Richard Gardner: Nuclear garbologist

Gardner investigates protein quality control and disposal in the nucleus.

The cell has a number of quality control mechanisms to ensure defective proteins get trashed. Most defects are detected and dealt with at the point of production to ensure that only quality proteins are released for use. But proteins can also become defective once they're at the job site. They can even be made to look bad by coworkers that want them destroyed, as Gardner discovered when studying sterol biosynthesis during his Ph.D. (1–3).

"It started to surprise me that very little was known about how protein homeostasis was maintained in the nucleus."

One of the most important job sites in the cell, where a protein's performance must be tightly regulated, is the nucleus. Rather surprisingly, however, no one had thought to look there for protein disposal mechanisms. So while studying as a postdoc at the Fred Hutchinson Cancer Research Center in Seattle, Gardner examined the fate of defective nuclear proteins (4).

Gardner has now started his own laboratory at the University of Washington, where he continues to sift through nuclear protein trash, to find out about disposal mechanisms and how these mechanisms might fail in some diseases.

SUNSHINE AND SCIENCE

Was there a defining moment when you thought, I want to be a scientist?

I don't know if there was one defining moment. As a kid growing up in rural Pennsylvania, I was always outdoors, either going fishing or capturing bugs and worms and butterflies. I think at that point I had decided I really loved ecology and was actually planning to pursue a career in ecology.

What changed your mind?

While I was studying biology at Cornell, I took a biochemistry class and became really fascinated by the idea of how molecules bring about all the important things that cells do. That really started to drive me toward wanting to understand the intricate internal mechanisms of life rather than the interactions of organisms in a larger environment.

I had taken some ecology courses and certainly loved the field trips! But I think I'm more of a gearhead in that I like to know how things work.

What influenced your decision to do your Ph.D. in San Diego—was it the lab or the location?

I picked the location first because Cornell, at the time, was going through a very harsh winter. The pipes in my apartment actually froze. I remember I was also thinking of coming to the University of Washington. I had flown in and interviewed with the biochemistry department and was very excited about it. But then I went home and went through two weeks of a terrible blizzard. When I flew to San Diego for the interview, it was 70 degrees and sunny. I said to myself, "You know what? I think I could spend five years in a warm climate."

I did also pick UCSD because it had a very broad biology department. I didn't know what I wanted to study when I left Cornell, so I wanted the opportunity to rotate in a lot of different labs.

You opted to work on yeast in Randy Hampton's Lab. What is it you like about yeast?

There's a couple of reasons I chose to focus on yeast. I like its simplicity—yeast is just a single cell, but it has to do most of the things that a metazoan cell does. Also, at the time, the yeast genome had just been sequenced. I realized that there was this powerful information sitting out there that would make yeast a great tool to study very important processes.

Randy was actually trying to understand how sterols were regulated, which is important for understanding heart disease, and I thought, "Wow, wouldn't it be great to take this simple system, yeast, and see if we could figure out mechanisms that might apply to humans?"

Richard Gardner

In fact, many of the things that I discovered during that time have now borne fruit, as people have started to look at them in mammalian systems.

What was your project in Randy's lab?

I was working on the rate-limiting step in the sterol biosynthetic pathway—the degradation of HMG-CoA reductase, which is the main target for the statin drugs.

The major discovery that I made was that the molecular signals that promote the degradation do so by somehow altering the structure of HMG-CoA reductase's transmembrane domain such that it mimics a misfolded protein. This then allows it to be targeted by the ER quality control machinery for degradation.

The interesting thing is that the ER degradation pathway normally picks up proteins that misfold as they are made, but HMG-CoA reductase was being made to mimic a misfolded protein, in a regulated way, after having been functional.

SETTLED IN SEATTLE

For your postdoc you moved to Seattle. Had you had enough of the sunshine by then?

The funny thing is, having grown up on the East Coast, I love changeable weather. San Diego is sunny all the time, so Seattle seemed like a great town. It rains in Seattle, but actually the quantity is only about the same as you get in Philadelphia, about 40 inches a year. It's just spread out over more days. It's like London, where it's misty all the time.

Text and Interview by Ruth Williams
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Ah, yes, I can see why you love it.
[*Laughs.*] Also, the winter temperatures are quite mild, which is perfect for me. I don't ever want to see snow again unless I go to the mountains.

You turned your attention to nuclear protein quality control. Why?
Realizing how important quality control degradation is in the cell, it started to surprise me that very little was known about how protein homeostasis was maintained in the nucleus. And that was surprising because, if you think about it, the nucleus is one of the most important organelles, where every critical function is driven by proteins.

