Using yeast to understand protein folding diseases: an interview with Susan Lindquist

Susan Lindquist is a founding editor of DMM, whose pioneering work in yeast has advanced our understanding of protein folding in disease, including Parkinson's, Huntington's and prion diseases. Here, she discusses her personal approach to model organism research and scientific leadership.

Protein shape is vital to its function. Pathological processes, such as neurodegeneration, stress tolerance and prion diseases, often result from the misfolding of proteins. Who could have imagined that research carried out in a unicellular organism, such as yeast, would yield discoveries relevant to the treatment of complex human neurological diseases? Susan Lindquist imagined that this simple eukaryote could reveal plenty about protein folding and pathology, and she was right.

What characteristics led you to focus on yeast as a model organism? I leapt from working on Drosophila to working on yeast literally the moment I heard about the technique devised by Terri Orr-Weaver, Jack Szostak and Rod Rothstein. They had figured out how to put a mutation into a piece of DNA and target it back into the genome, replacing the gene in the chromosome with what you wanted [Orr-Weaver, T. L., Szostak, J. W. and Rothstein, R. J. Yeast transformation: a model system for the study of recombination (1981). Proc. Natl. Acad. Sci. USA 78, 6354-6358] [using yeast as a model organism]. I thought, “Wow! It is just absolutely fabulous.”

We had been working on heat-shock proteins and using them as a system to study gene expression, asking ‘how does an organism rapidly and completely change its pattern of gene expression in response to a specific stimulus?’ We did a lot of satisfying and interesting work at a time when very little was known about how organisms can control what proteins they will make. We found that the expression of heat-shock proteins is regulated at every level you can think of. It’s not just regulated at the level of transcription, which was the hot thing at the time; it’s regulated at the level of preferential translation, selective RNA turnover, selective removal of polyadenylation, and preferential transport of RNAs from the nucleus. We uncovered a whole slew of regulatory mechanisms and found that they worked in an incredibly orchestrated way. That was great, but we didn’t know what the proteins were doing. At that point we needed to know what the proteins were doing and genetics is a powerful tool for that.

As soon as I heard about the new yeast methods, I signed up for the Cold Spring Harbor course on yeast. Gerry Fink [who developed baker’s yeast as a model for studying the fundamental biology of all organisms and is a Professor of Genetics at MIT and a Member at the Whitehead Institute] was one of the teachers. I remember at the time the senior faculty didn’t spend much time advising junior faculty. The only time someone did stop by to give me advice was after hearing I was going to start working on yeast – three years into my assistant professorship. He said: ‘that is absolutely crazy, don’t switch organisms, don’t do something completely different right in the middle of the tenure clock.’ But I went ahead and decided to do it. (As a woman, at that time, I hadn’t really thought it was likely I would get tenure anyway. So I thought it was too good to pass up.) I’m so glad I did. That capacity to go in and knock out a gene using site directed mutagenesis was just a transforming thing in terms of experimental elegance. Since then, and indeed before then too, many things were done to make the [yeast] organism more manipulable. It all started with brewers wanting to make better beer about a hundred years ago. But it continued because certain aspects of the organism are just wonderful for experimental manipulation. It can grow either as a haploid or a diploid, so you can cover or uncover the effects of mutations any time you want. It is very easy and cheap to grow. It has a small genome, so it wound up being the first higher organism to have its genome sequenced. Another great thing about it is that it actually does things in an amazing number of ways, just like human cells do. It is a simple, little organism but it has all of the cellular compartments – the endoplasmic reticulum, a membrane-bounded nucleus, vesicle trafficking – as well as chromatin structure, transcription factors, specific mechanisms for regulating the cell cycle, and many different types of signal transducers. All of these work in much the same way as they do in higher organisms. Bacteria do a lot of things quite differently. Don’t get me wrong, bacteria are great to work with, too. They grow even faster! And for studying many truly fundamental problems in biology they are terrific. But there are many layers of biology, and particularly many problems related to specific human disease mechanisms, that cannot be studied in bacteria. But in yeast,
protein trafficking – in and out of the nucleus, through the secretory pathway, into organelles – the control of growth and division, responses to diverse stimuli, mitochondrial respiration, all this is very similar.

There are obviously many differences between humans and yeast. It goes without saying: a yeast cell is not a neuron. But you can study much of basic eukaryotic cell biology in yeast. For example, we are studying neurodegenerative diseases. Why would anyone think to study a neurodegenerative disease in yeast? Many of the problems in these diseases derive from problems in protein folding and trafficking and that is largely the same in yeast as it is in neurons. To the extent that the problems are the same between yeast and other organisms it is really great to be able to study it in yeast because they are so fast to work with and because a host of very clever people have created such amazing tools to work with them. We’ve then been able to move from yeast into neurons largely through the help of collaborators, who have been wonderful. It is great having a group of people to interact with who have expertise in very different areas.

