© 2021. Published by The Company of Biologists Ltd | Development (2021) 148, dev200084. doi:10.1242/dev.200084

INTERVIEW

Transitions in development – an interview with Tom Nowakowski

Alex Eve*,‡

Tom Nowakowski is an Assistant Professor at University of California San Francisco (UCSF), where he uses single-cell sequencing technologies to study neurodevelopment. He is also a Chan Zuckerberg Biohub Investigator and a Next Generation Leader at the Allen Institute for Brain Science. We met with Tom over Zoom to hear more about his career, his transition to becoming a group leader and his plans for the future.

Let's start at the beginning. When did you first become interested in science?

Growing up in Poland, I quickly realized my strengths and weaknesses. I enjoyed problem solving using first principles, and therefore I gravitated towards subjects such as mathematics and physics in school. I was fortunate to have fantastic teachers who organized many after-school activities, especially in physics and biology. Learning the scientific method through hands-on experiments helped me learn how to formulate a hypothesis, design experiments, and collect, analyze and interpret data. I remember spending evenings in the kitchen trying to build a torsion viscometer in order to measure the viscosity of egg white under different denaturation conditions. Later, I volunteered at the Institute of Oceanology near my hometown of Gdansk, working on projects in hydroacoustics. Those early science projects were some of the best aspects of my early education, and I am grateful to all those who put in the effort to make sure that science seemed 'cool' and every student who was eager to learn more could do so.

You moved to Edinburgh, UK, for your undergraduate and postgraduate studies. Why did you decide to go there?

Poland had just joined the European Union, which made it possible for me to study abroad. I chose Edinburgh University because I wanted to learn more about stem cell research; I had just finished reading a book about genetic engineering and molecular cloning, and Edinburgh has such a long history of attracting scholars who focus on developmental biology and stem cell research. Conrad Waddington, Matthew Kaufman, Ian Wilmut and Austin Smith have all been part of this legacy. For all these reasons, I was excited to be admitted to Edinburgh. I owe my interest in developmental neurobiology to David Price, who delivered inspiring seminars about brain development that inspired me to pursue research in his lab. I applied for summer scholarships from the Wellcome Trust and the Nuffield Foundation, which enabled me to gain research experience in a molecular biology lab.

What did you research during your PhD?

When I was admitted into the 4-year Wellcome Trust PhD program at Edinburgh, I was unbelievably excited. I wanted to explore how organ-specific stem cells encode temporal hierarchies of differentiation, and what regulates their transitions between competence states. I have drawn inspiration from the classical work of Chris Doe in *Drosophila* neuroblasts, and Susan McConnell in ferrets. The developing mouse cerebral cortex seemed like a good place to ask these questions because cerebral cortical layers are generated sequentially, in an inside-out order, and at different timepoints during development, but the mechanisms are still unclear. I was inspired by the work on RNA interference by Victor Ambros, who had shown that microRNAs can regulate developmental timing in *C. elegans*, and I naïvely thought that maybe this pathway could control some aspects of developmental timing in cortical neurogenesis. My doctoral work implicated microRNA-dependent regulation in several developmental transitions in the cerebral cortex, including radial glia specification, intermediate progenitor cell specification and the overall neurogenic time-window (Nowakowski et al., 2011, 2013a,b). I was lucky to have supportive mentors throughout my PhD, particularly David Price, Tom Pratt and Veronica van Heyningen, who helped me realize the potential of my project while respecting my intellectual autonomy.

You moved from Poland to Edinburgh, UK, and from there to San Francisco, USA. What was your experience of moving between countries?

I moved to Scotland in 2004, having never visited the place. The move came with a 'culture shock', and it took me some time to adapt to the new country, and to get used to speaking in a foreign language round the clock. Thankfully, Scottish people were very welcoming, and

*Reviews Editor, Development

‡Author for correspondence (alex.eve@biologists.com)

A.E., 0000-0003-3577-4324

Development (2021) 148, dev200084. doi:10.1242/dev.200084

Edinburgh University attracts many international students, so I did not feel like an outsider. Instead, it was a great opportunity to learn to interact and work with people from very diverse backgrounds.

I moved to the United States in 2012 to work at UCSF (University of California, San Francisco), which has fantastic research resources and the infrastructure to enable any types of experiments I could imagine. There were some challenges I did not foresee. For example, because the UK PhD is much shorter than a US PhD, I had to apply for postdoc positions before my papers had been submitted or accepted. My CV only had one small paper in PLoS One. Understandably, without a Nature or a Science paper on my CV, I was not an attractive candidate. I was lucky that Arnold Kriegstein was willing to take the risk and created an opportunity for me. After joining his lab, I discovered another challenge, which was that, before publishing my other PhD papers, my track record was not strong enough for postdoctoral fellowships. Corey Harwell, who was a senior postdoc at the time, told me that I needed to develop a stronger track record of productivity. His feedback helped me to process the rejections without getting discouraged, which was a useful skill to learn.

