Sticking to it: Tracking the paths of integrin signalling

Martin Schwartz

In 1979, after completing my PhD in Physical Chemistry at Stanford University, I joined the hunt for the fibronectin receptor as a postdoc in Richard Hynes' lab. But I became fascinated by work from Judah Folkman and others showing that cell adhesion regulated cell growth, and thought that adhesion to fibronectin must regulate growth signals. Richard was tolerant of my chasing this then-heretical notion on the side while pursuing the main project. However, very little was known about signalling at that time, and I made no progress. In 1983, I was offered a principal investigator position at Harvard Medical School but postponed my start to visit Yossi Schlessinger's lab at the Weizmann Institute, to study the effects of cell adhesion on the epithelial growth factor (EGF) receptor. Again, I made little progress. Near the end of my time there, I noted that although EGF stimulated tyrosine phosphorylation of its receptor independently of cell adhesion, total cellular tyrosine phosphorylation was higher in adherent cells independently of EGF. But I was focused on the EGF receptor, and missed the clue.

In my lab at Harvard, while my students worked on 'safer' projects, I continued studying signalling on my own. In the mid 1980s, growth signalling was being rapidly unravelled and intracellular pH emerged as an important mediator. Around that time, integrins were also identified as the elusive fibronectin receptors. One turning point for our signalling studies came in 1987, when I was fortunate to team up with Claude Lechene, who had built a microscope for measuring pH in cells. Using his system, I saw that in NIH-3T3 cells, integrin-mediated adhesion elevated intracellular pH through the Na^+/H^+ antiporter. Later we saw that in 10T1/2 cells, integrins and growth factors synergized to activate the antiporter. Through a trail that led first to protein kinase C and then to phosphatidylinositol 4-phosphate 5-kinase, we eventually obtained evidence that Rho GTPases lay on the integrin arm of this pathway.

By 1999, I was at the Scripps Research Institute in La Jolla, California, where the connection between integrins and Rho GTPase was our major focus. It was an exciting time for both integrin signalling and Rho GTPases. The pull-down assays for Rho, Rac and Cdc42 GTPase activity had recently been developed; Rho in my lab, and Rac and Cdc42 by Gary Bokoch's group. We found that integrin-mediated adhesion changed the activity state of all three GTPases, a critical confirmation of our previous functional studies. That year, Gary Bokoch hosted a party where I met a new assistant professor named Klaus Hahn, whose background was mainly in chemistry and microscopy. He told me that he wanted to develop assays to visualize signalling events in cells, and wondered what signals to target. It was becoming apparent around that time that Rho GTPases regulated many important cellular functions. I made a strong case for Rac, which seemed likely to show interesting local regulation given its role in cell migration. Klaus developed a beautiful assay for visualizing Rac activity in live cells and published an important paper demonstrating high Rac activity in the lamellipodia of migrating fibroblasts. I was a co-author because my lab had provided some minor help, but I always felt that my major contribution was suggesting Rac in the first place.

Klaus helped Bill Kiosses, my very talented microscopist postdoc, to establish the assay in my lab. Bill's first experiment was successful, showing high activity with an active mutant of Rac, lower with wild-type Rac and lower still with inactive Rac. Klaus had already published these results, but I liked the images and pinned them on my bulletin board. There they sat for two weeks, until, one afternoon, I realised that something was wrong. The active Rac mutant should be fully activated everywhere, yet the assay showed a sharp gradient, with activity high in lamellipodia and low elsewhere. At that time, another exceptional postdoc, Miguel del Pozo, had discovered that integrin-mediated adhesion regulated not only Rac GTP loading ('activity'), but also independently controlled the translocation of active Rac from the cytoplasm (where it is held by RhoGDI) to the plasma membrane, where it interacts with effectors. Klaus's fluorescence assay was based on the binding of Rac to an effector construct, and thus might not have registered cytoplasmic Rac bound to RhoGDI. Indeed, Miguel showed that integrin-mediated adhesion drove the dissociation of active Rac from RhoGDI, its membrane translocation, and its binding to the effector construct in lamellipodia. The paper, published in 2002 in *Nature Cell Biology*, revealed a new level of spatial regulation for Rac, and set the stage for demonstrating that integrins control Rac localization to membrane binding sites by regulating caveolin-dependent trafficking of lipid rafts. Miguel has continued this work very successfully in his own lab, and Klaus also continued to make major contributions on Rho GTPases. My training by Klaus in fluorescence assays eventually led to our development of the tension sensors for measuring forces across specific proteins.

To my mind, these events illustrate the importance of the right collaborators (such as Claude, Klaus, Bill and Miguel) and of seeing the data without preconceptions. Had I seen the phosphotyrosine data more clearly in 1983, integrin signalling might have gotten off the ground years earlier. Since then, I have done my best to let the data speak for themselves. What they have to say is usually more interesting than any of my own ideas.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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