Career pathways, part 13

Alexis A. Jourdain & Feilong Wang

Following one's passion and curiosity are major drivers for a successful career in science, and finding the right mentors and collaborators is essential in this journey. In the thirteenth part of our Career pathways series, Alexis Jourdain and Feilong Wang share their experience.

Alexis A. Jourdain: the power of fruitful collaborations

For as long as I can recall, I have been drawn to science. I was the kid with a toy microscope, a telescope and a collection of rocks. Recently, my mother found a note I wrote when I was at school detailing my life's plans: priority number 1, become a biochemist; priority number 2, have my own TV and video games.

While I'm not sure where my attraction to science stems from, with no one in my family being a scientist or a medical doctor, I've been fortunate to have the unwavering support of my parents in pursuing my true interests, and with very little pressure to keep me from choosing my own career path. I was born in Brussels and moved to Geneva at a young age, where I followed the Swiss public school system until the time came for me to decide my career trajectory. Among my top choices were medicine, pharmacology and biology. Looking back, the strongest influence during that period came from my high school biology teacher, Madame Burki, whose programme introduced us to molecular biology and, most significantly, to the world of research.

The professor teaching my very first class at the University of Geneva was called Jean-Claude Martinou. Jean-Claude, as I would call him later, was himself a relatively new member of staff at the University, and he began by sharing his own career journey: his MD–PhD, his training in the US, his early career as a group leader in the pharmaceutical industry and his current research as a professor. Jean-Claude was working on 'cellular suicide' (or apoptosis), a crucial biological process intricately linked to mitochondria. I found his career path, which offered the opportunity to discover the world and explore fundamental questions through research, absolutely

fascinating. Becoming a professor rapidly became my academic goal.

Not surprisingly, I decided to join Jean-Claude's lab for my master's thesis, with the aim of looking further into the role of mitochondria in apoptosis. The lab had recently discovered that mitochondria fuse as a protective mechanism in response to stress. My project, which subsequently became my first PhD project, focused on uncovering the mechanism behind this fusion. We suspected it involved a kinase, as pan-kinase inhibitors could block fusion; but at the time, little was known about intra-mitochondrial kinases. And so I designed my first screen, using a list of hundreds of kinases and examining each for mitochondrial targeting sequences. However, I was lacking in programming skills, and manually checking for targeting sequences quickly became unrealistic. Fortunately for my project, at about this time a game-changing article was published from a Boston research group led by a scientist called Vamsi Mootha. They had used proteomics and integrative genomics to compile a much-needed inventory of mitochondrial proteins called 'MitoCarta'. With this resource, I could quickly cross-reference my list of kinases with the mitochondrial proteome, and voilà! I had my candidates.

As often happens, the candidate I ended up pursuing was not what I expected: the fas-activated serine/threonine kinase (FASTK) turned out to be neither a kinase nor to control mitochondrial dynamics. Rather, it was known to be a component of RNA granules in the cytosol, even though the Boston team had also located it in mitochondria. Using GFP-tagging and fluorescence microscopy, I confirmed the presence of FASTK in mitochondria, but oddly, the protein formed discrete foci within the organelle. What were these foci? It took us three more years to unravel the mystery. We found that they contained newly transcribed mitochondrial RNA, together with a set of proteins required for mitochondrial RNA processing and maturation, including GRSF1. Drawing a parallel to the previously established presence of FASTK in cytosolic RNA granules, we named these structures 'mitochondrial RNA granules'. This work led to my first first-author paper, which described the mitochondrial RNA granules and GRSF1, and which was published back-to-back with work from another laboratory working on the molecular biology of GRSF1 (refs. 1,2). A second manuscript describing our findings on the cryptic mitochondrial-targeting signal in FASTK and its function in mitochondrial RNA granules was published two years later.

Once again, I knew straight away that I wanted to join Vamsi Mootha's lab for my postdoc. I aimed to continue my work on mitochondria and also to acquire new skills, particularly for large-scale, hypothesis-free experiments. Vamsi's lab, shared between the Massachusetts General Hospital, the department of Systems Biology at Harvard Medical School and the Broad Institute of MIT & Harvard, seemed the ideal place for this. My route into Vamsi's lab proved to be longer than expected; but eventually, in 2015, I joined the lab as Broad Institute-based postdoc.

Vamsi had assembled an impressive team of talented scientists, with about 20 postdocs from diverse backgrounds, several highly experienced staff scientists and a handful of PhD students. I was immediately impressed not only by the diversity of skills and people in his team but also by the welcoming atmosphere. Contrary to my prior pessimistic expectations that postdocs in Ivy League universities would be hyper-competitive and unwelcoming, I soon found friends with whom I could discuss ongoing projects and meet outside the lab.

