Cole Haynes: On the trail of mitochondrial dysfunction

Haynes tracks the mitochondrial unfolded protein response and its ramifications for cellular health.

PHOTO COURTESY OF RICHARD DEWITT, MEMORIAL SLOAN KETTERING CANCER CENTER

Cole Haynes

Since graduate school at the University of Missouri, Cole Haynes has been preoccupied with how mixed up, misfolded proteins inside organelles affect the well-being of cells. At Missouri, he studied the classic pathway: how rumpled proteins are recognized in the yeast endoplasmic reticulum and ultimately targeted for degradation. As a postdoctoral fellow, he went a little more cosmopolitan, moving to David Ron's lab at New York University School of Medicine to investigate how cells adapt to the stress of unfolded proteins in mitochondria.

Working in *C. elegans*, the team began cracking the signaling involved in the mitochondrial unfolded protein response (UPR^{mt}) (1), a line of inquiry that Haynes brought with him to his own laboratory at Memorial Sloan Kettering Cancer Center in 2009. He identified both the matrix peptide exporter HAF-1 (2) and the import efficiency of the transcription factor ATFS-1 (3) as key signals for activating the UPR^{mt}. In 2012, his laboratory discovered that the kinase GCN-2 acts to slow protein synthesis in response to mitochondrial stress (4)—a pathway that complements the ATFS-1 pathway, which, among other things, sends more mitochondrial chaperones to the scene (3).

Currently, the Haynes lab explores the roles mitochondrial stress responses might play in aging, cancer, and innate immunity (5). He recently spoke with *JCB* about his longtime love of unfolded proteins and organelle maintenance.

DYSFUNCTIONAL FOLDS

Why do misfolded proteins fascinate you?

Maintaining the status of the proteome is clearly a basic and important problem. Protein-folding defects are at the heart of all sorts of mammalian pathologies. There are tons of examples of protein misfolding causing problems. It's an aging problem. It's a disease problem. The topic touches on various aspects of physiology that I think are cool.

Why use worms to study the UPR^{mt}?

Worms have about 20,000 genes and they are metazoan, so their mitochondrial functions probably resemble ours more closely than yeast's do. But the biggest advantage of the worm early on was that the RNAi library was available. That made it relatively easy to identify components of this mitochondrial-to-nuclear signaling pathway.

You can also use RNAi to knock down just about any respiratory chain or mitochondrial chaperone gene to induce mitochondrial stress in worms. Almost any sort of global hit to mitochondrial activity will induce the UPR^{mt}.

How did you discover that ATFS-1 is an important regulator of the UPR^{mt}?

David Ron's lab had worked out an assay to monitor mitochondrial-to-nuclear signaling. At that point, how mitochondrial status was conveyed to the nucleus was still a black box. So, I did a lot of RNAi work to identify components involved in this signaling pathway. In my last 10 minutes in David's lab, I found this transcription factor ATFS-1. I simply continued the project when I set up my own lab.

> "How mitochondrial status was conveyed to the nucleus was still a black box."

ORGANELLAR PROBE

Was it challenging to figure out that a reduction in ATFS-1's mitochondrial import is the signal for mitochondrial stress?

We knew ATFS-1 was in the nucleus during stress, but we wanted to know where it was in the *absence* of stress to see how it detected mitochondrial dysfunction. But we failed miserably for a long time.

We spent a fortune generating really good antibodies against ATFS-1 and we probably spent a year probing for it and never found anything tractable. We probably ran 50 or 100 Western blots trying to find the damn thing. In vivo we could never find it because it's got such a short half-life in the mitochondrial matrix. Then, we hashed out the idea to knock down a major mitochondrial protease, Lon. Once ATFS-1 was stabilized in the matrix, everything sort of fell together. And we stopped bad-mouthing the antibody company!

So, when all is well in the cell, ATFS-1's main purpose is to get shuttled into the mitochondria and be degraded?

It does seem a bit wasteful, I've got to say. But it seems to be a strategy that nature takes advantage of—it's essentially the same principle as p53 or NF-κB turnover. But the cool part for ATFS-1 is that its turnover is coupled with mitochondrial import.

What happens when there is mitochondrial stress?

I usually think about it as the nucleus sending out a probe in the form of this transcription factor. If it never comes back to the nucleus because it's sufficiently imported and turned over, then the pool of organelles is healthy.

