Carla Koehler: Small TIMs are a big deal

Koehler is investigating the mechanisms of mitochondrial protein and RNA import.

Mitochondria, the cell's powerhouse organelles, must import most of their component proteins from the cytoplasm. The multiprotein complexes of the TOM (translocase of outer membrane) and TIM (translocase of inner membrane) families are responsible for this process, with different TOM and TIM proteins working together to guide imported proteins to their proper locations within the organelle.

Carla Koehler has been fascinated by mitochondria since the start of her academic career (1). As a postdoc at the University of Basel's Biozentrum, she conducted some of the earliest studies characterizing a pathway dedicated to the import of proteins destined for the inner mitochondrial membrane (2), and since then her group has studied this pathway in detail (3). But there's more to learn and exciting new approaches to deploy (4, 5), as we heard when we reached Koehler at the National Institutes of Health, where she's currently on sabbatical from her lab at the University of California, Los Angeles.

FOCUS ON METABOLISM

You've worked on mitochondrial biology since the start of your research career…

I grew up in a rural farming community in Wisconsin with a typical farming life: driving tractors, milking cows, riding horses. By the time I was seven, I knew where cows came from, and I was heavily involved in animal husbandry and breeding to increase milk production from the herd. That's what piqued my interest in genetics. Growing up, I thought I'd be a veterinarian, so I decided to go to college at University of Wisconsin-River Falls, which had a high rate of placement into vet school—Wisconsin did not have a vet school at that time—and a really good biochemistry program. I majored in biochemistry.

> "We call these 'small TIM proteins,' and others call them 'Tiny TIMs.'"

Then I went to vet school at Iowa State for a year and realized that I was actually more interested in research. So after a year I left vet school and did a master's degree. That's where I first started working on mitochondria, studying the inheritance of bovine mitochondrial DNA.

Your graduate work was a bit of a departure, though…

I was looking at pseudohyphal growth and dimorphism in *Saccharomyces*, but I still was interested in mitochondria. And my PhD advisor at Iowa State, Alan Myers, had come from the lab of Alex Tzagaloff, who was one of the fathers of the yeast mitochondria field. By the time I got my PhD, I had decided that I was most interested in protein import into mitochondria, so Alan suggested I look into joining Gottfried Schatz's lab in Switzerland. I figured that I was probably going to have to go to the East Coast or West Coast for my postdoc anyway so I might as well go to Europe. And, lucky for me, Schatz was willing to take on a postdoc from a small Midwestern lab.

RACING IN EUROPE

How did you like working abroad?

I adjusted pretty well, partly because of my hobby, which was racing bicycles. I was racing at a high level in the US and really wanted to ride in Europe, so when I got there I joined a bike club. I started going on the club rides, and then I started racing. I became integrated into the Swiss culture because at the races they only spoke Swiss German. It was a fun opportunity and a good way to find some life balance for my lab work, which is what I spent most of my time doing. [Laughs]

In Schatz's lab, I started out working on the TOM complexes, which mediate protein transport across the mitochondrial outer membrane. But after my first paper on that subject, I switched to working on some mysterious proteins that our collaborators in the Schweyen laboratory had identified. I thought I could use my expertise from Alan's lab to make temperature-sensitive mutants to study these proteins. We figured out that these proteins—Tim10, Tim12, and Tim22—were part of a new mitochondrial protein import pathway. Of course, we weren't the only group working on this. A lot of other labs came out with the same story at that time.

PHOTO COURTESY OF PENNY JENNINGS

Carla Koehler

What is special about this pathway?

One major mitochondrial import pathway involves the TOM complex and an inner membrane complex that includes a protein called TIM23. In that pathway, proteins are brought through the outer membrane by TOMs, and then the receptor domains on the TOM and TIM23 complexes transiently associate to guide proteins across the intermembrane space.

The TIM22 pathway is different in that its substrates are limited. It imports carrier proteins, such as the phosphate transporter and glutamate/malate shuttles, and other TIM proteins including Tim17 and Tim23. Another thing that's unique to this pathway is that it includes two 70-kD chaperone-like complexes in the intermembrane space: the Tim8/Tim10 complex and the Tim9/Tim12 complex. We call these "small TIM proteins," and others call them "Tiny TIMs." They interact with target proteins that have been translocated across the outer

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

mitochondrial membrane by the TOM complex and then guide those proteins across the intermembrane space to the TIM22 complex, which mediates their insertion into the inner mitochondrial membrane. Interestingly, Tim8, also known as DDP1, was the first mitochondrial import protein found to be defective in a human disease.

Can you tell me a little more about the 70-kD complexes?

