People & Ideas

Ramanujan Hegde: The prion puzzle and protein translocation

Hegde uses prion protein as a model to explore how cells handle protein translocation and trafficking.

Protein translocation from the cytosol into the endoplasmic reticulum (ER) is generally considered a reasonably well understood, automatic process. But getting proteins into the ER is actually a highly regulated process that poses more of a conundrum for the cell than it seems, especially under challenging metabolic conditions like ER stress (1). What's more, aberrant or incomplete protein translocation can give rise to disease—which Ramanujan Hegde has shown can happen with prion protein (PrP) (2, 3).

Throughout his career, Hegde has worked through the puzzle of protein translocation. His laboratory at the National Institutes of Health focuses on trying to understand how cells regulate protein translocation (4, 5) and how certain proteins like PrP can illuminate these enigmas, bringing new insights to normal processes (1) and to the development of prion disease (3). In a recent interview, we asked Hegde how he thinks through these problems and how he's approached a career in research.

A GOOD FIT

Did you have any role models growing up?

My parents were both strong role models for me. My father's family comes from a small village in rural India, and they are all farmers. But my father injured his foot when he was very young and couldn't work in the fields, so his only option was to study—that's how he got interested in mathematics. He was self-taught at first, but eventually he went to a local college, and then my family emigrated to Saskatoon, Canada when I was about six years old so that he could pursue a PhD there. A couple of years later, we moved to DeKalb, Illinois.

It took a lot of internal drive to get him where he is today, which is one of the things I've always really admired about him. My mother, too, has that kind of drive, although she put aside a lot of her interests and education until her kids were grown up. She was a homemaker until my brother and I were about high school age, then she got a Bachelor's degree at the college in DeKalb and pursued a career in computer science. I think the fact that both my parents hold education so central to their lives, and also that it was central to our immigration to the West, was very inspirational for me. I feel that I have a special set of opportunities that I might not otherwise have had, so I try my best to take advantage of that.

With that background, were you pretty much predestined to go to college?

Yeah. I'd thought about majoring in physics or astrophysics because I loved stargazing as a kid in DeKalb. But when I got to college at the University of Chicago, I majored in biology, in part because I felt I was "supposed" to go to medical school—many first-generation immigrants seem to dream that their kids will become doctors. It's weird—I actually thought biology was pretty boring, mostly because of the way it was taught. It was just a bunch of things to memorize that really had very little to do with problem solving, which was what I really enjoyed.

> "You have to present a compelling case for something you want to study."

The summer after my first year of college, I worked in an insurance company doing data entry, and that was by far the most boring, horrible job I've ever had. That's when I decided I really had to find something more interesting to do. So I basically knocked on doors randomly and said I'd be happy to work in the laboratory. Clive Palfrey had space in his laboratory and took me on. That experience was really a key point in my career because that's where I realized what doing biology was actually about. I started to see that it was really all about problem solving: breaking a very complicated problem into smaller pieces, that then get approached and attacked in very specific ways. Then, when you learn more about why people are studying a particular problem, it becomes much more interesting.

IMAGE COURTESY OF MARYANN KIVIEN

Ramanujan Hegde

A PRETTY PUZZLE

But you still went on to medical school, didn't you?

I entered an MD-PhD program at the University of California, San Francisco and started taking medical school classes. But within a month, I realized that, while the subject matter in medical school is very interesting, the mode of teaching isn't problem solving–based. I decided I really missed the laboratory, and eventually I found a faculty member, Vishwanath Lingappa, who didn't mind having a med student in his laboratory. I soon got so wrapped up in my research with Vishu that I stopped attending classes—I would just show up for the exams.

Was it the problems the laboratory was working on, or research itself, that drew you in?

