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INTERVIEW

The people behind the papers – Ping Kao and Michael Nodine

The application of single-cell mRNA sequencing technologies to plant embryos promises to reveal the gene expression dynamics underlying cell-type differentiation. A new paper in Development reports the generation of high-quality transcriptomes from single embryonic nuclei without contamination from maternal tissues. To find out more about the story, we caught up with first author Ping Kao and his supervisor Michael Nodine, who recently moved from the Gregor Mendel Institute in Vienna to become Assistant Professor in the Laboratory of Molecular Biology at Wageningen University in the Netherlands.

Ping (L) and Michael (R) in front of the Belvedere in Vienna (image credit: Nicholas Nodine).

Michael, can you give us your scientific biography and the questions your lab is trying to answer?

MN: My first research position was at the Clemson University Genomics Institute in South Carolina starting in 2000, and then at the Arizona Genomics Institute with Professor Rod Wing. This was followed by PhD training in genomics, molecular and cellular biology at the University of Arizona in Professor Frans Tax's lab. I then joined Professor Dave Bartel's lab at the Whitehead Institute for Biomedical Research at MIT, where my interest in RNA biology grew and fused with the fascination with plant embryology and genomics I had developed as a student. In the summer of 2012, I established a research group at the Gregor Mendel Institute on the Vienna BioCenter (VBC) campus, where we investigated questions related to (epi)genetics/genomics, and molecular, cellular and developmental biology. Although we mainly study plant molecular embryology using *Arabidopsis*, it has been exciting to apply the methods we developed to other systems in collaboration with other groups. At the beginning of this year, I started as an Assistant Professor in the Laboratory of Molecular Biology at Wageningen University. I am thrilled to join my colleagues in Wageningen who have vast expertise ranging from basic to translational plant science research. We will continue investigating questions related to the RNA biology of plant embryos with a focus on how small non-coding RNAs regulate gene expression and associated developmental processes.

Ping – how did you come to work with Michael and what drives your research today?

PK: I am interested in the plasticity and diversity of plant cells in general. Many plant cells can be redirected to pluripotent or totipotent cells and re-differentiate into various cell types when given the correct stimuli in a process known as somatic embryogenesis. But, the initiating events and even the initial cells are hard to identify in somatic embryogenesis. On the other hand, the zygotic embryogenesis in *Arabidopsis* provides a great system to investigate the regulatory events related to both totipotency and differentiation. Since I joined Michael's group through the VBC PhD programme, we have been actively searching for new solutions to improve our knowledge of zygotic embryos. One of the goals is to generate a detailed expression atlas for *Arabidopsis* embryos, which is common for animals but not available for plant model organisms. Although we are not the first ones aiming for such goals, we were fortunate to overcome the technical difficulties and present this embryonic expression atlas. We hope this dataset can help plant embryologists in their projects and improve the overall understanding of plant embryos.

How has your research been affected by the COVID-19 pandemic?

PK: Fortunately, the COVID-19 pandemic had relatively little impact on this project as we had collected the single-nucleus RNA-sequencing (snRNA-seq) data before the first lockdown in Vienna. The VBC facilities also swiftly set up the remote systems so I could work from home analysing the sequencing data without difficulties. On the other hand, the wet-lab experiments were heavily limited by several anti-pandemic regulations, so we had to be more careful and selective about the RNA *in situ* hybridisation candidates. The *in situ* validations were essential because there was no detailed reference dataset and the knowledge of the representative genes for different embryonic cell types was limited compared with other tissues, such as roots. Thankfully, we managed to complete the *in situ* hybridisation experiments in time to publish this project.

MN: I completely agree with Ping. The coronavirus task force at the VBC did a superb job keeping us healthy with high-throughput COVID-19 testing, which also allowed research groups on campus to do wet-lab work. The information technology and high-performance computing teams of the VBC also increased the remote computing capacity very early during the pandemic, which allowed us to continue analysing data, accessing journals and

P.K. & M.N.: Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna BioCenter (VBC), Dr Bohr-Gasse 3, 1030 Vienna, Austria.
M.N.: Laboratory of Molecular Biology, Wageningen University, Wageningen 6708 PB, The Netherlands. Email: michael.nodine@wur.nl

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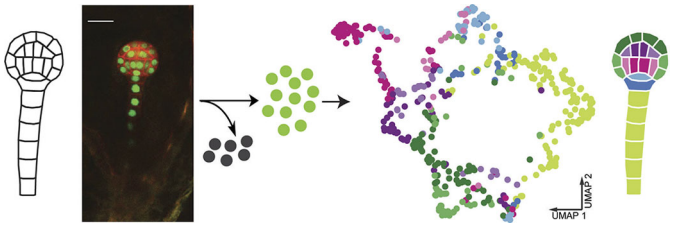

Resolving cell types in the *Arabidopsis* embryo.

writing papers. Although we had many virtual meetings, including some very long ones between Ping, Michael Schon and I for this project, I miss the personal interactions. Hopefully, we can all get back to talking face-to-face soon!

