

Chris Bakal: Look and learn

Bakal studies the signaling networks that control cell shape.

What can you learn about a cell just by looking at it? Quite a lot, as it turns out. A cell's shape can tell you about its behavior and its function in the body, just for a start. And, as Chris Bakal can attest, a cell's shape can also tell you a lot about the signaling pathways at work.

Bakal has been interested in cellular signaling pathways ever since his undergraduate days (1), but his approach to studying them (2) has evolved considerably over time (3–5). Today, in his lab at London's Institute of Cancer Research, Bakal's lab members use computational neural networks and advanced statistical methods to identify and analyze cellular features that inform us about signaling networks. We called him to learn about this approach to studying cell shape and to get a wider view of his career.

SKI RACER

Do you recall the interests you had while growing up?

To be honest, I wasn't particularly interested in science. Growing up in Calgary, I spent most of my time ski racing. I skied quite a bit; I think I barely went to high school. Then I took a year off after high school to ski full-time, and I did reasonably well. I was world ranked in the super giant slalom.

But eventually I decided I had to go to college. First, because I felt internally that it was important to go to school but also because both my parents were academics and took education seriously. My dad is a professor of psychology at the University of Calgary, and my mom also worked there in computer science. I think they really hoped I would go back to school. And I did, eventually. I went to The University of British Columbia at Vancouver as an English major.

Do you still ski?

Actually, these days I'm training for triathlons. I'm hoping to make the age group

world championships either next year or the year after. It sounds cliché, but I've always felt that I do better science when I'm peaking athletically.

How did you first encounter the sciences?

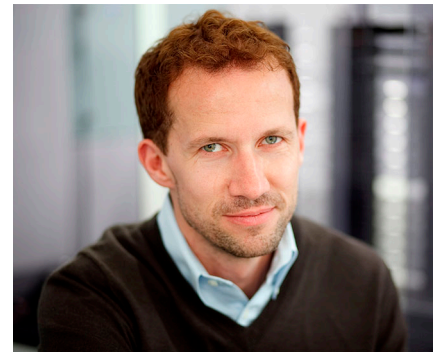
My dad eventually convinced me that I should consider trying to get into med school. And so I thought, "Okay, I'll at least get the prerequisites." I really struggled in the first year, but in the second year I took a course on microbiology and literally everything changed. I was absolutely fascinated by microbiology, and I had really excellent teachers. One of them was Julian Davies, and after my second year I started working in his lab, first as a dishwasher and then as a technician. He was working on *Streptomyces*, a soil bacterium, and at the time there was new evidence that certain elements of human signal transduction were also conserved in bacteria. He had a theory that signaling in bacteria would probably use tyrosine kinases. I thought his work was really exciting, and I decided I wanted to study signal transduction instead of going to medical school.

THE SIGNALING SLALOM

You did your graduate studies with Rob Rottapel at the University of Toronto...

That's right. When I started graduate school I wanted to become an immunologist and study signaling. I started out in Rob's lab working on a project about T cell anergy, but I also had a side project on a rodent protein called Lfc, which is known as GEF-H1 in humans. I started out by overexpressing Lfc in cells and saw that Lfc overexpression causes a very dramatic microtubule-bundling phenotype. That really opened my eyes to the power of cell imaging, and, from then on, I basically never left the microscope.

"Instead of studying one protein, I wanted to study all the proteins involved."



Chris Bakal

PHOTO COURTESY OF TYNAN STUDIO

Your publication record shows this is the last time you focused on a single protein...

I was studying Lfc, doing cell-based assays and finding that both RNAi knockdown and overexpression of the protein have a dramatic effect on cells. But I was also trying to make a knockout mouse. The first time I tried, I failed. The second time, it worked. But we saw no obvious phenotype! That's probably because Lfc is a Rho GTP exchange factor, or GEF—just one of more than 90 Rho GEFs in mammals—and the knockout animals had adapted to use one of those other proteins. Later on, Frieda Miller's group did identify a defect in neurogenesis, though.

I realized that a one-by-one approach wasn't going to be adequate for understanding signaling in the long run. Around that time, people like Tony Pawson and Charlie Boone in Toronto were getting into more systems-oriented approaches. I felt that was the route we needed to take to understand these complex systems.

So that was your aim when you joined Norbert Perrimon's lab for your postdoc?

