Thoru Pederson: Spotting novel roles for the nucleolus

Pederson's career has revolved around the nucleus, nucleolus, and the nucleoproteins that inhabit them.

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Thoru Pederson

Contrary to his name's Scandinavian appearance, Thoru Pederson is all-American. Long before the discovery of RNA splicing, he quips, his parents, Thorvald and Ruth, stitched their two names together. Although it sometimes causes other researchers to come up at conferences and begin speaking in Swedish, his name is just one of the special gifts his parents gave him. Their commitment to volunteerism influenced Pederson's own career as a researcher with a long history of service to the cell biology community. He has served on numerous award and editorial boards and has acted as treasurer, program chair, and served on the Minorities Affairs Committee for ASCB.

Pederson fell in love with the nucleolus in a graduate cytology course at Syracuse University. He did a postdoctoral fellowship at Albert Einstein College of Medicine and then, in 1971, he moved to the Worcester Foundation for Biomedical Research in Shrewsbury, Massachusetts, where he set up his own laboratory and eventually became president of the institute. In 1997, the Worcester Foundation became part of the University of Massachusetts Medical School, where Pederson is now the Vitold Arnett Professor of Cell Biology.

In 1998, Pederson proposed that the nucleolus was a site for more than just ribosome synthesis (1), and his group showed that, indeed, the organelle also hosted the assembly of the signal recognition particle (2, 3) that directs membrane and secretory proteins to the endoplasmic reticulum. More recently, his laboratory has shown that certain microRNAs co-reside with their mRNA targets in the nucleolus (4, 5) and that cell cycle progression is regulated by a nucleolar stress response (6).

Pederson recently spoke with *JCB* about his nucleolar preoccupation and his passion for essay writing.

> "You begin to get a sense, or to pick things up—I use the word zephyrs."

INSECURITY AND SEDUCTION

You grew up in New York State and stayed for college, graduate school, and your postdoc. Why stick so close to home?

I had a pretty strong case of insecurity and the imposter syndrome until I was about 45. I think this is more common among scientists than many people let on. I liken it to the scene in *A Beautiful Mind*, in which the economist John Nash realizes that the little girl in his life is a delusion because over several years she has not grown up. He uses that observation to crack through his psychosis. One day flying out to California to give a seminar, I said to myself, "You know this is hogwash. Why don't you just give up this crazy idea that you're no good?" And so I did.

How did you first get interested in the nucleolus?

In a graduate school cytology course, I had a lot of fun playing with various dyes and painting cells every color I could. Many of these dyes had a particular affinity for RNA, so the region that was often most brightly lit was the nucleolus.

I realize that's sort of the most "dumb-bumpkin" reason one could ever have, but it's the truth. I'm sure that there are people who, when they first saw mitochondria, went absolutely wild with excitement and expectation. The connection between the molecular species called RNA and cell structure and organization appealed to me. That's been a consistent footprint of my research. We do some RNA biochemistry and some molecular biology, but we always transport the results back to studies conducted in living cells.

Describe the nucleolus for those who have not stared at it through a microscope.

Nucleoli are not free-floating usually, but are anchored to the chromosomes that have the genes for ribosomal RNA (rRNA), which in human cells are on five of the chromosomes. You can think of them as cytological manifestations of the production of rRNA and its assembly into ribosomes.

They are also very dynamic. They go through cycles of fusion and dispersion during interphase and mitosis. There are proteins that are coming in and shuttling out.

A SUSPICIOUS MIND

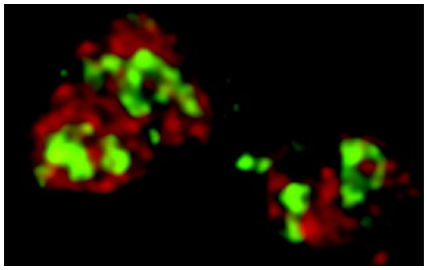
When did you first suspect that there was more going on in the nucleolus beyond ribosome synthesis?

It was about 1996. You begin to get a sense, or to pick things up—I use the word zephyrs.

