cellular responses. This field has had a major impact on our understanding of cell migration, cancer, microbial infection, developmental biology, and neurobiology.

Alan was at the forefront of this revolution, providing new insights into the role of RHO family proteins in cytoskeleton regulation, phagocytosis, cell polarity, and cell motility. He identified GAP proteins and effectors of RHO family signaling. He did not just lead through his experimental work; his many reviews and commentaries also provided critical evaluations of the field and highlighted the key questions.

So what made Alan so great? I believe there were several factors. First, he was highly intelligent. Second he had training in different disciplines: chemistry, enzymology, and molecular biology. This, I think, made him very adaptable and open to new ideas. This adaptability was reinforced by his expectation to move from laboratory to laboratory. He started his PhD in Oxford, then moved to Harvard, and went on to postdocs in Edinburgh and Zurich before coming to ICR London. He then moved across the city to University College London, followed by translocation to New York and the Memorial Sloan Kettering Cancer Center. Perhaps surprisingly to some reading this, Alan had no formal training in cell biology. In the early days of our collaboration, he was quite perplexed by what he saw as the imprecise nature of cell biology, and if you had said to me at that time he would become Editor-in-Chief of *JCB*, I would have been quite amazed. However the incisive nature of his intellect allowed him to him identify important questions, and his adaptability meant he was prepared to do the work to take them on. Coupled with his intellect was great warmth of spirit and generosity, perhaps derived from his Yorkshire background, where people are known not only for their directness but also their friendliness. These aspects of his character made him a great collaborator and a wonderful mentor of young scientists. He was always willing to discuss data and ideas with students and postdocs. Alan was devoted to *JCB*, and one of his last pieces of work for the journal was a wonderfully crafted piece with Kenneth Yamada discussing reproducibility in cell biology studies (Yamada and Hall, 2015). His passing has saddened many but his science and the memories live on.

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