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PEOPLE & IDEAS

Chii Jou Chan: The positives of being under "pressure"

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Chii Jou Chan investigates how tissue mechanics and fluid pressure regulate mammalian development, with a special focus on folliculogenesis and oocyte quality control.

Marty McFly did it, not once but twice. He traveled back to 1955, and then back to the future. Since he can remember, Chii Jou (Joe) Chan has been trying to replay Marty's steps. However, as he didn't have the support of Doc Brown to build a time machine, he decided to learn how to command time travel by himself. For that, understanding Einstein's theory of relativity was a must. Thus, Joe moved from Singapore, where he grew up, to Cambridge, UK, to study physics. He became fascinated by soft condensed matter physics, which later played a bigger role than he expected in his research, and did his master of science on non-equilibrium thermodynamics of liquid crystals, working with Eugene Terentjev at the University of Cambridge. Joe also did a brief stint in the Pincus lab at the University of California, Santa Barbara, USA, where he worked on the physics of polyelectrolyte brushes. However, his research interests pivoted toward the field of biological physics. And here is where one would think that Joe jumped straight to academia, but no, he didn't. He took a break of 6 yr! Everyone that knows Joe well could think he may have been trekking and traveling, which he loves—he used to do backpacking in Tibet as a teenager. But, far from this, Joe worked in industry during those years, and later managed to start a PhD. "I was initially skeptical that any lab will take me past age of 30 for a PhD starter, but I was wrong. It's never too late to 'go back in time' and do something you love, as long you have the drive and self-faith," he says. Joe joined the lab of Jochen Guck at Cambridge and later at Dresden to investigate cell and nuclear mechanics using optical stretcher and biophotonics. For this postdoc, he moved to EMBL Heidelberg to work with Takashi Hiiragi—a pioneer in mouse development—on the mechanobiology of mouse blastocyst development. In the Hiiragi lab, Joe made the seminal discovery that hydraulic pressure in the blastocyst lumen, as a result from an expansion of the fluid volume, played a critical role in controlling the embryo size and cell-fate specification (1). He then continued studying how tissue hydraulics regulate mammalian development, with a special focus on folliculogenesis and oocyte quality control, as a principal investigator (PI) at the Mechanobiology Institute (MBI), National University of Singapore (NUS)—his lab opened its doors in January 2021. Joe is also currently an assistant professor in the Department of Biological Sciences at NUS and has helped launched the Society for Cell Biology Singapore (SCBS; https://www.scbs.sg).

We talked with Joe to learn more about his scientific journey and current research projects.

Chii Jou Chan. Photo courtesy of Diego Pitta de Araujo.

What interested you about the tissue hydraulics of mammalian folliculogenesis and oogenesis?

In my PhD, I learned to build and apply various biophysical techniques to study the physical properties of single cells and nuclei, which was very rewarding. Toward the later phase, I was very much drawn toward understanding how physical forces guide multicellular dynamics, particularly in the context of animal development. That was when I decided to join the Hiiragi lab to study the role of fluid pressure in early mouse embryo development (1). As I started to wonder about the research themes for my future lab, I became interested in mammalian folliculogenesis, which is the maturation of ovarian follicle that houses functional eggs for future reproduction. If you look at a typical ovarian tissue slice, you can see many follicles surrounded by ECM and stromal cells, and within each follicle, the oocyte is surrounded by layers of

