

Reprogramming Stars #27: Drawing Inspiration from Reprogramming to Guide Stem Cell Differentiation: An Interview with Dr. Kyle Loh

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Dr. Pereira: Good afternoon. My name is Filipe Pereira, Professor at Lund University and editor-in-chief of *Cellular Reprogramming*. I'm very happy to bring you a new episode of *Reprogramming Stars*, our flagship series capturing the findings, projects, and ideas of the leaders in cellular reprogramming.

Today, we have Dr. Kyle Loh, an Associate Professor at Stanford University, USA, where he also conducted his PhD, supervised by Dr. Irving Weissman. After his PhD, Dr. Loh was appointed as a Siebel Investigator and, later, an Assistant Professor, a position he has held since 2018. Dr. Loh's work is focused on what extracellular signals need to be turned on or off to convert human pluripotent stem cells into a specific cell type. This is creating a developmental roadmap with exciting implications for fundamental research and regenerative medicine. Throughout his career, Dr. Loh received several awards, such as the NIH Director's Early Independence Award, Forbes 30 Under 30, Harold Weintraub Graduate Award, and the Hertz Foundation Thesis Prize. Dr. Loh, thank you so much for joining me today.

Dr. Loh: Thanks a lot. I feel a bit awkward, since I don't think I'm a star! But I'm happy to share our work at this venue.

Dr. Pereira: No, you are certainly a star, and it's our pleasure to have you featured in this interview series! Your lab is at the forefront of pluripotent stem cell differentiation into several lineages, studying differentiation routes in parallel. I was wondering how you began your journey in cellular reprogramming.

Dr. Loh: Our lab mainly works on stem cell differentiation, but we drew inspiration from the field of reprogramming. As you know, there's a broad concept of a lineage bifurcation where a progenitor cell forms at least two different



Dr. Kyle Loh

Dr. Kyle Loh is an Associate Professor in the Department of Developmental Biology and the Institute for Stem Cell Biology & Regenerative Medicine at Stanford University, USA. His lab strives to understand how human pluripotent stem cells develop into dozens of different cell types, in order to generate a roadmap for human development. Instead of focusing on the development of a specific cell type, the team is exploring many different lineages simultaneously, hoping to learn general principles underlying developmental biology. Research from Dr. Loh's lab has enabled the creation of nearly pure batches of human cells—ranging from blood to blood vessels to brain—that can advance disease modeling and regenerative medicine.

lineages. I was excited by pioneering work in the field of cellular reprogramming by people such as Thomas Graf and Harold Weintraub. Their work focused on the dual role of certain transcription factors (TFs) in lineage decisions. They showed that repressing the formation of alternate fates is equally important as establishing a new identity. The exclusion of alternative lineages is what sharpens lineage decisions; this fundamental principle was proven very clearly in the early decades of cellular reprogramming. For me, this concept was simple and elegant. Stem cell differentiation—a natural cell fate conversion rather than an artificial one during reprogramming—is governed by the same principle. Differentiating stem cells confront lineage bifurcations, and extracellular signals induce one cell type and repress another. My lab seeks to discover the signals that control each bifurcation during human pluripotent stem cell differentiation. We found a general principle. By adding the positive extracellular signal that induces one lineage (for instance, endoderm) and inhibiting the signal that instead induces the mutually exclusive lineage (let's say mesoderm), we could command stem cells to

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differentiate into a certain cell type with high efficiency and precision. In the past, if you typically added the positive signal but didn't block the negative signal, stem cells would tend to spontaneously differentiate into multiple cell-types at once. We hope to command differentiation more precisely, inspired by fundamental work in developmental biology and cellular reprogramming.

Dr. Pereira: Were you summarizing the studies you performed during your training with Irving Weissman? You published two seminal papers during that time, entitled “Efficient Endoderm Induction from Human Pluripotent Stem Cells by Logically Directing Signals Controlling Lineage Bifurcations” (Loh et al., 2014) and “Mapping the Pairwise Choices Leading from Pluripotency to Human Bone, Heart, and Other Mesoderm Cell Types” (Loh et al., 2016a).