We were injecting fluorescently tagged RNAs into the nucleus, including various known nucleolar RNAs. As a control my postdoc Marty Jacobson decided to try an RNA that we assumed would not become localized to the nucleoli. But it did. It was the RNA subunit of the signal recognition particle (SRP). Then we showed that five of the six SRP proteins are imported into the nucleus and assemble onto SRP RNA in the nucleolus. We found this in mammalian cells and it was soon confirmed by two other labs in yeast.

This really got me thinking about the possibility that the nucleolus might be doing other things as well. Jumping ahead

Text and Interview by Kendall Powell
kendall2@nasw.org



SRP RNA (red) and the pre-rRNA processing enzyme fibrillarin (green) show distinct localizations in the nucleoli of a rat kidney epithelial cell.

a few years, we demonstrated that, in many cells, the nucleolus seems to have a rather uncanny ability to monitor the progression of cells through interphase and to execute cell cycle arrests during nucleolar stress.

But these cell cycle arrests are actually unrelated to ribosome synthesis.
That's right. We used a low concentration of the drug actinomycin, which selectively inhibits rRNA transcription, to cause nucleolar stress. That halted the cell cycle at a very specific checkpoint. You might say, "Well, sure, the poor cells are trying to get through G2 and all of a sudden they run out of ribosomes!" But the fact is, in almost all growing mammalian cells, ribosomes are extremely long-lived, and the short bout of drug treatment we used would have hardly made a dent in the cell's store of ribosomes.

My suspicion is that the nucleolus can sense something is wrong and it sends out a signal to stop cell cycle progression. We don't have any idea of what that signal might be.

How did you discover that microRNAs brought yet another function to the nucleolus?
We were studying myoblast differentiation using a rat cell line and Joan Politz in my laboratory wanted to look at microRNA regulation of that process. She noted that one microRNA, called miR-206, displayed not only cytoplasmic localization, as expected, but was also prominent in the nucleoli. We then purified the nucleoli and found they contained large amounts of four other microRNAs as well.

Naturally, we began to ask whether certain mRNAs might visit nucleoli in order to be regulated by these microRNAs. That led to the paper we just published, showing that there are a number of mRNAs present in these rat myoblast nucleoli. The mRNA we chose to scrutinize, for insulin-like growth factor 2, contained predicted binding sites not only for miR-206, but also for the four other nucleolar microRNAs. That really made us sit up and take notice.

Why might certain microRNAs and mRNAs meet up in the nucleolus rather than the cytoplasm?
Our thinking is that there's some very important reason for keeping this mRNA out of the cytoplasm until it has been pre-docked by the microRNA regulating it. Why do this in the nucleolus? This might have to do with the fact that in the nucleoplasm you have all the competing nascent mRNAs and nascent microRNAs. And so the mature species get sent to the nucleolus where you don't have any spliceosomes or nascent RNAs around—there are no contending or competing activities.

> "This really got me thinking about the possibility that the nucleolus might be doing other things."

This is pure speculation. I'm going way out on a limb here and it's about ready to break.

FREEDOM OF EXPRESSION

What inspires your prolific publishing of non-research papers—book reviews, editorials, and researcher memorials?
My mother was a very good writer. When I was a boy she used to show me how to write a thank-you note: "Don't blurt out 'Thank you for the book' in the first sentence. You need an expository sentence." What 7-year-old knows what an expository sentence is?

I'm basically a storyteller. When I write a book review or a memoir, I feel like a million dollars. I think I operate in these non-research papers in the "essay" mode, which from the Latin means to try. It's something that's not routine. You have to try, and I enjoy that.

Your career spans almost five decades. What do you think were the biggest, field-altering discoveries in that time?
The advent of biological electron microscopy certainly gave us an appreciation of the cell that we had never had before. Also, once *E. coli* surrendered some of its precious secrets about molecular biology, being able to interrogate mammalian cells with the molecule in mind was really a transformation.

Finally, I think the ingenious use of biological materials that exemplify or amplify a particular phenomenon has been somewhat underappreciated. Joe Gall is one of my biggest scientific heroes because every one of his great discoveries—such as ribosomal gene amplification, in situ nucleic acid hybridization, and discovery of the telomeric DNA repeat—came from his astute instinct for using just the right material.

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Pederson chatting with John Gurdon, a nucleolus pioneer, at the 2014 Cold Spring Harbor meeting on Nuclear Organization and Function.