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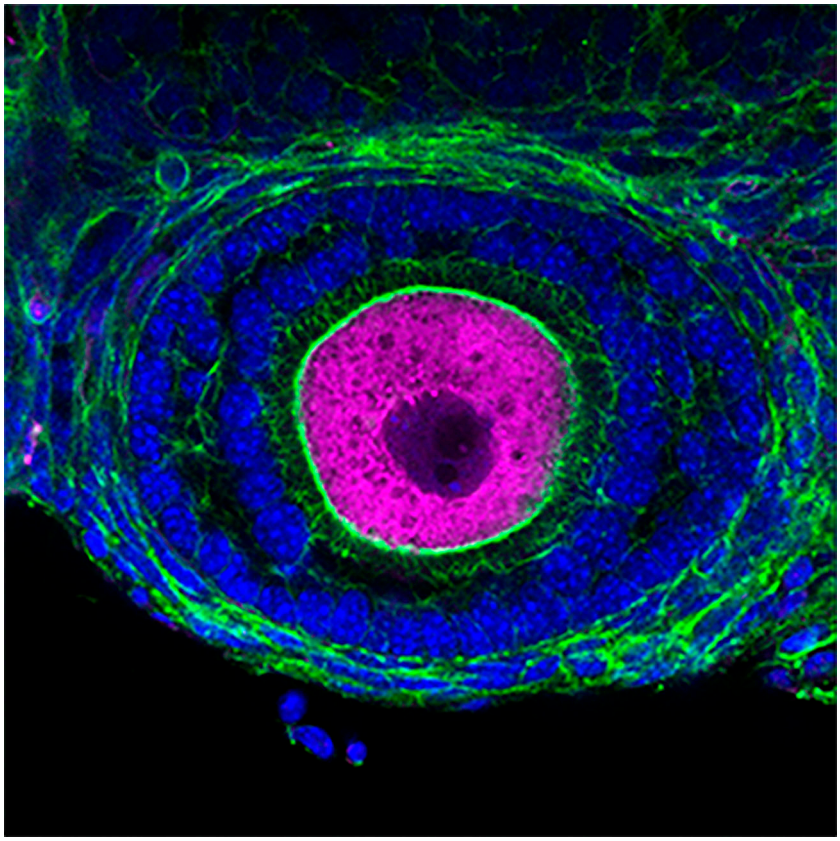

A secondary follicle showing actin (green), nuclei (blue), and an oocyte (magenta). Image courtesy of the Chan lab.

somatic cells that appear to be high packed. That led me to think that the follicle and oocyte growth must be regulated by some sort of mechanical feedback that acts across multiple scales. Yet to date we still have little knowledge of how the cells mechanically interact with each other. One of the reasons could be a lack of appropriate tools to measure and manipulate forces in ovaries or follicles. Another aspect of follicle morphogenesis that excites me is the formation of fluid-filled cavity during the antral follicle stage. Looking at it reminds me of a giant blastocyst! As the lumen expands in size, the follicle eventually ruptures and releases the oocyte into the oviduct for subsequent fertilization (ovulation). Hence it is plausible that the lumen exerts both mechanical and biochemical forces to regulate ovulation, which is a highly robust process, as we all know that only one egg is released in each reproductive cycle for humans. In general, I think there's a huge knowledge gap in our understanding of how fluids and hydraulic forces control oogenesis in reproduction.

What are the main research lines that you have set up to study the mechanical regulation of follicle and oocyte development?

We are currently investigating three different aspects across multiple scales. First, in regard to intra-follicle mechanics, we are studying how the outer follicle cells (theca cells) generate contractile forces and compressive stress to regulate follicle growth. We discovered that these theca cells are mechanosensitive, so another aspect is to explore how follicle growth itself regulate theca cell functions through cell stretching and ECM remodeling, and how this mechanical feedback loop leads to follicle size control. The second aspect we are looking into is follicle–follicle interactions. Previous work has shown that when two follicles are grown together, one tends to dominate in growth while the other remains arrested, but the mechanism remains unknown. We hypothesize this may be mechanically regulated, and we are currently investigating various mechanical signals that could explain this puzzling phenomenon. The third research theme concerns extra-follicle environment, which is dominated by the stroma cells and ECM. Here we are particularly interested in how the macrophages modulate their functions in response to the changing ECM environment during ovarian aging, which is accompanied by extensive fibrosis. Some of these questions are highlighted in a recent review published by our lab (2).

Do you have in mind other scientific projects for the future?

We would like to study more in depth the mechanism and functions of luminogenesis during antral follicle development and ovulation. I believe a fundamental understanding of this process will help us address ovarian aging, infertility, and various ovarian diseases such as polycystic ovarian syndrome where failed hydraulics and ovulation have been implicated. On a tissue level, we are very excited about new collaborations with experts in A*STAR and NUS to study spatiotemporal changes in the stromal cell transcriptome during ovarian aging using advanced single-cell RNA technologies. This is complementary to projects in collaboration with scientists in Australia to study changes in the stromal tissue stiffness during ovarian aging, using non-invasive approaches such as optical coherence elastography. Stayed tuned!

Knowing a bit more of your research, which seems to equally rely on physics and biology, I guess the approach you bring to your work is highly interdisciplinary...