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For my first project in the Mootha lab, I teamed up with another scientist, Jason Arroyo, to identify new genes essential for mitochondrial function using a genome-wide CRISPR–Cas9 screening approach. Our 'death screen', which involved isolating dead cells to investigate loss of viability in galactose compared to glucose, was one of the first of its kind and allowed us to identify approximately 300 genes essential for mitochondrial oxidative phosphorylation, including many with unknown functions. Drawing on my previous work on mitochondrial gene expression, I led the follow-up research, prioritizing genes with RNA-binding domains and discovering a protein module involved in mitochondrial RNA modification and ribosome assembly[3].

Being in a position to publish these findings within a year and a half of starting my postdoc was a testimony to the high-level scientific environment in the Mootha lab, and this co-first author publication became the basis, three years later, for applying for a faculty position.

Genetic screens never fail to surprise us. We had designed a screen to identify factors essential for mitochondrial function (characterized by low viability in the absence of glucose), but the screen also revealed many genes whose depletion seemed to enhance cell growth in glucose-limiting conditions. Strangely, many of these factors were associated with nuclear pre-mRNA splicing, and these were intriguing candidates, since it seemed they could potentially be involved in a natural, physiological shift between oxidative phosphorylation and glycolysis. Such shifts are frequently observed in cancers or during the immune response. Collaborating with the team led by Christopher Burge, just across the street at MIT, we discovered that subunits of the U1 snRNP, including LUC7L2, regulate the pre-mRNA splicing of metabolic enzymes involved at key cross-over points, such as PFKM in glycolysis and SLC7A11 in cysteine and glutamate metabolism. This work was published in 2021, alongside another study that also focused on the LUC7 family[4,5].

As much as I was enjoying my experience in the USA, by 2019 it became clear that it was time to return closer to family, and I began applying for positions in Switzerland. Western Switzerland seemed ideal, with institutes such as the University of Lausanne, the Lausanne Hospital (CHUV), the EPFL and my alma mater, the University of Geneva, all in close proximity. However, job offers for tenure track positions were rare, and the first advertised position I came across seemed somewhat outside my area: a department at the University of Lausanne, renowned for its discoveries in the field of inflammation and innate immunity, was seeking an experienced immunologist to lead high-quality immunology research, and I had never worked on immunology. However, one of the most fascinating aspects of mitochondria is their ubiquity and central involvement in almost all areas of biology. Over the years, from my master's work on apoptosis to my PhD on mitochondrial nucleic acids (recently recognized for their role in innate immunity) and my postdoc on energy metabolism shifts, aspects of immunology, particularly innate immunity, had been on my radar. I focussed on these topics in my job application, and happily, in April 2021, in the middle of the pandemic, I was able to open the doors of my new laboratory at the Department of Immunobiology, University of Lausanne. I had arrived in Boston as a 30 year-old postdoc, full of enthusiasm at the prospects ahead, and I was returning home six years later with a tenure-track position, exciting projects in mind and a wife!

Today, my lab investigates fundamental aspects of mitochondrial biology and energy metabolism, especially in the realm of immunity and metabolic disorders. Some of the scientific questions we address include mitochondrial biogenesis and the interplay between the nuclear and the mitochondrial genomes; the role of mitochondria-derived nucleic acids and mitochondrial RNA granules in innate immunity; and how mitochondria help to rewire metabolism during the immune response or when nutrients are scarce. To tackle these questions, we are using large-scale approaches, such as nutrient-sensitized CRISPR–Cas9 screening, metabolomics, proteomics and nutrient profiling. While I wouldn't call myself an immunologist (yet!), I feel fortunate to be part of the wider Lausanne immunology community, where the expertise within my group complements many aspects of on-going research in the area.

The first major paper from my new lab was published in *Nature Metabolism* in May 2023 (ref. 6). Starting in Boston and continuing in my own lab, the natural follow-up to our earlier nutrient-sensitized CRISPR–Cas9 depletion screen was to repeat the same protocol using an overexpression library. In this protocol we routinely add uridine to our media because cells with mitochondrial defects often struggle to synthesize pyrimidines, and uridine itself turned out to be a key molecule in energy metabolism. We showed that uridine-derived ribose can fuel glycolysis when glucose is scarce and when the appropriate uridine processing enzyme, the uridine phosphorylase (UPP1/2), is present. Screening around 500 diverse cell lines in collaboration with the PRISM Lab at the Broad Institute and with the team of David Fisher at Harvard, we found that the ability to grow on uridine was a feature of several cancers, especially melanomas. Mice, as well as primary cells such as macrophages, also exhibited the ability to catabolize uridine for energy production and even gluconeogenesis, and in all cases, UPP1 expression emerged as the primary determinant. Similar observations using pancreatic cancers were made in a joint publication from the labs of Costas Lyssiotis and Anguraj Sadanandam[7], further confirming the importance of UPP1 in cancer cell proliferation in vivo. Publishing this first paper from my new lab was an important milestone, which has undeniably increased the visibility of my young group and has provided the foundation for further research funding, giving us many exciting perspectives ahead.

