People & Ideas

Elizabeth Miller: Sleuthing the details of the secretory pathway

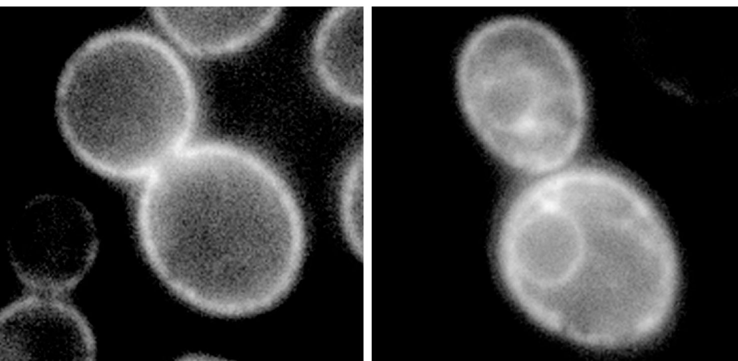
Liz Miller uses biochemistry and chemical and genetic screens in yeast to probe the mechanisms of intracellular protein transport.

Proteins destined for the cell surface or for secretion are first folded in the endoplasmic reticulum (ER) and then packaged into vesicles that carry them to downstream compartments. Exactly how proteins are selected for packaging into ER-derived vesicles for forward transport is a mystery.

"Randy came up with an idea that makes a postdoc want to run and hide."

Liz Miller has spent her career probing the processes and proteins involved in the secretory pathway. As a graduate student at La Trobe University (Melbourne, Australia) she studied the trafficking of defense proteins in tobacco (1). She then segued to postdoctoral work with Randy Schekman on the secretory pathway in yeast. Her work revealed how the COPII vesicle coat protein Sec24 helps select the cargo proteins that are deposited into ER-derived transport vesicles (2, 3, 4).

Now heading her own lab at Columbia University, Miller is focusing on how the ER discriminates between properly folded proteins, misfolded proteins, and ER resident proteins when choosing substrates for transport (5). We called her for a chat about her work and the many wonderful scientific and nonscientific uses of yeast.



A mutant form of Sec 24 cannot package its cargo protein (white) into vesicles, so the cargo accumulates in the ER (right).

MATHEMATICALLY INCLINED

What first got you interested in science?

When I started as an undergraduate at the University of Melbourne, I was mostly interested in psychology and mathematics. My dad's a mathematician, so I've always been comfortable with math, and it came easily to me. But then I had some fantastic biology professors in my first year at university. They really got me interested in biology, so I shifted my emphasis to zoology and botany.

In particular, I was interested in ecology and invertebrate zoology, but I felt like I didn't have the patience for the fieldwork that was required, especially for ecology research. So I became much more enamored with cell biology, and in particular in plant cell biology.

In Australia, the undergraduate degree is three years long, and then you have what's called an honors year, when you work in a research lab on a small, independent research project. That was my first real introduction to lab research. Again, I had some great professors who really made the internal workings of plant cells interesting to me.

What interested you about protein trafficking?

That year, I was working on how algae assemble their cell wall components, so I got to thinking a lot about how proteins move around in cells, and how cells move proteins around. That really appealed to me as a great biological problem. I decided when I was looking for a Ph.D. lab that I wanted to study protein trafficking. I looked around at various labs in Australia and settled on Marilyn Anderson, who was actually in the botany department at Melbourne, where I had been an undergraduate. She was just moving to take a position in the biochemistry department at La Trobe University.

Elizabeth Miller

I moved out there with her, and she had a great project that looked at the trafficking of defense proteins to the plant vacuole, which is like the yeast vacuole and the mammalian lysosome. It's a storage compartment, and these defense proteins are stored there in extremely high quantities.

My thesis work demonstrated that for a particular family of protease inhibitors (one kind of defense protein), there is a COOH-terminal domain that's required for delivery of their precursor protein to the vacuole. Once the precursor protein has been delivered to the vacuole, proteolysis occurs to release the fully active protease inhibitors.

THE TWO-BODY PROBLEM

What made you decide to move to the States?

A mentor who's been a great influence to me is Trevor Lithgow, who was also at La Trobe in the biochemistry department and is now at the University of Melbourne. He had been a postdoc with Jeff Schatz, who was one of the luminaries in the field of protein trafficking to mitochondria. Trevor was a local boy from the suburbs of Melbourne who went to the Schatz lab and had a fantastic time.