That's right. We combine biophysical, bioengineering, and molecular approaches to understand how mechanical inputs across scales impact oocyte functions and follicle morphogenesis. To tackle the challenge of multiscale complexities in vivo, we adopt bottom-up approaches such as ex vivo reconstitution and primary cell culture systems to dissect the mechanical interactions between the follicle cells and their microenvironment. We are establishing deep tissue imaging techniques and biophysical tools to map out cellular dynamics and mechanical stress pattern within the follicle. For example, our recent work using Brillouin microscopy revealed that the theca cells and granulosa cells within the follicle have distinct mechanical properties (3). We

are also applying tools such as micropipette aspiration and micropressure probe to study changes in cell contractility and fluid pressure during follicle development. We are also working closely with theorists to develop a biophysical model for folliculogenesis and collective dynamics in ovaries. We are aware of the saying that "what the cells can do in vitro do not always translate to what the cells do in vivo," so we always compare our ex vivo findings with in vivo staining of tissue slices where the stroma environment is preserved.

And what is the approach you follow to run your lab?

In our lab, each research theme is undertaken by a small team, typically a postdoc and student. This happened naturally as the lab evolved, and I realized that this helps to nurture learning and discussion. Research can be a lonely journey! I am a true believer in the power of 1 + 1 being >3—of course, with some mindful matching. Our lab is highly collaborative amongst the members, a culture I highly value and make a conscious effort to nurture. I also spent quite some time meeting students and postdocs on a weekly or bi-monthly schedule and conduct journal clubs myself. One thing I did early on as a PI is to hold regular "research skills seminars" once every few months during our group meeting, in which I shared with my lab members on topics like how to read a paper, how to present, how to design experiments, etc. I try to commit to these talks despite my busy schedule as I realize these skills are not taught at the university level, yet they are so essential for research. I also initiated a joint tissue mechanics meeting every month between various labs across the campus to provide a platform for the postdocs and students to share their research and to polish their presentation skills.

Is your way of doing science influenced by your PhD and postdoc experience?

In a certain manner, yes, especially by what I learned from my mentors. As I am leading my own lab now, I realize how much my PhD and postdoc advisors have shaped my research thinking and the way of doing science. One early advice from Jochen was to stay open-minded and "let nature speak for itself," and never try to look for nails that "fit" into the size of our hammer—aka our favorite hypothesis blind spot. He also taught me the importance of developing or applying new techniques, which often lead to discovery of new questions in biology. He was also very generous. I remember the day when I broke the optical stretcher in my first year of PhD, but he merely replied, "No PhD student graduates without breaking something in their journey; this is part of science." That was quite a reassuring moment and reminded me that failure is part of scientific journey. While Jochen taught me a lot about "how" biology works, my postdoc advisor Takashi Hiiragi taught me a lot about the "why"—why do biology behave the way they are, what are their functions? He taught me the importance of asking the right biological questions, and also showed by example how to mentor students and postdocs. He provided very fertile ground for scientific thinking to flourish in the lab, such as organizing joint retreats. He was also extremely generous—I recalled him offering me a chance to present my work, even though he was the invited speaker in a major conference. Both Jochen and Takashi gave me a lot of freedom to explore my research and allowed me to write my own manuscripts. As a PI now, I try to cultivate this kind of lab culture and spirit.

You said you regularly schedule journal clubs at your lab. They are often the place to discuss the most exciting advances in the field. Is there any recent development you would highlight in the developmental biology arena?

Of course, and not just because it touches my immediate research, I would highlight the increased recognition in the field that biological fluids are not just a mere by-product of developmental processes. Instead, they could play an active role by exerting shear stress and hydrostatic pressure to guide tissue morphogenesis. By changing the tissue geometry, the lumen can also modify the signaling profile in the tissue. Furthermore, the lumen can store signaling molecules, hence directly impacting tissue patterning. Recently, tissue hydraulics has also been implicated in mammalian reproduction such as oogenesis and spermatogenesis, thanks to new imaging techniques and ex vivo approaches. We recently wrote a review to highlight the progress and propose new questions for the field (4). Another exciting development in the field of mammalian folliculogenesis is the discovery of ECM-imposed mechanical stress in keeping follicles in their dormant state. In my view, these emerging studies are paradigm shifting as they tell us that it is not enough just to understand the genes and hormones that regulate oocyte functions—one also has to understand the environmental contexts of the oocytes, particularly the mechanical signals that get transmitted to the oocyte during development.