What did you study during your postdoc with Arnold Kriegstein at UCSF?

I became interested in identifying cell type-specific molecular mechanisms regulating cortical developmental using high-throughput gene expression analyses. Arnold was keen to identify genes unique to outer radial glia, a subtype of neural stem cell that his lab had described a few years earlier (Hansen et al., 2010). Soon after I started working on this project, we were fortunate to connect with researchers at Fluidigm, who developed microfluidic instruments for automated generation of single-cell sequencing libraries. The approach seemed like a good idea in this case, because asking questions of cellular heterogeneity were difficult using bulk tissue approaches. Taking a data-driven approach was key to identifying these major sources of transcriptional variation that distinguish outer radial glia (Pollen et al., 2015), neuronal subtypes (Nowakowski et al., 2017) and neural cells derived from human pluripotent cells (Bhaduri et al., 2020). Finally, I was excited to see that our single-cell data identified two distinct subtypes of radial glia. In addition to their role in neurogenesis, radial glia serve as a physical scaffold that guides postmitotic neurons to their terminal locations in the cerebral cortex. Using the new markers identified in single-cell RNA sequencing, I unexpectedly found that, at late stages of neurogenesis when radial glia generate mostly upper layer neurons, the vast majority – if not all – radial glia fibers contacting the pial surface originated from one of the subtypes: outer radial glia. Through a series of follow-up experiments, I confirmed this observation using orthogonal methods and found that the transition between what we called ‘continuous scaffold stage’ and ‘discontinuous scaffold stage’ occurs quite sharply during development. This finding has opened new areas of investigation, with important implications for our understanding of how the cerebral cortex develops.

You were one of the pioneers of single-cell sequencing at that time. Did you anticipate then how rapidly it would take off within the community?

There was no doubt in my mind that a technology that enables routine capture and whole-transcriptome amplification in single cells would be a ‘game-changer’. The question was how to do it efficiently, and at what scale. Fluidigm was the first major commercial vendor of microfluidic instruments for automated isolation of single cells and whole-transcriptome amplification. We were fortunate to work with the science team at Fluidigm to address the question of how much sequencing depth was necessary for unbiased cell-type classification. To our surprise, you didn’t need much, and with that came the opportunity to massively scale single-cell capture rates, which was accomplished using droplet encapsulation and later commercialized by 10X Genomics. The rapid pace of technology development was indeed astonishing. It was great to be a part of this revolution, and to contribute to demonstrating its potential through early applications.

Have you ever considered a non-academic career path?

In my second year of graduate training, I questioned my capacity for an academic research career. Despite working long hours, my project didn’t seem to be going anywhere, I didn’t have a story to publish. However, there weren’t very many opportunities for people with my training at the time. This was in 2009, and the world was just starting to recover from the financial crisis; there were not very many job opportunities overall. Looking back, I guess I was lucky that nobody tried to hire me. Without a tangible opportunity, I had no other choice other than to carry on with my project. In fact, I made a final push to generate a lot of data and, as I did that, my ideas started to come together to reveal new findings about cortical development, which have eventually resulted in three first-author publications. This journey was very personal; I had learned how to start an entirely new research direction in my mentor’s lab, to identify important questions and to carry it all the way through to publications that made conceptual contributions to the field.

At what point did you decide to go for independent research positions?

I applied about a year before I was ready. I had a couple of co-first author papers from my postdoc, but the bulk of my work had not yet been published. I reasoned that it was a good time to start exploring the process and what is involved, learning about the various departments at different institutions and, hopefully, also getting the chance to present my work. A lot of great schools had just advertised positions, so I had reasoned that the worst outcome would be that I would apply again the following year.

It was important in my early days as an investigator to identify mentors who were open to meeting at short notice and who had my best interests in mind

What was important to you when applying for different positions?

There were many important factors, but I would highlight two of them. First, it was important to me that the institution was prepared to make a commitment to support me and give me the resources I needed to start a lab. Although I was confident that I could get things up and running and get my first grants, starting a lab can be slowed down by so many factors, some of which can be difficult to predict. The need for support became so clear during the pandemic, when everything became complicated, especially for families with small children.

Second, I wanted to be at a place where I was surrounded by colleagues with whom I could write grants very quickly after I start my lab. Even before the pandemic, there was a lot of pressure and expectation of junior investigators to become productive within the first 3 years of starting a lab, otherwise it can be difficult

Development (2021) 148, dev200084. doi:10.1242/dev.200084

to secure funding. Three years go by faster than you think! So finding a place where I would have supportive colleagues who can involve me in their projects and grant applications was very important.

