People & Ideas

Carole Parent: Migrating cells relay the message

Parent studies cell motility in *Dictyostelium*, neutrophils, and cancer cells.

Cells are exquisitely sensitive to even the slightest of chemical gradients. For example, when deprived of nutrients, the amoeba *Dictyostelium discoideum* responds to the chemoattractant cyclic AMP by turning and migrating toward the source and recruiting other cells to follow. Similar behaviors are observed in cells of the immune system and are crucial to the ability to repel infections.

Carole Parent has been at the forefront of the cell motility field since her days as a postdoc in Peter Devreotes' lab at Johns Hopkins University. Moving from *Dictyostelium* (1, 2) to neutrophil (3, 4) chemotaxis, Parent's lab has elucidated the signaling pathways that drive cell migration. We called her at her lab at the National Cancer Institute (NCI) to get a glimpse inside her work and to hear about the directions she plans to go in now (5).

Carole Parent

NEW DIRECTION

Did you have any scientific role models when you were growing up?

I'm the only scientist in my family, but I remember that my science teachers in high school were very passionate. They were very animated about their subjects. Their passion—and the fact that I was learning so many new things—really grabbed me. Maybe that's why I ended up pursuing this career.

You first trained as a pharmacist…

Originally, my intent was to practice pharmacy in a hospital setting because I was very interested in drugs and the chemistry behind them. But the more I came to understand the mechanisms behind signal transduction, and how drugs mediate their effects, the more I became interested in the basic science of it all. That led me to pursue a master's degree, which I did in Montreal, where I was born and raised.

Then I moved to Chicago for my PhD, also in a pharmacy school. During my PhD I studied G protein–coupled receptor (GPCR) signaling in the context of platelet activation and thromboxane activity. But as I was doing this work, I began to feel that I was missing out on something. A lot of insight into signal transduction can be gained from genetic models, but of course platelets don't have a nucleus so I could not take that approach there. So I decided that for my postdoc I would look for a position studying G protein–coupled receptors in a system amenable to genetic approaches.

I joined Peter Devreotes' lab at Johns Hopkins, and that really changed my professional life, because that's when I was first exposed to a model system where it was possible to study conserved GPCR signaling but in the context of an amazing biological response, chemotaxis. I'm now more interested in chemotaxis than in GPCR signaling, although all of the responses I'm looking at are mediated by GPCRs, so I guess some aspects of my original interests have been conserved.

> "We both said, 'Oh my God.' We were just looking way too late."

MOVING FORWARD

What was known about chemotaxis at the time you started working on it?

Several groups of researchers had shown that cells could detect very shallow chemical gradients in their environment. We really had no clue how this was done, although we had some ideas about which proteins might be involved.

When I first joined Peter's lab, I was studying a molecular event downstream of GPCR signaling involving a key enzyme for *Dictyostelium* development, the adenylyl cyclase that makes cyclic AMP. Cyclic AMP is the main chemoattractant for *Dictyostelium*. In my first few years I was very focused on basic biochemistry, looking at the activation of adenylyl cyclase in response to ligand–receptor interactions.

We were also studying a PH domain–containing protein called CRAC, which is required to activate adenylyl cyclase downstream of GPCR activation in *Dictyostelium*. We suspected this protein would translocate from the cytosol to the plasma membrane when GPCR signaling was activated, and we set out to detect and define this translocation event using biochemical assays. I would add cyclic AMP to my *Dictyostelium* cells and then take my first time point at one minute, which at the time I thought was really fast. Then I'd isolate the cell membrane from the cytoplasmic fraction and look at whether I could detect CRAC localization biochemically. It kind of worked but was rather weak, and we were never really satisfied.

Then, three years or so into my postdoc, GFP technology appeared and changed our entire perspective. Now we had access to a tool to visualize these signaling events in live cells. We fused GFP tags to various proteins we were interested in, including CRAC, and we went under the microscope and added the attractant to *Dictyostelium* cells. And all of a sudden, within five seconds we observed the protein translocate to the plasma membrane and later to the leading edge of migrating cells.