The Chan Lab, July 2022. Photo courtesy of Chii Jou Chan.

What are the most pressing challenges of your field of research?

I think the field of ovarian mechanobiology is still in its infancy. Currently we have limited tools to probe mechanical forces in three-dimensional living tissues. The ovarian tissues are also highly scattering, which poses a challenge for live imaging of tissue dynamics. I also think this field will benefit more from interdisciplinary collaborations. I recently attended an annual conference on reproduction, and despite the large number of participants, there were only <10 posters discussing the biomechanical aspects of ovarian biology. Another challenge is the lack of theoretical and computational models in this field, which I think could be a powerful tool to study collective dynamics and tissue patterning during ovarian development.

Any suggestions on how to foster interdisciplinary thinking and collaborations?

This has to start from the undergraduate-/graduate-level education, as well as implementing workshops or symposiums to encourage cross-department talks. More curiosity-driven research that is not always geared towards translational impact only

would also be needed. A lot of times, true translational impact comes from fundamental understanding of how cells work, so basic science is as important as translational research, and collaborations here would benefit the advance of both.

You have helped launch the first official cell biology society in Singapore (SCBS). Can you tell us about what motivated you and the other founders to create this society and what are its main purposes?

While there is excellent cell biology research conducted in Singapore, in general, we feel there is a lack of a suitable platform for cell biologists (and others from related disciplines) across Singapore to interact and share the knowledge and expertise among each other. We also founded this society with the aim of promoting education and training for those interested in cell biology, through organizing regular workshops and symposiums, and supporting publications relating to cell biology. Finally, an important aim of this society is to support the early-stage researchers through awards, fellowship, and a mentorship network. This is something I am deeply passionate about, and I feel grateful to be part of the team to make it happen.

You became PI recently; what challenges have you faced as a new PI so far?

A biggest challenge for me so far has been moving to a new country to start my lab. There is a lot to learn and adapt to the new research culture, funding landscape, and the different administrative issues—the amount of administrative paperwork has been burdensome, although I think this happens everywhere, and it does not really improve over the years. But more arduous it has been the process of hiring! This is especially challenging from Singapore, which is quite disconnected from other countries. The pandemic has also made my first year of PI pretty challenging—yet as a PI we have to find ways to motivate ourselves, otherwise it is impossible to motivate and guide lab members on a daily basis. Another challenge for me has been grant writing, which is very different from manuscript preparation and requires a lot of perseverance, since rejection is the norm and there's often no clear feedback from the agency. As a PI we all have to juggle with many uncertainties, and I would say the ability to adapt is the key to a rapid and successful transition from postdoc to PI.

I am positive every new PI would love if limitations on research funding disappeared—no more grant writing! What would you do if you had unlimited funding?

I would use half of it to pay competitive salaries to hire more scientists to pursue the various projects we have in the lab. I would use the other half to build a core facility that houses all kinds of biophysical tools and technical support to probe mechanical forces from molecular scale to tissue level. This will be immensely beneficial for the local community here.

Any tips for a successful research career?

Explore your passion; figure out what really motivates you in your research. These days science is highly interdisciplinary, so it's important to stay open-minded and be willing to acquire new knowledge and approaches when the need arises. Despite my busy schedule, I still spend an hour or two every Saturday morning to catch up with the latest cool papers, very often outside my field of expertise. This helps me to stay creative and reminds me how fun science can be. To do well in science, it is also important to cultivate perseverance, as true discovery is often a combination of hard work and keen observation. Another thing is do not be afraid to "switch" field or questions if you have identified something that truly piques your interest. In my case, I have switched from theoretical soft matter physics (masters) to experimental biological physics (PhD) and mouse embryology (postdoc), and at every stage I tried to learn as much as I could about the field and to adapt to the new thinking and languages. I think all these have broadened my perspectives and help me identify new approaches and ways of looking at biological phenomena.

To finish with a more personal note, what has been your biggest accomplishment outside of the lab?

The answer is simple: my family. I wouldn't be where I am today without the unwavering support of my wife. Our 5-yr-old daughter is another gem who has brought so much joy and happiness to my life. They are definitely the anchor of my life, and I am truly blessed to have them by my side.

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