Dr. Loh: That's precisely right. I just want to add a shout-out to Thomas Graf, who has had a very big influence on me and many others. Although I had never met Thomas before, he had read something that I wrote a long time ago and, out of the blue, emailed me saying: “Dear Dr. Loh, I really enjoyed reading this.” I was very surprised to hear from him, because I had not even started graduate school yet. That experience of scientific comradery touched me: that a legend in this field would be reading the literature and corresponding with younger authors. After this correspondence with Thomas, I became fascinated about what cellular reprogramming could teach us about differentiation and mutually exclusive lineage decisions.

Dr. Pereira: That's certainly a very charming aspect of science.

Dr. Loh: A very heartwarming side of science. I think it's up to each of us to now pass on that kind of positivity.

Dr. Pereira: Yes, indeed. In direct reprogramming, we use TFs to kickstart identity changes, but there are also strategies to repress unwanted lineages. Is that also where you draw your inspiration?

Dr. Loh: Yes, and this is also highlighted in one of my favorite reviews by Thomas in *Cell Stem Cell* (Graf, 2011). For instance, some of Thomas's early work was very important in showing that PU.1 and GATA1 induce a certain hematopoietic subtype while repressing the starting lineage. Of course, there are also many preceding and subsequent examples.

Dr. Pereira: When differentiating pluripotent stem cells, you can also use small molecules or other modulators to achieve this.

Dr. Loh: That's right. A TF works like a Swiss army knife that can induce one side and repress the other. Extracellular signals can also tweak cell identities. In our early papers (Loh et al., 2014; Loh et al., 2016a), we found that TGF β induces endoderm and BMP induces mesoderm. To obtain endodermal identity, we simultaneously add TGF β and a small molecule inhibitor of BMP. If you figure out these

lineage bifurcations, the opposite also works — that's what is beautiful. So, if we add BMP and inhibit TGF β , we get reciprocal results and instead generate lateral mesoderm.

When people say that pluripotent stem cell differentiation isn't reproducible across cell lines or results in heterogeneous cell populations, my first instinct is to ask whether the issue is truly cell-intrinsic or simply a consequence of the differentiation methods. The field has now developed more refined methods, leading to more consistency and reducing differentiation variability across human pluripotent stem cell (hPSC) lines. We can avoid giving ambiguous signals to cells and sharpen lineage bifurcation to achieve pure populations.

Dr. Pereira: Not everything is about *plus*; we also have to repress the brakes. The cancer field also learned that message with immune checkpoint inhibitors.

Dr. Loh: Irving Weissman, my PhD advisor, told me about this funny line from a song—“accentuate the positive, eliminate the negative.” He thought it was a good summary of this differentiation approach.

Dr. Pereira: Great. I think that summarizes your early contributions. Then, you switched gears to one of the biggest problems with cell therapies, in your paper “Improving the safety of human pluripotent stem cell therapies using genome-edited orthogonal safeguards” (Martin et al., 2020). I believe this was your first contribution as an independent group leader. Could you describe the main findings?

Dr. Loh: Yes. I want to credit the two terrific PhD students who came up with the idea: my student Jonas Fowler and his friend Renata Martin from Matthew Porteus's lab, a pioneer in CRISPR and stem cell therapies. They were inspired by the question “How can we make cell therapies safer?”. Jonas and Renata sought to selectively kill undifferentiated pluripotent stem cells, which can form teratomas. Many people have had this idea before, but Jonas and Renata had an innovative approach: they implemented a kill switch with an inducible Caspase9 protein and introduced it into the endogenous *NANOG* locus. Whenever a cell transcribes *NANOG*, it will also express the kill switch protein, and it can be killed off by the addition of a small molecule. In this approach, we're directly linking the transcriptional state of the cell with the ability to kill it.

Dr. Pereira: *NANOG* is one of the downregulated genes during differentiation. But I believe there was also another component. Could you kill all the cells if wanted?

Dr. Loh: That's right. One safety risk is the persistence of undifferentiated pluripotent stem cells, but another safety risk is that a differentiated cell might become cancerous or infected by a pathogen. So, we created a so-called “panic button” that allows us to kill all the cells by knocking in a different inducible Caspase-9 into the *ACTB* locus, which cells must express to survive. This approach is useful because it directly links the ability of a cell to live (i.e., *ACTB* expression) with our ability to kill it.

Dr. Pereira: Have these approaches been used in any cell therapy moving toward the clinic?

