Keith Burridge: Cultivating knowledge on Rho

Burridge studies how Rho proteins regulate everything from focal adhesions to leukocyte migration.

Throughout his career, Keith Burridge has helped propel the burgeoning field of cytoskeletal research. Among other things, he's credited with discovering two major components of focal adhesions, talin (1) and paxillin (2). Despite these achievements, he wryly laments a few missed opportunities: for instance, as a postdoc in Jim Watson's lab, Burridge developed a method to use antibodies to detect proteins in polyacrylamide gels. But because he took some bad advice (someone told him nitrocellulose didn't hold proteins well), he was unable to simplify his laborious process and missed out on inventing the Western Blot.

Nevertheless, Burridge's laboratory at the University of North Carolina (UNC) continues to break new ground in cytoskeletal research. For the past two decades or so, much of their work has focused on understanding how Rho proteins are regulated (3) and how they, in turn, control the cytoskeleton in diverse situations (4, 5). We called Burridge to discuss his work and some of the better guidance he's received in his career.

ENGLISH GARDEN

Who were your role models when you were growing up in England?

My father had a strong influence on my career. He had been in the military, and when he retired from that he became a tomato grower. With the cloudy, cold climate, it's pretty hard to grow good tomatoes in England, but it was his passion, and he had a very scientific approach to it. He was always trying out new things to get better results, and I learned a lot from his efforts. He also encouraged my interests in natural history, from ornithology to just looking at bugs in the garden.

Another person who had a large influence on me was my teacher at Canford School, Peter Dawkins. He mentored some of my earliest attempts at experimentation and really encouraged me to pursue those interests.

Did you know from the start that you wanted to pursue a career in research?

Early on, I was quite interested in doing research, but I realized that doing well at that would depend on academic success. So when I went to the University of Cambridge I enrolled as a medical student as a sort of fallback. Fortunately I did well enough in my degree that I didn't need to go on to do clinical training, and instead I went to do a PhD at the Laboratory of Molecular Biology. That was an amazing place; there were four Nobel Prize winners working there at that time, so you can imagine I had a bit of an inferiority complex. [Laughs]

> "[It] was quite a missed opportunity, but it made me realize that the focal adhesion is a very interesting structure."

AMPLE HARVEST

You parlayed your graduate work into a postdoc with James Watson at Cold Spring Harbor…

Actually, I didn't originally go there to work with Jim Watson. As a graduate student in Dennis Bray's lab, I had shown, using peptide mapping, that there were two different myosins in nonmuscle cells. Having worked on the cytoskeleton already, I decided that being a postdoc was the one chance I'd have to move to a completely different area. So in fact, I went to Cold Spring Harbor to study with Joe Sambrook, who worked on the SV40 virus. But I'd only been there for literally a couple of days when I met Jim Watson and he said, "Keith, you should think about continuing to work on the cytoskeleton." Well, who was I to argue with Jim Watson?

PHOTO COURTESY OF PATRICIA SALING

Keith Burridge

At the time, Watson was enamored with images of cells, and particularly with immunofluorescence, but most of the people in his little cell biology group had left or were leaving. He wanted someone to carry on that work, so suddenly there I was: Jim Watson's new postdoc.

You were working on focal adhesions before they were called focal adhesions…

When I arrived at Cold Spring Harbor, both I and a graduate student, Elias Lazarides, had individually made antibodies against α-actinin. Our antibodies showed this beautiful distribution of α-actinin along stress fibers, and, at the fibers' end, the staining terminated in a sort of patch. Together, we published a paper about this in *Cell*. (This was back when *Cell* was really slim and struggling for publications, and the editor actually begged us for more images to fill out the journal.) In that paper, we talked about the patches being the point where the stress fiber terminated at the plasma membrane, and that's where we left it.