How do you approach the great distance between a yeast cell and a mammal?
The major way we’ve done it is with collaborators. With one of the diseases we work with, which is Parkinson’s disease, the neuronal cell lines do not seem to be to be very good for studying [mechanisms of the disease]. I think that neurons are not meant to have continuous replication and when you have a neuronal cell line in culture derived from a tumor, it’s just not really normal. They’ve lost a lot of their apoptotic mechanisms. So the neuronal cell lines have not been as useful as one would hope for studying some problems. That’s not to say that there are not some fabulous things done with cell lines! There have been, but some responses to protein misfoldings and mistransferring may actually be more similar in yeast than in cultured tumor cells. In any case we’ve translated some of our yeast findings to neuronal models in other ways. For example, we collaborated with Chris Rochet to use differentiated primary neurons taken from the brains of embryonic rats, and with Guy Caldwell to study dopamineergic neurons in nematodes. We take genes that we find in yeast to serve as genetic suppressors or enhancers and clone their human homologues. We send [our collaborators] expression clones or viruses, which are then cloned into these other systems. [This process] has validated the effects of some of our genes on toxicity due to α-synuclein. So I think this is really exciting – that’s one billion years of conservation for a basic cell biological process.

How do you think recent technical advances, for example genomics, will influence the future model organisms?
Technologies are incredibly enabling. Certainly the genome sequencing that has been done has opened up many more organisms to study, it’s absolutely tremendously empowering. Invertebrates, vertebrates, and other fungi become powerful and you get access to doing things with pathogens that were [previously] very difficult. I think now we need to develop other tools to work with these organisms. There are many things that you might want to study in another organism. For example, [let us take] a pathogenic organism. We have been doing some looking at Plasmodium falciparum, one of the organisms that causes malaria. It’s very difficult to manipulate genetically. It’s hard to grow [and] it has a very complicated life cycle. But there are certain aspects of the biology of P. falciparum that one might be able to study by transposing its proteins into a yeast cell. For example P. falciparum has a very weird proteome. It has a lot of asparagine-rich proteins in it and many other simple sequence repeat proteins as well. I think this may create a particular vulnerability with respect to protein folding that could potentially (this is really a ‘pie in the sky’ thing) lead us to entirely new therapeutic strategies. It happens that my lab knows a lot about protein folding, and especially about the folding problems of simple sequence proteins. So we are thinking about taking some of the P. falciparum proteins that look like they would have a hard time folding and putting them into yeast cell to study.

Another thing that we are thinking about is doing is to work with Rudolf Jaenisch on making IPS [induced pluripotent cells] and, as we start to unravel some of the genetic processes that are causing PD [Parkinson’s disease], actually use cells from patients to discover what strategies are most appropriate for that individual. [Since] different patients will have different genetic predispositions, we hope to take such cells and try to test different combinations of therapeutic strategies initially derived from genetic analysis in yeast, nematodes and rat neurons.

These are the big scheme things that I would like to do and they are enabled by the fact that this pluripotency technology [the ability to manipulate cells into discrete mature lineages] has come about. It lets us make a wide leap. New imaging techniques are also fabulous. We have not yet been on the forefront of imaging, but I’d like to get there. One of my postdocs is attempting some amazing things in collaboration with Matt Lang’s group here at MIT.

Another thing that is terrific is the entry of different types of people into this field. The physicists have brought in all kinds of interesting ideas in terms of technologies. Engineers too – tissue engineering and microfabrication – so that you can study processes in parallel in large numbers much more rapidly. Thinking about the circuitry of the cell, engineers bring a whole different perspective to it. It’s a phenomenal time for biology right now and it’s such a shame that we – and by this I mean the entire scientific community – are so pinched in terms of funding.

If you were to begin a completely new project, what would it be?
I would want to tackle the malaria problem. I just told you about, because it is such a dreadful disease. And with global warming, I think it is going to get worse. A fresh and unique inroad into it might be the unusual nature of its protein folding problems, because its genome encodes proteins that are simply so weird. But whether I will do that or not, I don’t know yet.

What would I get into if I was really starting fresh – if I was a postdoc or grad student trying to decide what to do? I find that I love so many different aspects of biology that I would just try to find something that gets me excited and move out from there.

Certainly one of the great mysteries and great frontiers is the brain. How does our brain work and store all of our memories? We have a bit of a toehold on this and are working on it with Eric Kandel and Kausik Si. Crazy as it sounds we are actually working on this in yeast. This springs from other work we are doing on prion biology. The name comes from this awful, horrible disease where a protein changes shape and then it creates a conformational chain reaction where one
protein after another undergoes a change in shape. It’s all the same protein but it can have different shapes. And one of those goes and reacts with another protein of the same type and gets it to change its shape too. It causes a horrible disease by a process we still don’t understand. A similar process can occur in yeast cells, but in that case it doesn’t cause a horrible disease, it just changes the function of the protein. Importantly, that change in shape and function perpetuates itself, from protein to protein. So the change in function is transmitted from one generation to the next generation in the yeast cell. You actually have a heritable element that is based upon a change in protein shape, not a change in nucleic acid.