Dr. Loh: That's a great question. We have not used it directly, but I have heard that companies are considering kill switch approaches. The downside to the kill switch is that you're removing the therapeutic cell product as well, in some cases.

Dr. Pereira: In your opinion, what is the biggest concern about these cell therapies with hematopoietic stem cells (HSCs)? Ensuring safety or tackling the lack of engraftment?

Dr. Loh: Lack of engraftment, which translates into lack of efficacy. I personally think that the kill switches we described would be easy to implement, at least from a scientific perspective. In theory, the inducible Caspase-9 kill switches are not immunogenic because they are comprised of human proteins, and after you knock them in, you can perform genome sequencing. So, I don't think there's any intrinsic barrier to using a kill switch. However, we want to show first that wild-type cells can engraft and then move one to genetically modified versions carrying kill switches.

Dr. Pereira: Understood. Moving forward, in 2022, you published a paper where you generated human artery and vein cells from pluripotent stem cells and explored arterial tropism in the context of the dangerous Nipah and Hendra viruses (Ang et al., 2022). I think this was a nice way of bringing stem cell biology into the field of viral biology. How did you come up with this idea?

Dr. Loh: It all started with this amazing non-fiction book I read in second grade, *The Hot Zone*, that takes place in a Biosafety Level 4 (BSL-4) lab during a viral outbreak. My dad was reading the book and told me it was too violent for me. Naturally, I secretly read the book when he went to sleep. I ended up loving the book so much that I brought this story to present in class as part of "show and tell" and read a dramatic scene to the other kids. There's a strong female scientist in the book, Colonel Nancy Jaxx, who dissects a monkey that's been infected by Ebola. She gets a hole in her outer glove when dealing with the monkey's blood and initially thought she might have been contaminated. I always had this obsession with BSL-4 viruses, which is the initial reason I got into science.

Dr. Pereira: So, you started your life, began working on cell differentiation, and suddenly remembered that those viruses infect endothelial cells.

Dr. Loh: Yes. At the time, I was working with my close colleague at Stanford, Lay Teng Ang, who is a co-corresponding author of the paper. We both work on cell differentiation, but we were very lucky to collaborate with a BSL-4 biologist, Joseph Prescott, from the Robert Koch Institute, Germany. Although I read that book about Ebola a long time ago, I remembered there was this deadly virus that Joe worked on, Nipah virus. During the COVID-19 pandemic, I recognized there were many people working on BSL-3 viruses such as SARS-CoV-2, but few people were working on these BSL-4 viruses. I wrote to Joe, and we formed a consortium between the three labs to explore this together. I'm really grateful to Lay Teng for all her

work on developmental and stem cell biology and to Joe for teaching me a lot about virology.

Dr. Pereira: Before we dive into the scientific details, could you summarize the findings of this paper for our audience?

Dr. Loh: This paper has two parts. First, we developed methods to rapidly and efficiently differentiate human pluripotent stem cells into the two principal types of blood vessel cells, artery and vein endothelial cells. The second part of the study focused on the deadly Nipah virus, which has a ~59% fatality rate, has no known vaccines or treatments, and is the only BSL-4 virus known to spread airborne between humans; it's like the worst combination. Together with Joe and Lay Teng, we found that Nipah virus doesn't attack all blood vessels equally: it attacks arteries but not vein cells. It also presents this peculiar infection biology, because Nipah causes infected cells to form massive multinucleated syncytia with up to 23 different artery cells fused together. Nipah virus is part of the *Paramyxoviridae* family, and it's well known that Nipah and other paramyxoviruses induce cell fusion. One thing that Joe Prescott's lab did in this paper, which really impressed me, was to perform live imaging at BSL-4. To my knowledge, this was only the second time in history that live imaging was performed at BSL-4, something truly difficult and unusual. He saw that when Nipah-infected artery cells fuse together, they don't remain static. Their nuclei creep toward the center, forming this massive grape-like aggregate, and cells die catastrophically. I wish more people could work in this area, because anything you discover in BSL-4 biology tends to be quite new.

Dr. Pereira: And your system is very useful to separate the biological aspects of the cells from the infection by the virus. I want to ask another question. If you express the virus receptor in differentiated vein cells, will you get syncytia or similar morphological observations?

