Ulrike Eggert: Big things from small molecules

Eggert uses RNAi and chemistry to develop novel tools for investigating cytokinesis.

You can learn a lot if you have the right tool to help your investigations along. But where those tools don't exist, you may have to work on a different question. Ulrike Eggert isn't about to let a little thing like the lack of a tool get in her way—she'll just make her own.

Since her postdoc in Tim Mitchison's lab at Harvard (1), Eggert has used a combination of RNAi and small molecules in phenotypic screens to identify new ways to interrogate biological pathways (1, 2). She's chosen to focus on cytokinesis, a biological process that happens so quickly it's been difficult to study by other means (3–5). We called her at her new lab at King's College, London, to discuss the advantages she's enjoyed and the hurdles she's surmounted in her efforts to add to the scientific toolbox.

NEW FIELDS

You came to biology from a chemistry background?

My undergraduate and graduate degrees are in chemistry. I did my PhD in Daniel Kahne's lab, who was then in the chemistry department at Princeton. He was interested in applying ideas from chemistry to biological research and was just getting started on that. I think that's what attracted me to his lab, apart from the fact that he's a really great mentor. Another student had found that if you attach a glycolipid moiety to the antibiotic vancomycin, it becomes active against vancomycin-resistant bacteria. My project was to study how that happens. For both of us, it was a journey into biology.

I think that's really when my interest in what's now a big part of my research started. That's also why I chose to do a postdoc with Tim Mitchison, because he was one of the pioneers in the field of using small molecules to probe biology. Since I'm a chemist and I have never taken a biology class, I thought it would be good for me to learn biology in a hardcore biology lab that still had an understanding of chemistry.

In Tim's lab I collaborated with a postdoc in Norbert Perrimon's lab, Amy Kiger, to do one of the first visual full-genome RNAi screens in *Drosophila*. It was a good opportunity because I also did a small molecule screen in parallel, and it gave me a chance to combine the two approaches. This got me thinking about how small molecules and RNAi are in some ways complementary and how one can take advantage of both the similarities and differences between them.

What are some of the advantages and drawbacks of using small molecules to probe pathways?

The biggest advantage of small molecules is that they act very quickly, so they can be used to study dynamic systems. Also, their action is often—but not always—reversible, so you can add a small molecule and then wash it out and observe the effects. The main disadvantage of small molecules is that we do not have a small molecule that binds to every protein that we're interested in, and the process of getting them is very difficult. That's in contrast to RNAi, which you can use to knock down pretty much any protein you want to, as long as you know the sequence of its mRNA. But I think the disadvantage of RNAi is that, because you're depleting a protein by destroying its mRNA, it takes time to completely take effect, and cells can sometimes adapt to gradually decreasing amounts of a given protein.

> "The biggest advantage of small molecules is that they act very quickly."

Sometimes the phenotypes caused by small molecules and RNAi can be different because with RNAi the protein is gone but with small molecules the protein is still there and you're inhibiting maybe just one function of the protein. So, often the phenotypes of small molecules and RNAi are the same, but sometimes they aren't, and I think that's interesting.

PHOTO COURTESY OF SAIED EZZATI

Ulrike Eggert

NEW TOOLS

How do you go about developing a new small molecule tool?

There are two ways in which one can find a small molecule tool: a protein-based screen or a phenotypic screen. The protein-based screen is basically what most of the pharmaceutical industry does, where you purify a protein for which you have some kind of functional assay, then do a screen to find small molecules that inhibit that protein. It's possible to screen hundreds of thousands of compounds using a plate reader, but the main limitations are that one can only target proteins with detectable activities and that you don't know how specific your small molecule will be for its desired activity.

Some basic researchers instead use a phenotypic screen, where you add a small molecule to cells and you look for a phenotype that suggests your compound inhibits your pathway of interest. This is often done using a high-throughput microscope, and you can take pictures of treated cells in multi-well plates. In that approach you know you're getting a specific

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

small molecule because you're looking for a specific phenotype. But the huge, huge challenge—and the reason why the pharmaceutical industry doesn't use this approach as much—is that it's difficult to find the target of the small molecule that gives rise to the phenotype that you're interested in.

What approaches can be used to identify a small molecule's target?

One approach is the one I initiated during my postdoc, which was to compare the phenotypes between small molecules and RNAi. The idea was that if a small molecule and RNAi phenotype are the same, then in many cases they would target the same protein (although there are the caveats that I mentioned earlier). We used this approach to identify small molecules that target proteins involved in cytokinesis, which is a very fast process that is difficult to study using RNAi or other genetic methods because many of the proteins that are involved in cytokinesis are also involved in the step before, mitosis.

