Mapping Parkinson's

"I'm trying to understand steps in the biochemistry of Parkinson's disease," says Dr Alban Ordureau, a postdoctoral research fellow in the cell biology department at Harvard Medical School. "It's a competitive field but there is still so much more to figure out and discover."

Every day, Dr Ordureau uses biochemistry techniques he learnt during his PhD and combines the results with data generated by quantitative mass spectrometry. His aim is to visualise the cell signals, protein activity and interactions that when impaired can ultimately lead to the neurodegenerative disease we know as Parkinson's.

Dr Ordureau completed his PhD in Professor Sir Philip Cohen's lab at the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC PPU) at the University of Dundee in 2011. He also holds a Masters of Biology in Biotechnology and Therapeutic Research from the University of Nantes in his native France. In 2012 he found a lab with the perfect facilities to enable him to pursue his research interests and so began his current postdoc in Boston.

The strength of the mass spectrometry skills in the Harper and Gygi labs at Harvard Medical School drew Dr Ordureau to the US: "The lab was the thing that most influenced my move to Boston. Because they specialise in mass spectrometry and ubiquitin signalling, I thought it had the perfect set up. I did a lot of biochemistry applied to cellular signalling in Dundee focused primarily on how innate immune pathways are governed by cross talk between phosphorylation and ubiquitylation, but I was really interested in learning more about what mass spectrometry can reveal when combined with biochemistry. I think if I hadn't been accepted here I might have gone to do a post-doc in industry rather than academia, but this was really the perfect lab for me."

Driven by competition in the field Dr Ordureau works long hours and is usually in the lab at the weekend, he says: "I like the competition and I think our lab is out near the front. Competition is stimulating because there are different things happening all the time. Yes, it can be stressful but at the same time it's clearly beneficial for the field. If another lab is able to reproduce the same result you get it's a positive way to validate your findings."

Dr Ordureau is particularly interested in what he calls the cross talk in conversations between proteins that happen when mitochondria, the power house of the cell, are damaged. The tremor and frozen motion symptoms of Parkinson's disease are rooted in the way the cell deals, or fails to deal with, damaged mitochondria. More generally, he says, this may also be what underlies many other neurodegenerative diseases.

"What we, and others, have found is that cells possess a very specific and elegant process to get rid of damaged mitochondria called mitophagy. This is very important, if it doesn't happen then damaged mitochondria generate what we call toxic species that can spread to healthy mitochondria and ultimately kill the cell. Two of the proteins that are mutated in an early-onset form of Parkinson's disease are key players that get rid of these damaged mitochondria. Our research and that by others, including several labs from the MRC PPU, have shown that once a mitochondrion is damaged it starts a cascade of signalling events

that involve phosphorylation and ubiquitylation. There is an essential cross talk between these two processes – these two post translational modifications work hand in hand to lead to recycling of the damaged mitochondrion," Dr Ordureau says.

When he started his postdoc three years ago the general outline of this process was known but the molecular details were not. Dr Ordureau wanted to understand how cell signals could target damaged mitochondria without also targeting healthy versions.

"We found that when mitochondria get damaged, a kinase called PINK1 accumulates on the outside of the damaged mitochondria but not the healthy ones and that in turn activates a ubiquitin ligase called Parkin that starts to build chains of ubiquitin molecules anchored to the surface of unhealthy mitochondria. Those newly synthesised chains of ubiquitin become new substrate for the kinase PINK1 itself. Once that chain is phosphorylated it acts as a molecular glue to stick the ligase Parkin to the phosphorylated chain. So we've figured out why Parkin, that is normally located in an inactive form everywhere in the cell body, sticks so strongly only to the surface of damaged mitochondria and never to the healthy ones," he says.

He didn't stop there. Ubiquitin can form eight different shapes of chains. Each of them has different specificity and they dictate the fate of the protein they are attached to. So next, Dr Ordureau used mass spectrometry to figure out what type of chains are put on mitochondria and so what the cell signal is for the next step in the mitophagy process. Overall he found a type of chain called K63 works like a scaffold to then attract receptors of another piece of cell machinery called the autophagosome that breaks up and recycles the damaged mitochondria.

The hard work is worth it

"When we figured out that the role for phosphorylation of the ubiquitin chain was basically to act as a glue to bring Parkin in and retain it specifically on the damaged mitochondria, that's when everything started to make sense. It was worth all the hard work." says Dr Ordureau. "We had all these little bits of pieces of information from different experiments and that's when it allowed us to fit everything together to create a working model of the biochemistry of mitophagy in Parkinson's disease."

Untangling the cross talk doesn't mean there's an automatic translational link however: "In theory we can use this model to identify a biomarker that could diagnose Parkinson's but the cause of Parkinson's disease can be so diverse at the molecular level that the road to a biomarker will be a long one. So this isn't helpful for living patients yet. We do have ideas about a biomarker candidate that we are looking at in stem cell derived neurones but that work isn't published yet. Any potential translation is a very long way away, I think. Right now it really is about basic biochemistry, to improve our molecular understanding and make new discoveries." he says.