He really encouraged me to find the best lab that you can and do an amazing postdoc and have a great time, because it's a really

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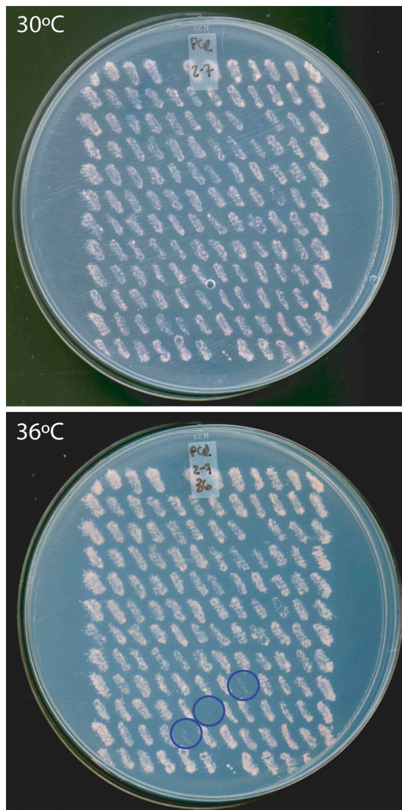

A screen of mutagenized yeast reveals temperature-sensitive mutants of Sec24 (circled) that do not sort cargo properly into secretory vesicles.

fantastic time of life, and it's an opportunity that you don't necessarily appreciate at the time. I looked at labs in Europe and the US, and I settled on Randy Schekman's lab in Berkeley for a couple of reasons.

First, I thought the yeast system was fantastic because of its great genetics and the ability to do both biochemistry and genome-wide analyses. Then, obviously, Randy is at the top of his field and is also a great guy. It was a great environment to work in. An added bonus was that Randy also offered a postdoc position to my husband (who is in a similar field), which solved our two-body problem of two scientists trying to get jobs together.

With Randy, you studied the secretory pathway in yeast?

We had reason to believe that a coat protein called Sec24 might be involved in selective capture of newly synthesized proteins into vesicles that leave the ER and deliver proteins to downstream compartments. I first studied isoforms of Sec24 involved in cargo capture. Later, Randy came up with an idea that makes a postdoc want to run and hide. He wanted to try alanine-scanning of the surface of Sec24. It's a 105-kD protein, meaning there are a not-insignificant number of residues to target on the surface that might be cargo-binding sites! He convinced me to do it, so we picked charged residues to target because we knew that some proteins used ER export motifs that are charged. I generated a library of about 20 alleles of Sec24 that had been mutagenized in these charged residues. We've now published two of them that correspond to specific cargo-binding sites. That work has dovetailed really nicely with ongoing structural work from Jonathan Goldberg's lab that also defined these two sites.

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MANY USES OF YEAST

You're Australian, so I have to ask: are you for or against Vegemite?

Growing up, actually, I was very anti-Vegemite. But then you can't work with yeast and be anti-Vegemite, so now I'm incredibly pro-Vegemite. I'm pro-beer also. All yeast products are good to me [*laughs*].

Have you ever brewed beer?

Well actually, brewing beer is something my husband and I got into when we were in Randy's lab. A friend from Australia set us up with a little home brewing system. I should point out, we don't use our lab strains. We really do use proper brewing yeast. We had done it in Berkeley quite a bit, and when I moved to New York, I decided I really wanted to brew a Christmas ale for the department Christmas party. I thought I'd do it in the lab so everybody could come down and see fermentation in action, and everybody in the department could have a connection to this beer.

The result of having this brewing yeast in the lab, which is more robust than our lab strains, was that we ended up with a terrible contamination of the diploid brewing yeast that took over some of our plates. For about a month, things weren't making sense anymore; strains were growing really well when they shouldn't be.

So we bleached everything in sight, threw out all the suspect stuff, and my students and postdocs made me promise to never brew beer in the lab again! I still brew for the department Christmas party, but I do it at home now.

And what about the yeast science in your lab?

The project that I've taken with me from Randy's lab to start on my own here at Columbia is expanding our understanding of how that cargo/coat recognition occurs. In particular, the question that we're interested in now is whether the folding of the cargo influences its interaction with the coat proteins, and if so, how. This question stems from an observation—although one that's probably not universally true—that misfolded proteins are not packaged into ER-derived transport vesicles.

One possible reason is that the ER contains a whole host of chaperones that help these proteins fold. Once you've removed secreted proteins from the ER environment, they can't fold properly because they no longer have access to those chaperones. If misfolded proteins escaped the cell, they could be toxic, so there's a real necessity to retaining them.

The question is, What is the mechanism by which misfolded proteins are recognized and prevented from gaining access to a vesicle? We just published our first paper on this topic in September, so that was a real landmark and really exciting for all of us. Things are moving along. And it really is a lot of fun. JCB

1. Miller, E.A., et al. 1999. *Plant Cell.* 11:1499–1508.
2. Miller, E., et al. 2002. *EMBO J.* 21:6105–6113.
3. Miller, E.A., et al. 2003. *Cell.* 114:497–509.
4. Miller, E.A., et al. 2005. *Mol. Biol. Cell.* 16:3719–3726.
5. Pagant, S., et al. 2007. *Mol. Biol. Cell.* 18:3398–3413.