Most people were studying degradation in the cytoplasm and the ER because these are protein-folding compartments, where you anticipate you're going to need to have quality control systems around to handle mistakes that happen during translation or folding. The nucleus isn't involved in protein synthesis, so perhaps we didn't really expect it to be in the nucleus.

So why would the nucleus need its own degradation pathway?
Certainly, most quality control happens at the production level. But you can imagine that a protein might be made correctly, shipped into the nucleus, and is happily going about its business, when all of a sudden it's denatured by heat or reactive oxygen species. And once it's in the nucleus, the cytoplasmic degradation systems can't access it anymore.

In many of the neurodegenerative diseases that are associated with protein aggregation, the aggregates form in the nucleus, causing nuclear toxicity. This got me really interested. I thought, "Do these diseases show nuclear aggregation because the nucleus doesn't have degradation systems? Or does it have degradation systems, and they're simply failing?"

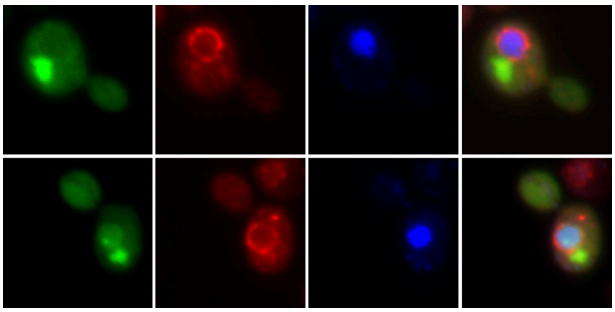
What did you find?
From the ER studies in yeast, I had realized that most temperature-sensitive mutant proteins are degraded by quality control pathways in the ER. But from reading the literature, I discovered that a nucleus-specific protein called San1 suppressed a number of nuclear temperature-sensitive mutant proteins.

San1 had been discovered back in '89 in Jasper Rine's lab, but people thought, "Well, maybe it's just a negative regulator of the normal nuclear proteins." But I thought, maybe San1 doesn't regulate the normal versions of these proteins, but instead it's actually degrading the mutant versions of these proteins because they are misfolded.

We found that San1 is a ubiquitin ligase and indeed it is degrading the nuclear temperature-sensitive proteins—the mutant forms, not the wild-type forms. And, if we take San1 out of the nucleus, its substrates become stable, fitting the definition for a nuclear quality control system.

We're now characterizing San1's function, and our studies suggest that San1 does protect the nucleus from some very toxic, aggregation-prone proteins.

Another thing that came out of that study is that we realized that there were some temperature-sensitive proteins that weren't completely stabilized in the absence of San1. So there's at least one other degradation pathway in the nucleus that functions in quality control.


With San1 (top), the nucleus (blue surrounded with red) is kept free of aggregating mutant protein (green).

Is there any evidence for faulty nuclear protein disposal in the neurodegenerative diseases that are characterized by nuclear inclusions?
There was a recent report about a protein called PML-IV that has been shown to colocalize with the polyglutamine aggregates that form in Huntington's Disease.

PML bodies are thought to be sites of protein destruction in the nucleus. It's known that the proteasomes are localized to PML bodies, and the PML proteins themselves are ubiquitin ligases. Interestingly enough, if you take the human PML-IV sequence and search for a match in the yeast database, the first homologue that comes out is San1.

An interesting thing about the human aggregation diseases is that they all develop later in life. So what we think is going on is that, when you're young, your burden of aberrant proteins in the nucleus is relatively low. But, as you get older, you produce more and more misfolded and damaged proteins. Eventually, a barrier is crossed after which the degradation systems can't cope, and the mutant proteins accumulate and aggregate and cause all sorts of problems in the nucleus.

Any idea how the other nuclear degradation pathways you mentioned might work?
We think that one of the other pathways is proteasome independent and that it could involve something called piecemeal autophagy of the nucleus. This is a phenomenon that David Goldfarb's lab recently discovered. They showed that there's a route from the nucleus to the vacuole in yeast.

We'd now like to see if any of the misfolded proteins that we are studying in the nucleus are targeted to this autophagy pathway. It's pretty exciting. This year is going to be a very big year for us, I think. We're poised to answer some very deep questions in the quality control field in general and in trying to understand how the nucleus defends itself against these very bad, abnormal proteins. JCB

1. Gardner, R.G., and R.Y. Hampton. 1999. *J. Biol. Chem.* 274:31671–31678.
2. Gardner, R.G., and R.Y. Hampton. 1999. *EMBO J.* 18:5994–6004.
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4. Gardner, R.G., et al. 2005. *Cell.* 120:803–815.