© 2019. Published by The Company of Biologists Ltd | Journal of Cell Science (2019) 132, jcs232926. doi:10.1242/jcs.232926

FIRST PERSON

First person – Anthony Tran

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Anthony Tran is the author of 'The N-end rule pathway and Ubr1 enforce protein compartmentalization via P2-encoded cellular location signals', published in JCS. Anthony began the research described in this article while a PhD student in Davis Ng's lab at the National University of Singapore. He is now an Automation Engineer in Molecular Diagnostics at Siemens Healthineers, Berkeley, CA, USA, investigating the quality control mechanisms that cells use to deal with errors in protein folding, trafficking and compartmentalization, and how disruptions to these intrinsic quality control pathways may be linked to various diseases.

Anthony Tran

How would you explain the main findings of your paper in lay terms?

For cells to survive, millions of proteins must co-exist within a small space and be able to perform their unique functions without interfering with each other. Part of this ability in eukaryotes is enabled by the compartmentalization of different protein types into organelles, such as the endoplasmic reticulum (ER) and mitochondria, which serve different cellular purposes. Often times, however, proteins, which are made in the main compartment of the cell, the cytosol, are unable to reach their intended sub-cellular destinations. This may be due to a variety of causes such as environmental stress, mutations, or the intrinsic error rates that are associated with moving proteins around inside a crowded cell. All proteins are made up of a string of amino acids. In my work, I discovered that the second amino acid residue of the majority of proteins encodes a signal that tells the cell whether they are in the right place or not. When a mislocalized protein in the cytosol presents this signal, and at the same time has issues folding properly, the cell recognizes it and facilitates rapid destruction of the protein so that it cannot accumulate and potentially interfere with other cellular activities. The discovery of this mechanism is important because it is possibly involved in how diseases such as Alzheimer's develop. If proven to be the case, it could lead to new approaches to diagnosing and treating them more effectively.

Were there any specific challenges associated with this project? If so, how did you overcome them?

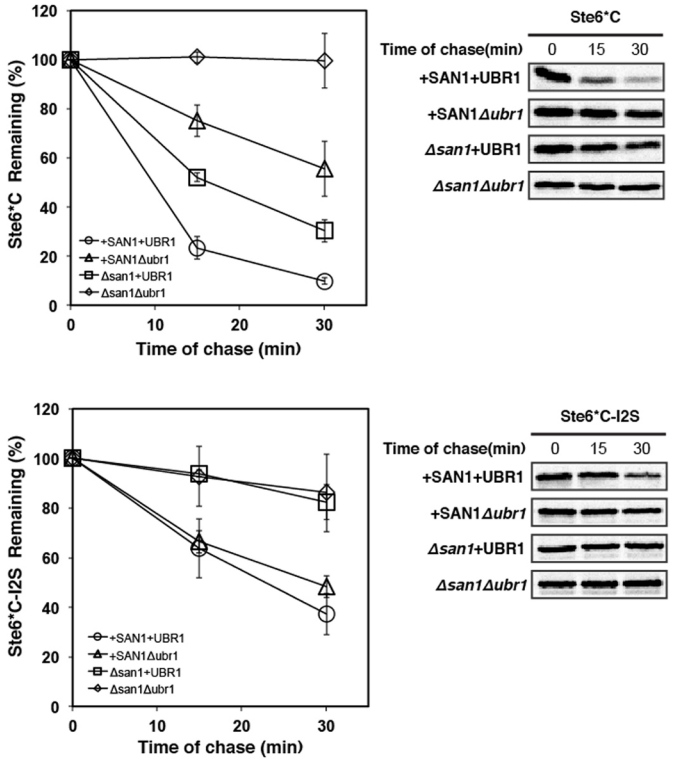
During my PhD studies, I had accumulated a substantial amount of biochemical and bioinformatic data that supported my hypothesis. However, after my departure from the lab, I did not have the means to perform additional bench-based experiments to gather more *in vivo* data that would strengthen the evidence for my proposed model of cytosolic protein quality control. At that point, I could only rely on designing and conducting additional convincing bioinformatic analyses on existing published yeast protein localization data, as well as performing an extensive review of literature to obtain critical statistics on secretory proteins, in order to further substantiate my theory. This took quite a bit of working on-and-off over a span of several years, as I also had jobs in industry and could only afford to do research as a side-project. In retrospect, the extra time it took was a blessing in disguise. It allowed me to discover, on a global scale, how the P2 residues of different protein types are biased towards specific amino acids in such a way that they can be used as a tool for cells to enforce the cellular organization of proteins, degrading aberrantly localized ones through a pathway called the N-end rule, while protecting normal ones from inadvertent degradation. In addition, an analysis I performed on a smaller published set of ER proteins helped me to make sense of why the P2 residues of some proteins didn't conform to the model, which in the end helped to support it even more definitively. One take-home lesson was that a lot of useful data are already out there – you just need to figure out the best way to use them, and how to apply it to your project. The second take-home lesson was to not give up on something you think is important, even if it takes longer than expected.