From my childhood fascination with science to my current role as head of a research laboratory, I have been extraordinarily fortunate to have had the guidance and support of my family, teachers, colleagues and mentors. I am deeply grateful for the inspiration they have provided. Now, as a freshly minted mentor myself, I look forward with my team to continuing our exploration of the world of mitochondrial biology and energy metabolism at this fascinating interface between immunity and metabolic disorders.

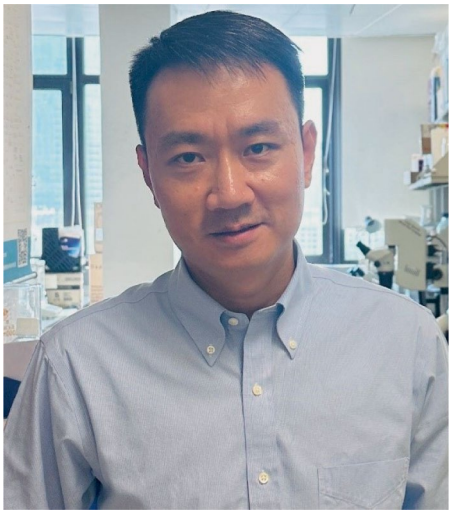
Feilong Wang: my journey to becoming a physician-scientist

Like in many countries, being a physician in China is a rewarding profession due to the opportunity to make a difference in the lives of patients. After graduating from medical school and completing residency training, I became a critical care physician with a passion for taking care of critically ill patients. Indeed, caring for patients with life-threatening illnesses brought me satisfaction and joy, while in the process, I learned new multidisciplinary skills and approaches required in clinical care. During my work in the intensive care unit, I was amazed by how quickly sepsis can progress to a life-threating condition, which led to my curiosity for understanding how the human immune system responds to invading microorganisms. With infection by the same bacteria, why do some patients remain stable and even have self-limited courses, while other patients encounter intense immune responses and cytokine storms that ultimately lead to organ

dysfunction and even death? With this in mind, I realized that a more in-depth understanding of immunology would be essential in fulfilling a career with a broader perspective and that lacking basic research and experimental skills would be a major shortcoming.

In September 2013, I was highly fortunate to join the laboratory of Joerg Herrmann at the Mayo Clinic to receive postdoctoral training. Whilst I had experience in clinical studies, I barely possessed the knowledge and skills of basic research; however, despite my inexperience, Herrmann gave me immense kindness, encouragement and support in pursuing my dream of becoming an independent physician-scientist. My initial project was to study the role of immunoproteasomes in the regulation of macrophage differentiation as well as in the development of atherosclerosis. But an 'accident' led us to enter a new area. One day, as usual, I stimulated Raw264.7 cells (an immortal murine monocyte-macrophage cell line) with lipopolysaccharide and expected many of these cells to start floating after 24 hours, an indicator of losing viability, as I had consistently observed this phenomenon many times. To my surprise, however, all the cells grew well and remained adhered to the dish, and no dead cells were observed the next day. I went back to check the medium and found that different medium was used for this experiment. For whatever reason, our technician ordered cell culture medium with high glucose instead of low glucose (the latter was what I intended to use, as per usual). I was astonished by how different nutrient supplies could affect the fate of immune cells. I was planning on resuming my original culture protocol and continuing to explore the immunoproteasome project after telling Herrmann what happened during this experiment. But Herrmann told me that great discoveries often come from accidents and encouraged me to continue studying the interaction between metabolism and immunology. Since this serendipitous and pivotal experiment, I changed my project and entered the field of immunometabolism, which was a new area I had never even heard of.

Upon reflection, I feel extremely lucky that I had the to study the role of metabolic reprogramming in the activation of classical and alternative macrophages kindly offered to me by Herrmann. He provided great guidance but simultaneously gave me the freedom to explore the projects that interested me most. In the next couple of years, we found that glycolysis is required for the activation of both LPS and IFN-γ-stimulated macrophages, but is not



necessary for the polarization of IL-4-driven macrophages[8,9]. My training experience in the laboratory of Joerg Herrmann has impacted me hugely – not only did it give me the ability, independence and confidence to conduct basic research, but it also set an example for me on how to mentor trainees in my own lab.