One thing we know is that the two different complexes have different substrate specificities. That might be why the Tim9/Tim10 complex is more important than the Tim8/Tim13 one. In humans, there's a disease associated with Tim8/Tim13, but if there were a mutation in Tim9/Tim10 we would probably expect it to be lethal.

Another thing we know is that the Tim8/Tim13 and Tim9/Tim10 complexes require intra-protein disulfide bonds to form. We've found another protein, called Hot13, that we think is involved in quality control for the formation of these disulfide bonds. And we've recently moved into using small molecules to try to target these components more directly in cultured cells. For instance, there's a protein called Erv1, which we and others have shown is involved in this disulfide bond formation pathway. We have new data using a small molecule inhibitor showing that Erv1 also plays an important role in stem cell biology. An interesting thing about this Erv1 inhibitor is that it selectively kills pluripotent stem cells but not differentiated lineages. This suggests that Erv1 is a stem cell survival factor, so now we're interested in finding out how mitochondria might influence stem cell differentiation.

PHOTO COURTESY OF CARLA KOEHLER

Koehler racing at the Encino Velodrome in Los Angeles.

SMALL TIMS, SMALL MOLECULES

What other small molecules have you designed?

I have a strong background in genetics, but I think we've taken classical genetics as far as we can. For example, say you'd like to shut down expression of a pathway at a specific time. You can use RNAi, but it takes several days to take effect. By that time, all of the secondary defects that you associate with losing mitochondrial function also come into play. On the other hand, you can put small molecules on and within 10 minutes see an effect. So now we're combining genetic approaches with small molecules.

> "We can actually target RNAs for mitochondrial import."

We're screening compounds first in yeast, with the aim of taking them into higher organisms such as zebrafish. In addition to the Erv1 inhibitors, we have some that target Tim10, and we've done a screen on the whole import pathway, so we've got TOM and TIM23 inhibitors, too.

Right now, I'm actually on sabbatical in Richard Youle's lab, where we're working to design small molecules that modulate the accumulation of Pink1 on mitochondria. The hope is we could then use these to manipulate Pink1-mediated recruitment of Parkin in neurons, which would be interesting because Pink1 and Parkin mutations are observed in some inherited forms of Parkinson's disease.

You're also working on hacking mitochondrial RNA import…

Yeah. That's actually a really big story. We found a new pathway that imports RNA into mitochondria. Through a collaboration with Michael Teitell, who is in the Department of Pathology at UCLA, we identified a protein called PNPase, which is an RNA-processing enzyme that is present in the intermembrane space. That really had us scratching our heads; why would an RNA-processing enzyme be in the intermembrane space?

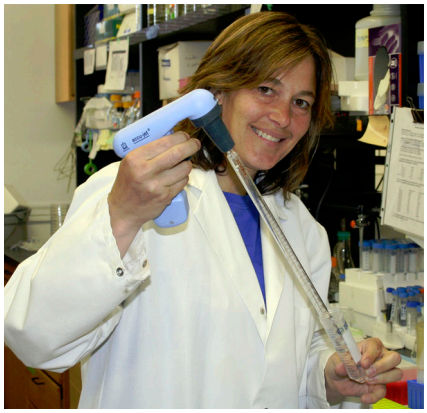

PHOTO COURTESY OF PENNY JENNINGS

Koehler uses yeast as a model system for studying mitochondrial protein import.

We started doing RNA import assays and figured out that it is able to bind RNAs and mediate their import. In a follow-up paper, we showed that we can actually target RNAs for mitochondrial import by tacking a specific RNA import sequence onto them. Now we'd like to look at whether, in patient cell lines with mutations in the mitochondrial DNA, we can target corrective RNAs to mitochondria. We'd also like to know how these RNAs get across the outer and inner membranes.

Do you still race bicycles?

No, I haven't raced since about 2003. I just ride to stay in shape, because it's not very practical to have a lab and to race. Besides, at some point, you get old, and you don't want to race against younger women. [Laughs] So maybe it's better to just do some coaching.

When I first came back to the US, I wasn't going to race. But then I started training again and began riding with a group from Pasadena. The goal of the team was to develop younger women to become as close to pros as they can, so I was involved with their program mentoring younger women. I also got involved in mentoring younger riders at the Encino Velodrome, which has a really big junior program. It is especially fun watching the young kids race their bikes on the track.

1. Koehler, C.M., et al. 1991. *Genetics*. 129:247–255.
2. Koehler, C.M., et al. 1998. *Science*. 279:369–373.
3. Curran, S.P., et al. 2004. *J. Biol. Chem.* 279:43744–43751.
4. Hasson, S.A., et al. 2010. *Proc. Natl. Acad. Sci. USA*. 107:9578–9583.
5. Wang, G., et al. 2012. *Proc. Natl. Acad. Sci. USA*. 109:4840–4845.