What have been the challenges of *Arabidopsis* early embryo transcriptomics before your paper?

MN, PK: Profiling the transcriptomes of *Arabidopsis* early embryos is a challenging endeavour because they are small and deeply embedded within maternal seed tissues. Previously published early embryonic transcriptomes either had limited spatial resolution or were contaminated with RNAs from the surrounding maternal seed coat.

Can you give us the key results of the paper in a paragraph?

MN, PK: We developed a method that allows the generation of high-quality transcriptomes from individual nuclei of early *Arabidopsis thaliana* embryos that can then be computationally assigned to their corresponding cell type. This scRNA-seq method and associated computational analyses, which were developed together with Michael Schon, who is another PhD student in the group, enabled us to observe gene expression variations across cell types associated with differential evolutionary trajectories, epigenetic mechanisms and transcriptional programming.

Why do you think that individual cell types didn't come out from the initial unsupervised clustering?

MN, PK: We suspect that unsupervised clustering could not readily resolve individual embryonic cell types either due to the relatively low number of snRNA-seq libraries included in the clustering (n=486) or because the cell identities are just emerging at this early developmental stage. Other variations, such as differential cell cycle activities, may also make it more difficult to resolve different cell types from one another.

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

PK: There are two particular moments that I believe were essential to the success of this project. The first eureka moment was the decision to go for the more accessible nuclei instead of whole cells. As scRNA-seq became more popular, we wanted to utilize such techniques to reveal the spatio-temporal transcriptional dynamics in developing embryos. However, dissecting early embryos is technically challenging and dissociating plant embryos presents another layer of difficulties. These limitations made it difficult to acquire large amounts of samples from plant embryos and limited the utilization of high-throughput single-cell methods. Inspired by published nuclear transcriptomes and the Div-Seq protocol, the decision to make snRNA-seq libraries paid off when we found that the preliminary snRNA-seq libraries were complex and the identities of individual nuclei can be differentiated.

The second eureka moment was the introduction of the cell-type scoring system for clustering. As mentioned above, conventional scRNA-seq unsupervised clustering was not sufficient to reveal the differences among cell types in our dataset. Guided clustering with the curated marker genes was not informative either, as the variations of individual markers blurred out the spatial resolution. Inspired by some reasoning games that I played, the idea of making a scoring system and viewing the cell type-specific markers collectively came into my mind. With help from Michael Schon, we managed to develop the cell-type scores based on hypergeometric tests. *In silico* validation showed that clustering based on cell-type scores was the most informative and this was further supported by RNA *in situ* hybridisation validations. Once we saw the results from the initial set of RNA *in situ* hybridisations, we knew we were on the right track!

And what about the flipside: any moments of frustration or despair?

PK: Scientists investigating new research directions are familiar with frustrations on a daily basis. Jokes aside, the most impressive moments of frustration for this project were the moments before the eureka moments mentioned above. The thought that the problems were insurmountable, and the project may fail, came into my mind a few times while facing such major difficulties. Fortunately, with encouragement, I kept searching for solutions and all the efforts eventually paid off.

What next for you after this paper?

PK: In my opinion, this paper is just the beginning of understanding at a deeper level the regulatory events in developing embryos. To pursue my interest in plant totipotency and differentiation, I am joining Minako Ueda's group in Tohoku University in Japan after acquiring my PhD degree. Minako's group specialises in embryo morphogenesis and intracellular dynamics, and I anticipate that we can reveal the regulatory systems in early embryos by combining our expertise.

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Where will this story take the lab?

MN: In addition to following up on epigenetic and transcriptional mechanisms that apparently vary across early embryonic cell types, the methodology developed for this project provides a roadmap for how single-cell genomic methods can be effectively applied to plant embryos. We are just at the beginning of an extremely exciting era of developmental biology and the application as well as integration of various single-cell methods promises to yield insights into the gene regulatory mechanisms underlying pattern formation in plant embryos. There is much to do and, together with other plant embryologists, it will be exciting to see how our field progresses in the near future.

Finally, let's move outside the lab – what do you like to do in your spare time in Vienna and Wageningen?

PK: I am a relatively indoor type of person, but I also really enjoy strolling on the historical streets of Vienna and visiting various museums as you can feel the rich history of the empirical capital. The private cafes in Vienna are also great places to spend a few hours on weekends to think or relax. I also love the seasonal events

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in Vienna, such as enjoying the Donau river in summer, the Oktoberfest and the Wintermarkt.

MN: I also enjoy all of the aspects of Vienna that Ping mentioned and am just getting to know Wageningen and the Netherlands. In general, I like spending time with my family and travelling around Europe to enjoy the rich history and diverse cultures.

Reference

Kao, P., Schon, M. A., Mosiolek, M., Enugutti, B. and Nodine, M. D. (2021). Gene expression variation in Arabidopsis embryos at single-nucleus resolution. *Development* **148**, dev199589. doi:10.1242/dev.199589