When I started, I had no idea what Vishu worked on, and to tell you the truth, I don't think it was especially important to me. I just wanted to be in the laboratory doing experiments. Slowly I learned that the area he was working on was how proteins get either translocated into the ER

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

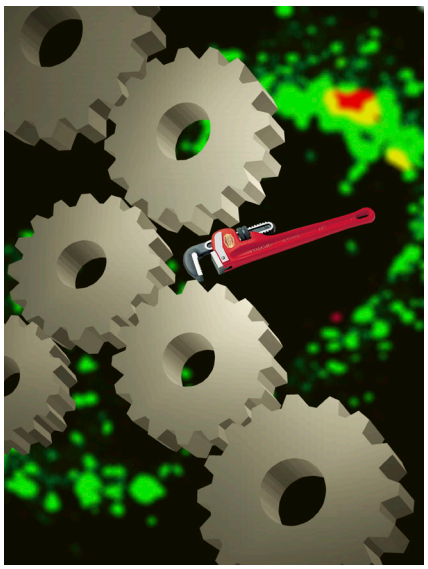

IMAGE COURTESY OF OISHEE CHAKRABARTI AND RAMANUJAN HEGDE

Mislocalized proteins (wrench) can interfere with unrelated cellular machinery (gears).

lumen or inserted into the ER membrane. Vishu wanted to understand how this process was regulated and how defects in these problems could lead to disease. He was ahead of his time, though, because people then were still trying to identify the core components of translocation.

One of Vishu's early observations was that a small proportion of PrP, instead of being translocated completely across the membrane into the lumen of the ER, either winds up getting released into the cytosol or gets inserted into the ER membrane as a transmembrane form of the protein. I found this interesting, but because this observation was made in an in vitro translocation system, it seemed like an artifact to everyone else. In fact, my graduate committee rejected it when I proposed to study it as my thesis project!

From that I learned that you have to present a compelling case for something you want to study; you have to demonstrate the relevance of it before you start investigating the molecular details. So what I wound up doing during my graduate work was to determine if this transmembrane form was relevant to anything. We turned to transgenic mouse studies and showed that mice with higher levels of this transmembrane form of PrP suffered neurodegeneration.

THE NEXT PIECE

You took some of these same problems with you straight into your own laboratory…

I went ahead and finished medical school, but it wasn't a difficult decision for me to pursue research full time. I was fortunate to be chosen for the National Cancer Institute's Scholar program, which basically meant I skipped doing a postdoc and set up a laboratory right away.

In the beginning, we started working on what I had proposed to do for my qualifying exams: trying to understand at a more molecular level how prion protein translocation is regulated, what parameters influence how efficiently it is translocated into the ER, and how this transmembrane form (that's associated with disease) gets made. Since then, we've expanded our interests to other areas of protein translocation that we've slowly come to realize are very poorly understood.

> "Mislocalization of very small amounts of prion protein… can be very detrimental."

How is protein translocation tied to things like ER stress?

Probably every process in the cell is regulated. The trick is to try to think of conditions where regulation of a particular process would be advantageous to the cell. With respect to protein translocation into the ER, we realized fairly early on that one condition in which the cell would want to control the subset of proteins that are allowed to enter the ER would be when the environment in the ER is less than optimal. This can happen when the ER is overburdened with proteins that need to be folded, or with certain toxic insults to the cell.

Prion protein translocation turned out to be regulated during such stresses, probably because it's a fairly difficult protein to fold and because its misfolding can really cause problems for the cell in terms of aggregation. So we're continuing to study how protein translocation might be regulated in a general sense, using prion protein as a model to try to understand what determines whether or not a protein is translocated. Protein translocation could be regulated at the level of the translocation machinery (the translocon), in combination with differences in intrinsic protein features like signal sequences. These processes have been very challenging to understand, in part because they're all happening at a membrane.

With respect to prion biology, it was very illuminating to us when we realized that mislocalization of very small amounts of prion protein, and probably of other proteins as well, to the wrong part of the cell can be very detrimental. This might be because when proteins wind up in the wrong place, they can interact with various factors that they would never normally see. So that raised the question: how does the cell recognize and deal with mislocalized proteins? It's possible that defects in these pathways could contribute to certain diseases of protein misfolding and neurodegeneration—so that's something we're interested in following up on.

1. Kang, S.-W., et al. 2006. *Cell.* 127:999–1013.
2. Hegde, R.S., et al. 1998. *Science.* 279:827–834.
3. Chakrabarti, O., and R.S. Hegde. 2009. *Cell.* 137:1136–1147.
4. Hegde, R.S., and S.W. Kang. 2008. *J. Cell Biol.* 182:225–232.
5. Stefanovic, S., and R.S. Hegde. 2007. *Cell.* 128:1147–1159.

IMAGE COURTESY OF RAMANUJAN HEGDE

Hegde spotted the comet Hale-Bopp at perihelion while stargazing on his birthday.