Dr. Loh: Outstanding question. We did not do that experiment. We showed that if we delete *EPHRIN B2*, the receptor for Nipah virus, Nipah cannot enter artery cells efficiently. But we never did the reciprocal to show that *EPHRIN B2* is sufficient to enable Nipah infection. However, others have overexpressed *EPHRIN B2* in other cell types and showed increased susceptibility to Nipah infection.

Dr. Pereira: Is your lab following up on this topic?

Dr. Loh: Yes. My lab members always say that BSL-4 viruses are my passion project. Maybe that's straying a bit far from cellular reprogramming... but you use viral vectors to deliver reprogramming factors, so maybe it all comes together in the end. With Joe Prescott and Lay Teng Ang, we're trying to work on many BSL-4 viruses, including Ebola, Marburg, Nipah, Hendra, and Crimean-Congo viruses. There's a whole universe of hemorrhagic viruses to explore.

Dr. Pereira: Also, I think using pluripotent stem cell products to model viral infection is a unique angle. Thinking about developmental progression, are there any viruses that preferentially affect certain developmental stages? You can also use pluripotent stem cells to model this.

Dr. Loh: Thank you, that's a good question. Unfortunately, there are certain viruses that can infect the fetus; Zika, for example. Sadly, pregnant women infected with Ebola often lose their pregnancy. Not much is known.

Dr. Pereira: It's fantastic to see how productive you've been in your independent group. I want to address a study you carried out about the origin of blood, which is very close to my heart. In 2024, you published a paper in *Development Cell* entitled "Lineage-tracing hematopoietic stem cell origins *in vivo* to efficiently make human HLF + HOXA+ hematopoietic progenitors from pluripotent stem cells" (Fowler et al., 2024). Could you summarize the main results of this study?

Dr. Loh: Of course. On the one hand, this paper focuses on the *in vivo* origin of HSCs in development, since there's a long-standing question of where HSCs come from. Here, we used genetic lineage tracing to demonstrate that artery endothelial cells give rise to HSCs. On the other hand, our study describes our attempts to differentiate human pluripotent stem cells into HSC-like cells. We could differentiate human pluripotent stem cells into artery endothelial cells *in vitro*, and we used that as a stepping stone to make HSC-like cells. These cells express HSC markers such as *HLF* and *HOXA5-10*. Unfortunately, they have very weak engraftment potential and that's what we and many others in the field are trying to improve.

Dr. Pereira: A persistent problem addressed by the work of Andrew Elefanty, Ed Stanley and Elizabeth Ng. I'm looking forward to following new papers coming out in this field. Returning to the topic: is it fully mature arterial endothelium that transdifferentiates into HSCs *in vivo*, or is it a hemogenic endothelial progenitor with arterial features, identifiable by lineage tracing markers, that gives rise to blood? At that developmental moment, lineages are so intertwined that I find it very difficult to see the distinction.

Dr. Loh: That's a great point. In our paper, we used three lineage tracing models, and we can claim that cells expressing arterial markers give rise to HSCs, which is an important distinction. Specifically, we used two artery drivers, *Cx40-CreERT2* and *Efnb2-CreERT2*, to show that artery-marked cells generate HSCs; up to 90% of HSCs come from those cells, suggesting they are the exclusive source. In contrast, a vein marker, *Apj-CreERT2*, did not efficiently label HSCs. So, as you said, a conservative interpretation is that cells expressing arterial markers make HSCs. Whether that cell is actually an arterial endothelial cell is a good question to ask.

Dr. Pereira: Your paper definitely shows that these cells are arterial and not venous. I think that's a consensus now. It's just difficult to see the picture in the context of other developmental processes, where you have a mature endothelium that's embedded in the tissue and suddenly switches lineage. I'm not saying that's not possible—we know there is a lot of flexibility in these tissues. It remains an open question.

Dr. Loh: Exactly. The biggest problem is that at E11 there is approximately one functional HSC formed in the dorsal

aorta. So, it's difficult to identify what exact endothelial cell gave rise to that single cell.

Dr. Pereira: A single HSC defined by function, right? But it could be that other cells are there, we just don't have a marker to identify them, or they are not fully mature, or they lack competence for engraftment. Although the question remains to be addressed, I think it's good we are reaching more definition to identify what these lineages are expressing.