Is that what attracted you to staying at UCSF, because you already had that network around you?

Knowing my way around the organizational structures at UCSF was helpful. However, I moved to a different campus, where I did not know many investigators. With that came the challenge of finding myself in a new scientific environment, but also the opportunity to develop new collaborations and new relationships, almost as if I was moving to a different institution entirely.

Would you advise other early career researchers to move and keep making new connections?

Challenging oneself to be surrounded by new environments can be beneficial in many ways, inspire new ideas and lead to new collaborations. Moving between countries can be particularly exciting because of the additional challenge of embedding oneself in a society with a different way of thinking. For me, these have always been positive experiences. However, these transitions take time, and therefore can be risky when emphasis is placed on meeting productivity milestones.

I try to ensure that trainees in my lab come from diverse backgrounds and are eager to contribute unique perspectives

How was your experience transitioning to a group leader position?

The first year was both exciting and terrifying. Starting a lab involved having to learn – quickly – how to be organized and prioritize among the many responsibilities: writing protocols, grants, talking to administrators, mentoring trainees, ordering, fixing equipment and tracking down packages. On top of that, there is the added challenge of not having senior lab personnel and having to get accustomed to a high turnover of project responsibilities. It was important in my early days as an investigator to identify mentors who were open to meeting at short notice and who had my best interests in mind. Mentors are very important, especially when they tell us things we need to hear, but do not always want to.

I worried about striking the balance between wanting certain projects to happen and encouraging my trainees to come up with their own ideas. Throughout my career, my mentors let me be very independent. For better or for worse, I believe that approach contributed to my success, and so my goal has been to create a similar environment for my trainees, as much as possible. One way of accomplishing this was to work with early trainees on fellowship applications, and tailoring their projects to their interests. Some of these were successful, and that was very rewarding.

What advice would you give to other researchers that are starting their own lab?

I think that, surprisingly, many people struggle to really believe in themselves. It can lead to not making sufficient investments in the infrastructure and potential projects. Many junior investigators, including myself, feel anxious about running out of money. But, the reality is that you have this once in a lifetime opportunity to start a lab. Holding back is not a good idea.

What has been your approach to hiring new team members?

Recruiting talented people to join the team is probably the most crucial aspect of running a lab. Every candidate brings their own unique story, and a reason why they want to pursue an academic career. I want to be their ally and help them be successful. I try to make sure that everyone feels comfortable and is eager to collaborate. I try to ensure that trainees in my lab come from diverse backgrounds and are eager to contribute unique perspectives. The opportunity to interact with people from different cultures and backgrounds is one of the aspects of academia I enjoy the most. Finally, I try to make sure that the coffee machine has enough beans!

What are the research themes of your lab now?

We are interested in identifying the cellular and molecular underpinnings of cell type diversity and tissue homeostasis in the brain. Our central goal is to develop a quantitative framework for understanding how cell types emerge during development and enable complex functions of the nervous system. The problem of cell type classification is not new in neuroscience and biology more broadly. Single-cell technologies have accelerated the discovery process of proteins, genes and gene regulatory elements that allow us to group cells. Translating these predictions into testable hypotheses about developmental mechanisms is very exciting. For example, the epigenomic states of cells can reveal a lot about the mechanisms that got that cell to that state, and also help us predict the potential of what that cell type might become in the future – one of the foundational concepts proposed by Conrad Waddington in ‘the strategy of the genes’. Our most recent work using single-cell epigenomics has provided novel insights into the developmental origin of cell types in the cerebral cortex, demonstrating that chromatin accessibility differences foreshadow divergent transcriptomic trajectories during neurogenesis.

We are also interested in understanding how tissues maintain homeostasis through cell-cell interactions during development. Understanding the logic of cell-cell communication could teach us a lot about the first principles of tissue organization, and help us predict the consequences of genetic or environmental perturbations, not just on cellular development but more broadly on tissue development. For example, we are using this strategy to understand the role of myeloid cells in brain development and how their abnormal activation could lead to pathology.

Finally, we seek to understand how the genetic mutations associated with neurodevelopmental or neuropsychiatric disorders can shape or disrupt the fundamental processes of brain development. This comes back to my high school experience, when I volunteered at a hospice. I interacted closely with patients with psychiatric conditions, including schizophrenia. It was a powerful experience that made me realize how little we understand about the human brain and mental states, and an experience that probably influenced many of my career choices. I am excited that advances in medical genetics have identified many candidate genetic variants associated with psychiatric traits, and I am excited to have the tools to start probing their roles in brain development.

What excites you most about your field right now?