Yes. Instead of studying one protein, I wanted to study all the proteins involved in Rho GTPase signaling: all the Rho GEFs, all the Rho GAPs, and all the Rho GTPases themselves. To do this, I needed a readout of the pathway.

Many of these proteins are involved in regulating cell shape. For instance, if you activate the GTPase Rac in a cell, the cell becomes huge and flat; whereas if you activate Cdc42, the cell becomes very spiky.

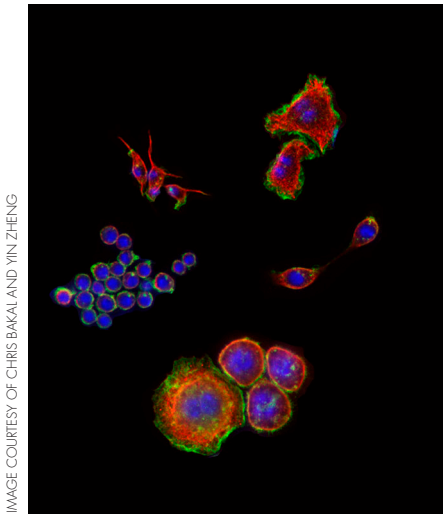


IMAGE COURTESY OF CHRIS BAKAL AND YIN ZHENG

***Drosophila* Kc cells adopt one of five typical shapes.**

I had the idea that, if I knocked out a GEF or GAP involved in regulating these proteins, then this may have similar effects on cell shape as their downstream targets. So that's where I started. But while I was working on those genes, I decided I might as well assay a few hundred others. [Laughs]

Once we analyzed the data, it became clear that we could establish gene–gene interactions or even protein–protein interactions just by looking at the phenotypes. By clustering the mutant phenotypes and examining the literature, we could show that genes in the same cluster tend to physically interact or be present at the same time or place within the cell. This is important because it allows us to predict the function of other genes, where in the cell they are, and what other proteins they may interact with, solely based on looking at their shape phenotype. It was a nice demonstration of the power of this approach.

HOW THINGS SHAPE UP

You've since elaborated on this approach quite a bit...

If you're a biologist, you'll realize that, if you're looking at complex populations such as cells in a plate, the mean or the average score of a given feature might not represent the underlying variability or heterogeneity in the population.

There could be subpopulations in there that are being missed or misclassified if you only consider the mean. This is particularly important when you're talking about a complex phenotype like cell shape.

So, we began thinking of ways to look at the heterogeneity of cell shape within a population. First, we had to define the heterogeneity of the population. And what we found is that an untreated population of, for example, *Drosophila* Kc cells is very heterogeneous but that most cells simply explore variations on one of five different shape categories. Using statistical methods, we could show that these five shapes are discreet, meaning they aren't continuous variations of another. And then we could also infer that cells make fast, switch-like conversions between these shapes. There are intermediate forms, of course, but cells don't spend any time in those shapes because they're unstable. We also found this is true in cells from other organisms. For example, human melanoma cells have a different baseline heterogeneity, but they also exhibit switch-like behavior.

Finally, we showed that knocking down kinases and phosphatases often changes the frequency that cells appear in one or more of the basic initial shapes. It was very rare that knocking down a protein produced an entirely new, stable cell shape. So basically what we were doing was enriching for cells already in one of the original states. I think the results of several kinds of small molecule screens—for example, drug-resistance screens—could also be interpreted as enrichments of preexisting baseline states.

These kind of analyses aren't limited only to questions on cell shape...

Think of all the different antibodies or dyes you could apply to cells. Each one of those provides a different readout, and each one of those readouts is high-dimensional in nature; you can stain for just one

protein and then analyze 400 features about it. The trick is to be sure you are measuring the right features and you're measuring them in enough cells to be certain your findings are statistically significant. And if you are, then you have to find a way to extract those data from these large data sets. That's one area where we've made some big contributions: taking these large datasets and getting some sort of biological sense out of them.

What sense are you extracting now?

We want to understand how many shapes there are. I think this is a grand question. Can we categorize all these shapes? Can we understand a cellular shape space

completely? Can we describe it quantitatively? That's a very big question, I know, but we've already done it with one cell type.

We'd also like to understand how cells change their shape to do specific things—such as migrating or undergoing cell division—and

how the networks that regulate cell shape change in normal versus metastatic cells.

"We want to understand how many [cell] shapes there are."

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Bakal is training mind, body, and neural networks to better understand cell shape.