It is an interesting mechanism to create a ‘molecular memory’ in a sense, because once you change conformation, there is a self-perpetuating loop to keep it going. The propagated change in shape is associated with a change in function that passes from generation to generation in yeast. But it is an interesting way to think about how other types of memory might be encoded – even neuronal memory. Everyone I mentioned this to thought I was crazy [for this idea of a protein-derived memory process] until Eric Kandel came into my office and asked: “Do you think prions might be involved in learning and memory.” I nearly fell off my chair. He had a gifted scientist in his lab, Kausik Si, who had found a protein that is involved in learning and memory and is located at the end of the synapse. It had sequences on it that looked just like our yeast prions. We collaborated with them to study its prion-forming capacity and to try to identify the regions of the protein that are involved in making its conformational change. We do see [their protein of interest] undergo a conformational change when we look at it in yeast and it also self-perpetuates that change. And that perpetuates a change in function that would be expected to help maintain the synapse.

Now we are looking for other yeast prions and we think that we have found several that are completely unrelated proteins. The next phase might be to look for them in other organisms, such as humans, now that we’ve learned how to work with them using a high throughput – or moderately high throughput – system in yeast.

You have an unusual breadth of experience in both high level administrative and scientific roles and you have gained a reputation as someone who creates positive working environments. In your opinion, is there an identifiable infrastructure or type of organization that provides the most supportive environment for scientific research?

I think there are several things [that enable people to do their best work]. In terms of my own laboratory, I have two types of criteria. First, that people be bright and creative and rigorous…. Good scientists. But every bit as important, a very high priority in bringing someone in is knowing that they are generous, open, want to share information, like to help other people, and are willing and ready to accept advice from other people. People don’t get into my lab anymore unless they are like that. Still, there are a lot of misunderstandings that will happen and I honestly think that one of the most important things is to be open about things. I ask people when they come into my lab to be [open]. I have a wonderful lab manager and when someone has a problem they first should talk to her and if it cannot be resolved in an easy way then they can come talk to me. I had an Italian mother and a Swedish father and they were stereotypical in their ways of dealing with the world. They were constantly misunderstanding each other. I loved them both so dearly and I saw them have these misunderstandings that were simple and it drove me nuts. I kept having to mediate between them when I was a kid and I think I still have some of that left in me now. I can understand that people say things in different ways and they just need to get it out into the open and talk about it. I usually find that difficulties that happen in the laboratory are just simple misunderstandings.

In terms of institutions, I think some of those lessons apply to an institution as well. You can’t, as Director of the Whitehead, screen everyone to see if they are going to be good colleagues; that just doesn’t happen. You can try to put in place support structures that let people know that you really care about them and you care about creating an environment where they can do their best work. One of the first things I did when I was Whitehead Director was raise the postdoc stipend because I thought it was too low and create a system to reward people who get their own fellowships by giving them a little extra funding that they could use at their discretion to help support their career. I’m very happy to say that David Page, who is the current Director, has taken that on as one of his major priorities. In fact he has been able to do far better than I. The postdocs do feel that people care about them and that we [the faculty] remember when we were there.

We have a wonderful, long tradition at the Whitehead of a retreat. We go up to New Hampshire for a few days, all of the students, all of the postdocs, many of the technicians and, importantly, every single faculty member. There are many places that have retreats, University of Chicago used to have one and I attended about half the time. Terri Orr-Weaver, when she was away on sabbatical in Greece, came back for the [Whitehead] retreat. That kind of commitment to having a community, and realizing that the whole is much greater than the sum of its parts, is really important. At our retreat the faculty are there the whole time, talking to people, looking at their posters. It really takes that level of commitment. Last year two people in my lab didn’t go, both were having babies. Now that is a good reason not to come. People take it very seriously and they do so because they know the faculty does too and that they will be there.

While at an institutional level, we cannot go around to see how collegial every student and postdoc is but what we do is make very careful decisions about our faculty. The Whitehead faculty has lunch together every Thursday afternoon. We really know each other, we talk to each other and we interact a lot. When we hire a new faculty member, we are looking for the most brilliant minds we can find but we are also looking for people that we want to interact with and who we think will be part of the community. You really can establish a community by creating a set of operating principles.

Another thing that an institution can do, if they have any financial resources at all to do it, is to provide seed funding for crazy ideas that would be high risk, high payoff ideas. The Whitehead has a tradition of doing that and it is very central to its mission.

The DMM staff greatly appreciates Susan Lindquist’s candor in relating her interesting personal story and sharing her insight about creating lab and institutional environments that enable productive science. Her creative research demonstrates the value of model organisms in understanding and treating human disease. We feel that her work and personal example provide a fitting subject for the first ‘Model for Life’ article in this new journal.

Susan Lindquist was interviewed by Kristin Kain, Associate Reviews Editor.