With the determination to become a physician-scientist, in 2018 I returned to China to join the Department of Pulmonary and Critical Care Medicine at Shanghai East Hospital. It was relatively straightforward for me to return to the physician track, but establishing an independent lab to continue my research career was challenging. While the research ecosystem for physician-scientists, including funding opportunities at the national level, as well as position availability and time assignment at the hospital level, is well established in the USA, there are no such avenues for clinicians in China. Due to the limited space and resources available, which is usually prioritized for full-time researchers, even the simplest experiments could not be conducted for me in the first two years. I had to find alternative ways to continue my research. I conducted several clinical research projects that have turned out to be successful in hindsight. I read papers extensively in the metabolism area and thought in-depth to identify the most fundamental questions in the field of immunometabolism, which would form the basis of my research program once I established a lab. Based on previous studies from our own and other groups, glycolysis is the metabolic signature of classically activated macrophages. However, how glycolysis links to the activation of macrophages remains unclear. As the last product of glycolysis, pyruvate can either enter the mitochondria via mitochondrial pyruvate carrier (MPC) to support the tricarboxylic acid cycle or be converted to lactate by lactate dehydrogenase A (LDHA). Targeting these two pathways would be helpful to elucidate the underlying mechanisms. To this end, I started to breed mouse models with a myeloid-specific deletion of MPC1 or LDHA, which allowed us to explore these metabolic routes in depth in the following years.

Fortunately, with generous support from the hospital and our department, I was able to gradually establish my laboratory, starting in the middle of 2020. Although it was in the midst of the pandemic, seeing the lab grow was a big relief. I have also been very lucky to recruit some talented and hardworking students and postdoctoral researchers since then, who are key elements for our lab to succeed. With great support from and cooperation with Herrmann and Song Zhang of Mayo Clinic, our work using a genetic depletion model demonstrated that mitochondrial pyruvate carrier-mediated metabolism is dispensable for the activation of M1 macrophages. This not only challenges the previous notion but also reveals unknown off-target effects of UK5099 – the gold standard inhibitor of MPC. It was a big moment for us when the reviewers' comments came back from *Nature Metabolism*, showing that we had a chance to improve our paper. In the meantime, however, I felt nervous as our lab had been forced to close due to the pandemic, and I myself had also contracted COVID-19. In addition, our plan to breed new genetically modified mouse models, which were required to address the points raised by the editors and reviewers, was also disrupted. Thanks to the editors who gave us a flexible timeframe and kind guidance through the revision process, and the reviewers who provided fair and constructive comments, we were able to cross the finish line and our paper was finally published in *Nature Metabolism*[10]. This publication was a milestone for our lab, giving me more confidence to pursue my dream and demonstrating that we, as physicians, can do beautiful science as well. It also sparked media coverage and public interest in our research projects and was essential to subsequent grant applications and trainee recruitment.

The work published in *Nature Metabolism* was an important project for our lab, but it was not the only focus. Over the past three years, our lab has expanded extensively, enabling us to explore more intriguing scientific questions. We've started investigating if and how the same metabolic pathway

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could differentially affect the activation of bone marrow-derived macrophages and tissue resident macrophages such as alveolar macrophages. In addition, we found that targeting glycolysis has distinct impacts on the function of classically activated macrophages and $CD8^+$ T cells, despite both types of immune cells switching to glycolysis after activation. In the coming years, our group will focus on this exciting yet rapidly evolving area and will strive to find novel targets for the treatment of human diseases based on the use of emerging technologies.

The path to becoming a physician-scientist and an independent investigator is never easy. Apart from clinical duties, we have endless grant applications, manuscripts to write, administrative responsibilities and so much more, which inevitably leads to less time spent with our family and friends. However, this unique position is also incredibly rewarding. In addition to having the freedom to explore what we are most interested in, it is a great privilege for us to bridge the gap between clinical practice and biomedical research. Seeing young trainees grow and develop in terms of confidence and self-esteem is also very satisfying. With an awareness that the road ahead might still be challenging, I will stay on track with my will unbending.

My first piece of advice for late-stage postdocs or early-career principal investigators is to stay focused on the questions that motivate you the most, do the experiments and let the data speak for themselves. Second, think critically. While it is with gratitude that we, as researchers, are to be able to build our work on the shoulders of giants, we also need to be aware that previous studies can sometimes be misleading, especially in an emerging area. I also have a piece of advice for those who are pursuing a career as a physician-scientist. Although there is a popular idea that physician-scientists should do clinical problem-driven research, it is not necessary for us to limit ourselves to this path. I agree that basic research originating from clinical practice is important, but solving the puzzles that you are most curious about will inspire greater perseverance, which is a key quality required for a scientist to succeed.

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