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

I experienced one eureka moment when I saw that changing the P2 residue of a model misfolded protein virtually eliminated its degradation by the Ubr1-dependent pathway (see accompanying figure). At that point I knew I was on to something, but realized that if I wanted to unravel the significance of this preference for certain P2 residues, I would have to perform a systematic analysis involving all the possible amino acids. Another eureka moment was when I saw the clear bias in P2 amino acid usage frequency in secretory and mitochondrial proteins as compared to cytosolic proteins. Not only was there was a clear preference for most of the Ubr1-compatible P2 residues in ER and mitochondrial proteins, cytosolic proteins were biased in the opposite direction for most of the Ubr1-incompatible residues. The final eureka moment was when I had discovered that most of ER proteins that don't carry the expected P2 residues are also membrane proteins, which can be degraded by alternate pathways, while soluble ER

Anthony Tran's contact details: Siemens Healthineers, 725 Potter St, Berkeley, CA 94710, USA.
E-mail: anthonytran17@gmail.com

Journal of Cell Science (2019) 132, jcs232926. doi:10.1242/jcs.232926



Metabolic pulse-chase analysis performed on model misfolded proteins to assess their degradation rates in *S. cerevisiae* strains with (+UBR1) and without (*Δubr1*) the Ubr1 ubiquitin E3 ligase. Mutation of the second position amino acid residue of Ste6*C (Ste6*C-I2I) from isoleucine to serine (Ste6*C-I2S) completely abolishes its degradation via the Ubr1 pathway.

proteins almost always have the expected P2 residue. Those were the final pieces of evidence that really made me confident in my model and ready to publish it.

Why did you choose Journal of Cell Science for your paper?

The Journal of Cell Science has a tremendous history of publishing scientific discoveries dating back to 1853. The thought of being a part of the journal's longstanding history was exciting, and led me to aim for a publication in JCS. It is also one of the most widely read and cited journals out there, which is really helpful for getting research out in the open to allow other scientists to critique it or utilize it in their own research.

"…the rules governing biological processes are very often close to perfect, but typically come with exceptions with logical causes behind them"

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

I would like to thank my former PhD advisor, Dr Davis Ng, for his invaluable guidance during several key junctures in the project and for always giving me the freedom to pursue my ideas. In addition, he was encouraging and supportive of me continuing on in academia, which boosted my confidence in my ability to perform independent research. One particular point he made that stayed with me is that the rules governing biological processes are very often close to perfect, but typically come with exceptions with logical causes behind them, some being more obvious than others. Thus, one of the jobs of a scientist is to figure out the reasons behind those exceptions so that the rules can be elucidated and accurately characterized. I feel that this advice was very applicable to the project.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

Living organisms are the most interesting things in the universe. Learning more about the inner workings of cells helps us to not only understand ourselves at a very intriguing, fundamental level, but also enables us to fix real-world problems such as disease. For those reasons, I gained a strong interest in cell biology. That started me on my journey to acquiring the skills and knowledge needed to make contributions to science and medicine. I am still on that journey and hope to stay on it for as long as possible.

What's next for you?

Recently, I have been heavily invested in industrial laboratory automation and utilizing it to enhance the speed at which molecular diagnostics can be carried out. I hope to one day use the skills and knowledge I have gained in industry to perform in-depth, high-throughput, global genomic and proteomic studies in yeast and mammalian cells with a focus on protein quality control mechanisms, such as P2 residue-based cellular location signaling, and how they play into the progression of common diseases.

Tell us something interesting about yourself that wouldn't be on your CV.

I am currently getting an education on how to take care of a baby boy. I have a 6-month old son named Calin who has been keeping me and my wife very busy when I'm not working. He has taught me that milk is infinitely more important than any research I might be doing at the moment!

Reference

Tran, A. (2019). The N-end rule pathway and Ubr1 enforce protein compartmentalization via P2-encoded cellular location signals. *J. Cell Sci.* **132**, jcs231662. doi:10.1242/jcs.231662