If import traffic is slowed down, then ATFS-1 accumulates in the cytosol and gets sent back to the nucleus via its nuclear localization signal. If it comes back—and it comes back in a way that's proportional to the amount of stress inside the organelle—then it turns on the UPR^{mt} pathway. That pathway induces mitochondrial chaperones, antioxidant factors, and mitochondrial

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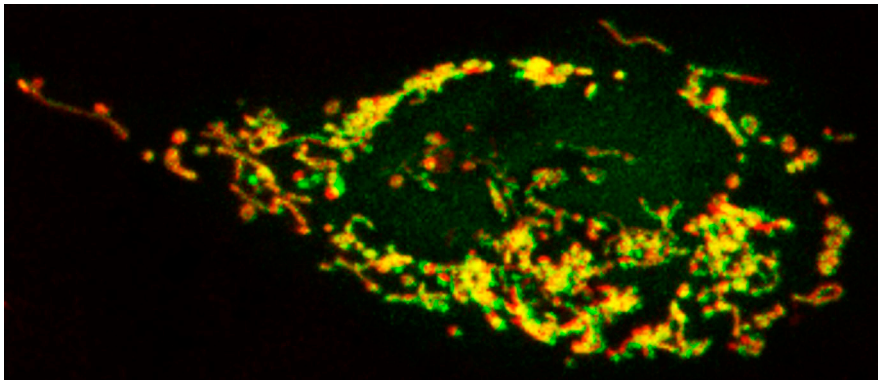

IMAGE COURTESY OF YUCHEN XIE AND CHRISTOPHER FIORESE

ATFS-1 (green) is imported into mitochondria (red), where it is subsequently degraded. If mitochondrial dysfunction results in impaired import, ATFS-1 traffics to the nucleus where it activates the UPR^{mt}.

translocase components. It's a way to constantly monitor the pool of organelles, all at the same time, and turn on a protective response when it's needed.

MITOCHONDRIAL HEALTHCARE

Why does it behoove the cell to monitor mitochondrial health?

By monitoring import efficiency, the cell gets a readout of the health of the whole mitochondrial collective. Something like 99% of the proteins in mitochondria are imported—so it makes sense to monitor import. But toxins and defects in mitochondrial DNA also activate mitochondrial stress pathways.

I think the cell probably benefits from not eliminating every organelle that has just a modest problem. It's worthwhile salvaging these things considering how much energy went into making them. But once you have a dead organelle or one that's depleting chemicals from the cell that could be used elsewhere, it's probably best to get rid of it.

Does finding a kinase like GCN-2 open the door to potential therapeutics?

It suggests that you might be able to find a drug that activates a mitochondrial protective pathway without actually needing or causing more mitochondrial dysfunction. Also, there are a number of tumor types that have clear mitochondrial dysfunction. In that case, a kinase inhibitor could be the way to go, to force more mitochondrial dysfunction and push the mitochondria over the edge to kill cancerous cells.

How did your lab take a turn into the realm of innate immunity?

When we did microarrays to find what genes ATFS-1 turns on, the two biggest categories were innate immune genes and xenobiotic detoxifying genes—which seemed kind of odd. Why couple a mitochondrial protective response and an innate immune response via the same transcription factor?

> "I think about it as the nucleus sending out this transcription factor as a probe."

Every mitochondrial poison we use in the lab comes from some sort of bacteria that lives in the dirt. So the hypothesis was that this mitochondrial stress pathway can detect or respond to those pathogens, like an early alert system. We gravitated toward testing this idea using *Pseudomonas aeruginosa*, which makes cyanide, an inhibitor of the electron transport chain.

What happened when you fed the pathogen to the worms?

We wanted to know if the pathway, in addition to maintaining mitochondrial function, also had any sort of bactericidal activity. When we pre-activated the UPR^{mt}, *Pseudomonas* didn't accumulate in the worms' gut. The worms were either killing the bug or clearing it faster and were therefore resistant to infection.

I think the worms are essentially gambling that mitochondrial dysfunction is caused by a pathogen. The mitochondrial stress pathway turns on immune genes as well. This may be another way the body knows to activate an immune response.

Mitochondrial dysfunction reminds me of Madeleine L'Engle's* A Wind in the Door. *What's the last book you read?

[*Laughs*] We have two-year-old twins, a boy and a girl, so we read "Elmo Does Bath Time" or some such. The first eight months were a fog—it was sort of "you watch that one and I'll watch this one" and hope we're still kicking in the morning.

Prior to life with twins, did you have hobbies outside of lab?

I play tennis. I played in college and I still try to play it as much as I can. Being in New York City makes it a little more complicated. We do go out and watch the US Open in Flushing Meadows every year.

What's next for your laboratory?

We're actively looking for mammalian versions of ATFS-1 and we think we've found such a thing. So we're looking at it in cancer cells.

And then in worms, we are looking at ATFS-1's role in basic biology—in development, as opposed to stress. It almost certainly plays the same role. Rapid mitochondrial biogenesis is probably fairly error prone and likely to involve transient stresses.

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3. Nargund, A.M., et al. 2012. *Science*. 337:587–590.
4. Baker, B.M., et al. 2012. *PLoS Genet.* 8:e1002760.
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PHOTO COURTESY OF CHRISTINA PARISSE

Haynes's two-year-old twins, Georgia and Weston.