When I started my lab at the Dana-Farber Cancer Institute and Harvard Medical School, we began working on another approach: combining RNAi and small molecules in a different way to identify the small molecules' targets. We wanted to find small molecules that target Rho signaling during cytokinesis. The Rho GTPase pathway is really important in regulating many aspects of the cytoskeleton, but there are not many small molecules available to study proteins in that pathway. It would be nice if we could target Rho directly, but people haven't found any small molecules that target small GTPases because GTP binds very tightly to them and it's difficult to interfere with that interaction. We can't compete with huge pharma companies that have tried for decades to find small molecules that bind to GTPases specifically, so we thought we'd look at the actual pathway itself. The way we did that is that we sensitized cells by doing partial RNAi of Rho GTPase, and then we looked for small molecules that either increased the RNAi phenotype or suppressed it. And by doing that, we found a number of small molecules that we know target Rho signaling in cytokinesis. We don't know yet precisely which proteins in the Rho pathway most of the compounds bind to, but that's one thing my lab is working on right now.

> "Discovery-driven research involves... experiments where you can't predict what the outcome will be."

NEW DIRECTIONS

What else are you working on right now?

I'm currently moving my lab to King's College, London, but I have two postdocs who will be staying at my lab at Harvard, and they're both working on aspects that are connected to membrane trafficking and the role of the membrane in cytokinesis. One is working on a small molecule we discovered that's a very potent inhibitor of cytokinesis and that also induces endocytic tubules. These are novel structures that stain positive for a number of different endocytic markers and almost look cytoskeletal but that don't associate with any cytoskeletal features that we've looked at. It's got us looking at connections between endocytosis and cytokinesis.

The other postdoc is looking at the nature of the lipids involved in cytokinesis, using lipodomics and lipid profiling, asking questions such as whether there are changes in the lipid composition of dividing cells versus interphase cells. In a warm-up experiment for that, we used different small molecules to inhibit different lipid biosynthetic pathways, and we found that, if you inhibit ceramide biosynthesis, then you also inhibit cytokinesis.

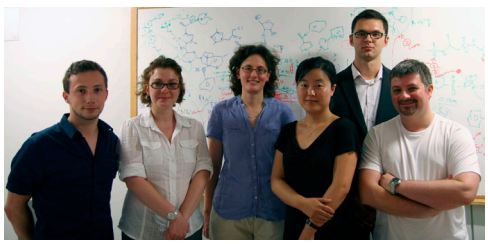

PHOTO COURTESY OF WENCHAO WANG

Eggert, with members of her Harvard and King's College labs.

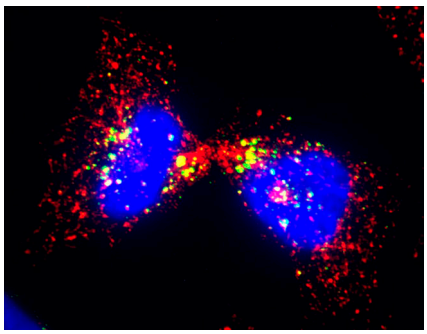

IMAGE COURTESY OF XIN ZHANG, EGGERT LAB

Early endosomes (yellow) and recycling endosomes (red) accumulate at the cleavage furrow in a dividing HeLa cell.

Is your approach to your work different than that of your biologist colleagues'?

Chemistry as a field is much more interested in discovery-driven research, whereas in biology there's more emphasis on hypothesis-driven research. Discovery-driven research involves screening or doing experiments where you can't predict what the outcome will be or where you can't hypothesize what the outcome will be. I think, in general, the more traditional biologist prefers experiments where people say, "I think that protein X does this, and I will test it in the following ways." That's a valuable approach, but I think using a less hypothesis-driven approach to generate new tools that can then provide new insights is also valuable.

1. Eggert, U.S., et al. 2004. *PLoS Biol.* 2:e379.
2. Castoreno, A.B., et al. 2010. *Nat. Chem. Biol.* 6:457-463.
3. Atilla-Gokcumen, G.E., et al. 2010. *ACS Chem Biol.* 5:79-90.
4. Smurnyy Y., et al. 2010. *ACS Chem Biol.* 5:1015-1020.
5. Atilla-Gokcumen, G.E., et al. 2011. *J. Am. Chem. Soc.* Jun 14 Epub ahead of print.