What Dr Ordureau is most proud of so far is having found a way to combine the biochemistry he learnt in Dundee with mass spectrometry to better understand cell signalling: "We work closely with the lab of Steven Gygi next door," he says, "they are world

experts in mass spectrometry, I basically have all their knowledge to draw on whenever there is a problem or need advice."

The overall advantage of mass spectrometry is that Dr Ordureau can use it quantitatively to measure global changes. It allows him to look at multiple biochemical events at the same time. "Instead of looking at monitoring one event semi-quantitatively, for example, the phosphorylation of ubiquitin, we are now monitoring hundreds of events happening in cells simultaneously in one single sample," he says.

He also uses quantitative mass spectrometry as a tool to measure how much ubiquitin is phosphorylated and where this happens: "It's important to know the stoichiometry in cells. Is it point one percent, one per cent or 100% of ubiquitin that is phosphorylated? The only way to measure this is using quantitative mass spectrometry. We've been able to show that overall when mitochondria get damaged there is up to 10% to 20% of the ubiquitin that is attached to damaged mitochondria that is actually phosphorylated which is a lot and this will recruit the whole pool of Parkin present in the cell."

When working with such detail, Dr Ordureau has found that moving forward sometimes means taking a break: "During my PhD with Philip I did some large yeast two hybrid screening assays and thinking about it retrospectively, I did get some nice results but I couldn't really make sense out of it at the time. It was only in the last two months of my PhD, before I started writing-up, that I made a connection with a protein called DEAF1. I'd found that protein interacting with the protein I was studying at the time called Pellino in a screen before, so it actually all made sense. After my PhD I started working on it again and we got a paper out of it before I moved to Boston. Sometimes you just have to take a step back and look at your work, try to get a global picture to see if it's going anywhere. In the short term, if something isn't making sense you might need to put it to the side and work on a different way to answer your current question, then go back to it when you have a new idea or something new comes up in another experiment."

Of his time in Dundee, Dr Ordureau says: "Overall I really enjoyed working in Dundee, it was beneficially personally and everything that I learnt in the lab – it'll be useful where ever I go or whatever I do in the future. Dundee is quite unique, it's a small city, everyone is in the same building and there are people from all over the world working there, so you learn a lot and you make very close friends most of which I'm still in contact with. Almost all of the students and postdocs were international, surprisingly I found it was more international in PPU than it is here in Harvard."

Final word

"We know that mutations in various genes have been linked to Parkinson's disease and that each one could potentially affect different cellular pathways. If we already understand how everything works at a molecular level then we can understand how each pathogenic mutation affects a specific pathway and maybe find, not a global cure, but something for one specific type of mutation versus another one. Even in the past two years there are already so many things that we've learnt about two genes that are mutated in an early-

onset form of Parkinson's disease that we just didn't suspect when I began my postdoc. There is definitely more to learn" Dr Alban Ordureau.

Career Highlights
MRC Prize Studentship (PhD) - (2007- 2011)

Edward R. and Anne G. Lefler Fellowship (Post-Doc) - (2015-2017)

Top publications

Dr Alban Ordureau picks his top three papers and tells us why he has chosen each one**.**

1)*The IRAK-catalysed activation of the E3 ligase function of Pellino isoforms induces the Lys63-linked polyubiquitination of IRAK1.*

The Biochemical Journal, 409(1), 43–52.

Ordureau, A., Smith, H., Windheim, M., Peggie, M., Carrick, E., Morrice, N., & Cohen, P. (2008).

"This paper was published during my first rotation in Philip's lab. It introduced me to the importance of interplay between post-translational modifications in enzyme regulation and in driving cell signaling. It also led to me doing my PhD in Philip's laboratory."

2) *Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis.*

Molecular Cell 56, 360-375.

Ordureau, A., Sarraf, S.A., Duda, D.M., Heo, J.M., Jedrychowski, M.P., Sviderskiy, V.O., Olszewski, J.L., Koerber, J.T., Xie, T., Beausoleil, S.A., Wells, J.A., Gygi, S.P., Schulman, B.A., and Harper, J.W. (2014).

And

Defining roles of PARKIN and ubiquitin phosphorylation by PINK1 in mitochondrial quality control using an ubiquitin replacement strategy.

Proceedings of the National Academy of Sciences USA 112, 6637-6642.

Ordureau, A., Heo, J.M., Duda, D.M., Paulo, J.A., Olszewski, J.L., Yanishevski, D., Rinehart, J., Schulman, B.A., and Harper, J.W. (2015).

"Here I was able to combine biochemistry assays that I had just learnt during my PhD with exciting mass spectrometry techniques. Together they allowed us to comprehend a signaling pathway involved in Parkinson's disease in great detail."

3) *The PINK1-PARKIN Mitochondrial Ubiquitylation Pathway Drives a Program of OPTN/NDP52 Recruitment and TBK1 Activation to Promote Mitophagy.*

Molecular Cell, 60(1), 7–20.

Heo, J.-M., Ordureau, A., Paulo, J. A., Rinehart, J., & Harper, J. W. (2015).

"I really enjoyed working on this paper as it illustrated again very well the importance of crosstalk between phosphorylation and ubiquitylation. It also allowed me to work again on two proteins I researched during my PhD, though here they were involved in a totally different pathway."

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This PPU alumni profile was written by Hazel Lambert of sciencestory.com