The big breakthrough on focal adhesions came later when Benny Geiger discovered vinculin and showed that it was also present at these sites. What's funny is that I and another postdoc, Jim Feramisco, had independently stumbled onto vinculin around the same time. Neither of us had got around to making antibodies to it, though, because we didn't know anyone

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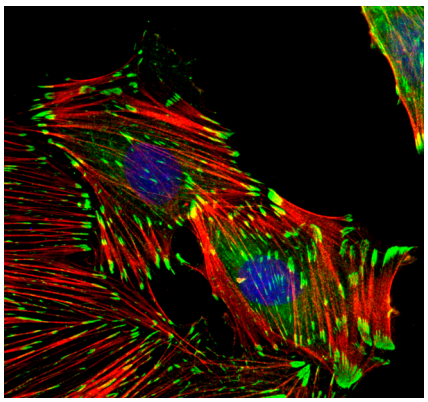

IMAGE COURTESY OF RAFAEL GARCIA-MATA

Actin stress fibers (red) terminate in focal adhesions (green). Nuclei are in blue.

else was working on this protein. That was quite a missed opportunity, but it made me realize that the focal adhesion is a very interesting structure.

You didn't miss paxillin or talin, though…

I realized that lots of other proteins must be involved in this complex, so in my own lab at UNC I—by which I mean several brilliant students and postdocs—went looking for them. That's how we discovered talin. We had no idea what talin's function was, but we generated an antibody against it, and, lo and behold, it stained focal adhesions.

Paxillin was a different story. John Glenney had made some monoclonal antibodies against proteins that were tyrosine phosphorylated in Src-transformed cells. Several of these antibodies stained focal adhesions, but he wasn't very interested in pursuing the proteins, so we struck up a collaboration with him to do that and ended up identifying paxillin.

FERTILE GROUND

What have you focused on since then?

For the last 20 years, since Anne Ridley and Alan Hall first showed that focal adhesions are regulated by the GTPase RhoA, a lot of my lab has been focused on the Rho family: what regulates them and how they regulate adhesions. This has been a fertile area of study for us, and it's taken us into many other areas because it's in the core of the signaling field. For instance, we got interested in Rho guanine nucleotide dissociation inhibitors (RhoGDIs). These are a group of three proteins that hold Rho-family GTPases in an inactive conformation in the cytosol, away from the membrane. We focused on the most abundant one, RhoGDI1, and realized that it's about equimolar with the sum of RhoA, Rac, and Cdc42. Those three proteins compete for binding to RhoGDI1, so, if you overexpress one of them, you'll displace any of the other family members that were bound to RhoGDI. That causes them to be quickly degraded, which in turn has some pretty strong implications for experiments that rely on overexpression of individual Rho family GTPases; the effects you observe may in fact be due to degradation of the other family members.

You've also studied other phenomena where Rho is involved…

> "[Studying Rho GTPases has] taken us into many other areas because it's in the core of the signaling field."

One related area we've studied is cell–cell adhesions, which obviously have some similarities to cell-matrix adhesion. A lot of the lab is now working on how leukocytes interact with endothelial cells. This work was, again, started by a talented postdoc, and what she found was that, if she inhibited Rho in monocytes, the cells would get stuck as they went across the endothelial barrier. But endothelial cells also respond to the interacting leukocytes, and we've found this also involves Rho proteins.

Another focus of the lab is mechanotransduction. For the past couple of years we've had a great collaboration with a physicist here named Richard Superfine. He has a magnetic tweezer system coupled to a microscope that gives us the ability to apply forces to adhesion proteins over a much greater range than is possible with other technology such as laser tweezers.

Is your lab your main hobby?

For many years it was. I used to work horribly long hours. I don't do that so much now, partly because of age.

These days I enjoy spending time with my family. My wife used to be a cell biologist, but she retired about six years ago and now has a new career as a potter. I also have a daughter who's a history major here at UNC. In addition, true to my English heritage, I'm an avid gardener. I love growing vegetables, and we eat a lot of things that I grow myself.

The other thing I like—and I'm nervous to mention this—I'm very into the theater, and I actually write plays. Unfortunately, I've discovered that getting plays published or put on is even harder than getting research papers published or grants funded! But when I retire, if I'm still able to think, I would love to write more.

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PHOTO COURTESY OF WILL OWENS

Former and current lab members threw a symposium to celebrate Burridge's 60th birthday. (Rick Horwitz, former collaborator and guest speaker, is on the right of the second row.)