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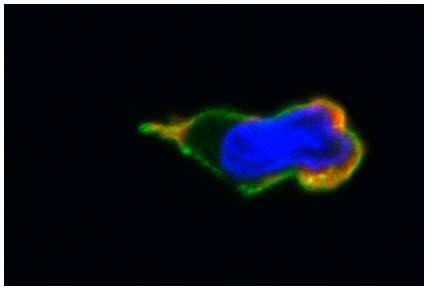

IMAGE COURTESY OF DR. KOSTAS MOISSOGLU

A migrating HL-60 neutrophil expressing the LTB4 receptor fused to GFP (green) and stained for actin (red) and DNA (blue).

I remember looking at Peter, and we both said, "Oh my God." [Laughs] We were just looking way too late.

Later you looked at adenylyl cyclase itself, which doesn't go to the front…

Yes! [Laughs] That was completely shocking. This took place in 2001, just after I joined the NCI to start my own lab. I wanted to go down the pathway from CRAC, and I decided to look at where the cyclase is. I really didn't expect much. I expected it to be on the membrane and that this would maybe bring me back to my roots, toward the pharmacological and biochemical aspects of cyclase activation, which are really interesting.

But then we found this totally unexpected distribution: it went to the back of the cell, far away from CRAC. We ended up showing—after doing a bunch of control experiments and making sure we were not pursuing some silly idea—that having the cyclase at the back of the cell is essential for the cells to align in a head-to-tail fashion when they're migrating. We call this behavior streaming, and it is how *Dictyostelium* transitions from single to group migration. We proposed that the cyclase at the back of the cell generates a compartment where cyclic AMP is spatially generated to relay the chemotactic signal and attract neighboring cells to migrate in a head-to-tail fashion.

We continued to study this, and now we know much more about it. One interesting thing we've found is that cyclic AMP is released from the back of the cell within very small membrane-bound vesicles called exosomes that form from multivesicular bodies.

So the idea is these exosomes act as a breadcrumb trail?

Right. We're currently working on understanding how cyclic AMP is released from these vesicles. One possibility is that these vesicles burst after they are secreted from the cells. Another possibility is that there could be regulated active transport of the cyclic AMP out of the exosomes. At this point, we're not sure what's happening, but we're looking at these questions now.

RELAYING THE MESSAGE

***You study other motile cells besides* Dictyostelium…**

When I first came to the NCI, I was determined to look at neutrophils because they behave similarly to *Dictyostelium*, but they're much more complex cells. We were in a good position to apply what we know from the simpler system to ask what could be conserved or what we could investigate that would give us some insight. It was a lot of work to set up the system. Although some people were using neutrophils when I first started, they're much more challenging to work with than *Dictyostelium*. But early on I chose to focus on the lessons we learned from the relay system in *Dictyostelium* and to try to apply them to neutrophils. Of course, neutrophil migration isn't mediated by cyclic AMP, but neutrophils respond to many attractants that are synthesized cell-autonomously and by neighboring cells. When they detect an attractant, they will make additional attractants to amplify responses.

> "When I first came to the NCI, I was determined to look at neutrophils."

Very early on I picked the leukotriene B4 (LTB4) pathway to study in neutrophils, mainly because I was looking for a relay signal that could be generated very quickly and LTB4 fit the bill very well. Much like cyclic AMP, LTB4 is generated enzymatically, so it can be rapidly made available to these dynamic, fast-moving cells. We quickly realized that it works in a very similar fashion to cyclic AMP. It's essential to amplify responses that are initiated by primary attractants; when neutrophils lose the ability to synthesize this secondary attractant, it dramatically inhibits the long-distance recruitment of neutrophils to an inflammation site. We've pursued this at the signal transduction level, and we found that there are particular pathways that are responsible for this. Now, much like we did in *Dictyostelium*, we want to go to the molecular level and look at the enzymes that make LTB4 to see if we can learn how this is regulated. Recently, we've also begun working on breast cancer metastasis to see if what we've learned from simpler systems can be applied to disease states.

What's your life like outside the lab?

[Laughs] Well, I have two kids who keep me busy. My husband and I adopted one child from Vietnam in 2002 and another from China in 2008. I think my scientific career, combined with parenting two wonderful kids and having a very busy family life, completes me as a person.

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PHOTO COURTESY OF CAROLE PARENT

Complete: Parent and family