Three things. One, I am excited to see how spatially resolved transcriptomics will help to contextualize cell-cell interactions in tissues. These approaches are starting to gain momentum and will have a transformative impact on the types of questions that we can answer. Two, I am excited to learn the logic of gene expression

Development (2021) 148, dev200084. doi:10.1242/dev.200084

regulation. In particular, the combination of genome-engineering approaches with single-cell genomics will be a transformative and scalable approach for conducting functional experiments at scale. Many of these technologies can be applied to non-model organisms, making it a lot easier to study these processes in diverse genetic backgrounds and in many species, including human. I think this will teach us a lot about how the genome encodes both developmental and cell-type instructions, and potentially also reveal shared vulnerabilities to mutations. Last, I am excited for the many unexpected findings that are going to be enabled by the exponentially growing datasets that are being made available openly through many exciting research consortia, including the BRAIN Initiative, the Human Cell Atlas and the psychENCODE. High-quality datasets can serve as a platform for hypothesis-driven research. In-depth analyses of those datasets can be an important source of ideas.

In 2018, you were elected to the Next Generation Leaders Council at the Allen Institute for Brain Science. What does this role entail and how did you get involved?

The Next Generation Leaders Council is selected annually through an open competition. Council members participate in the Showcase symposium, which is an annual event that features many of the latest research discoveries at the Institute. During the pandemic, the Institute and its resources have also been a great source of learning and skill development. For example, the Next Generation Leaders helped to develop a series of 'open for (neuro)science' webinars that promoted the research, tools and technologies, as well as data resources created by the Institute across the world. I hope that some of these datasets, combined with properly tailored educational resources could be leveraged to promote more equitable science education in high schools around the world.

Would you encourage others to get involved in similar initiatives?

Absolutely. Being a Next Generation Leaders Council member has been an overwhelmingly positive experience. It has inspired me to think very broadly about neuroscience, and about the 'team science' and 'open science' culture that researchers at the Institute have been promoting since its beginning. I would encourage anyone who has new ideas for how the Institute could achieve a broad scientific and societal impact to apply.

Finally, is there anything Development readers would be surprised to learn about you?

In high school, I aspired to become a theoretical physicist. At Edinburgh, I sought to double major in biology and physics. I was particularly drawn to the quantum theory of subatomic particles as fundamental building blocks of the universe. However, the orthogonal class schedules in mathematics and biochemistry did not allow for this to happen, and that was when I decided to focus on studying the fundamental building blocks of life: genomes and cells.

References

Bhaduri, A., Andrews, M. G., Mancia Leon, W., Jung, D., Shin, D., Allen, D., Jung, D., Schmunk, G., Haeussler, M., Salma, J. et al. (2020). Cell stress in cortical organoids impairs molecular subtype specification. *Nature* **578**, 142-148. doi:10.1038/s41586-020-1962-0

Hansen, D. V., Lui, J. H., Parker, P. R. and Kriegstein, A. R. (2010). Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* **464**, 554-561. doi:10.1038/nature08845

Nowakowski, T. J., Mysiak, K. S., Pratt, T. and Price, D. J. (2011). Functional dicer is necessary for appropriate specification of radial glia during early development of mouse telencephalon. *PLoS ONE* **6**, e23013. doi:10.1371/journal.pone.0023013

Nowakowski, T. J., Fotaki, V., Pollock, A., Sun, T., Pratt, T. and Price, D. J. (2013a). MicroRNA-92b regulates the development of intermediate cortical progenitors in embryonic mouse brain. *Proc. Natl. Acad. Sci. USA* **110**, 7056-7061. doi:10.1073/pnas.1219385110

Nowakowski, T. J., Mysiak, K. S., O'Leary, T., Fotaki, V., Pratt, T. and Price, D. J. (2013b). Loss of functional Dicer in mouse radial glia cell-autonomously prolongs cortical neurogenesis. *Dev. Biol.* **382**: 530-537. doi:10.1016/j.ydbio.2013.08.023

Nowakowski, T. J., Bhaduri, A., Pollen, A. A., Alvarado, B., Mostajo-Radji, M. A., Di Lullo, E., Haeussler, M., Sandoval-Espinosa, C., Liu, S. J., Velmeshev, D. et al. (2017). Spatiotemporal gene expression trajectories reveal developmental hierarchies of the human cortex. *Science* **358**, 1318-1323. doi:10.1126/science.aap8809

Pollen, A. A., Nowakowski, T. J., Chen, J., Retallack, H., Sandoval-Espinosa, C., Nicholas, C. R., Shuga, J., Liu, S. J., Oldham, M. C., Diaz, A. et al. (2015). Molecular identity of human outer radial glia during cortical development. *Cell* **163**, 55-67. doi:10.1016/j.cell.2015.09.004