Dr. Loh: Gordon Keller asked the same question, so you're in very good company.

Dr. Pereira: Building on that, I also think the way you used that information to improve human pluripotent stem cell differentiation establishes a very interesting parallel. Would you like to share any current research projects?

Dr. Loh: Thank you. I would like to highlight our preprint "Two parallel lineage-committed progenitors contribute to the developing brain" (Dundes et al., 2025). My lab is interested in the early developmental progenitors that form different organs. The textbook view is that the brain is a single organ that comes from a common progenitor, the neural ectoderm, which can form the forebrain, midbrain, and hindbrain. However, using both *in vitro* and *in vivo* approaches, we showed that during gastrulation, there exists not one, but rather two parallel brain progenitors: one that gives rise to the forebrain and midbrain, and another that gives rise to the hindbrain. Although the brain appears as one organ, we believe it is a composite organ coming from two separate progenitor populations. This has interesting implications for stem cell differentiation *in vitro* and evolution.

Dr. Pereira: Given these findings, what do you see as the evolutionary implications?

Dr. Loh: The origin of the brain is one of the biggest questions in evolution. Because the hindbrain controls life-sustaining functions like breathing, heartbeat, sleep, and wakefulness, most intuitively think it emerged first. Yet, we found these dual brain progenitors were conserved across 550 million years of evolution, from humans to mice, chickens, zebrafish, and finally, even acorn worms. The work of Christopher Lowe, my colleague at Stanford, revealed that these dual brain progenitors also exist in the acorn worm, which was really amazing to me. Acorn worms diverged from humans 550 million years ago: the last time we shared a common progenitor was before the supercontinent Pangea, and this brain progenitor split had already occurred.

Dr. Pereira: How did you identify these two progenitors?

Dr. Loh: By the expression of *Otx2* and *Gbx2*, which mark the two progenitors. This has taken us in a very different direction in the lab, in collaboration with Chris Lowe, who has been very interested in when these progenitor domains were established evolutionarily. Chris has a paper showing that acorn worms seem to have territories that express forebrain, midbrain, and hindbrain markers, based on the markers established in vertebrates (Pani et al., 2012).

Whether these are functionally analogous to their mammalian counterparts is very complicated to answer. But the progenitor domains were set up a long time ago.

Dr. Pereira: That’s fascinating. And you were able to recapitulate that during pluripotent stem cell differentiation, correct? Could you tweak the differentiation into one or the other progenitor?

Dr. Loh: Yes. Just like this lineage bifurcation logic we talked about before, we could figure out the extracellular signals that induce the anterior neural ectoderm, which makes the forebrain and midbrain, or the posterior neural ectoderm, the second population that makes the hindbrain. By figuring out those signals we could command hPSCs to generate both. What was quite remarkable to me was that this fundamental split is made so early: in 48 hours *in vivo*.

Dr. Pereira: So, it’s one of the first steps in differentiation.

Dr. Loh: Precisely. In the current model, cells first become neural progenitors and only afterward do they undergo anterior-posterior patterning. In the model we propose, when cells gain brain-specific identity, they simultaneously gain anterior-posterior patterning. There’s also work from James Briscoe and other people that broadly supports this idea. I would say that this is still very difficult to prove this model definitively, because in developmental biology it’s hard to pinpoint when cells become restricted in identity.

Dr. Pereira: Maybe epigenetic profiling at earlier stages could help.

Dr. Loh: You’ve read my mind. We found that before cells gain their anterior *versus* posterior restriction, their chromatin landscapes already prefigure that. I feel this is an interesting contrast to cell reprogramming, where we are trying to open up the potential of cells by synthetically expressing factors. We are working on the reciprocal in development, trying to understand how cells become canalized.

Dr. Pereira: Very good. I would also like to touch on one of your reviews “Building human artery and vein endothelial cells from pluripotent stem cells, and enduring mysteries surrounding arteriovenous development” (Loh and Ang, 2024). You discuss the developmental specification of arterial and venous identities. Could you comment on the main challenges in this context?

Dr. Loh: As you know, you can often reprogram cells into a certain cell type, but generating a finely grained cellular subtype tends to be harder. Now, people have progressively more elaborate TF combinations and media conditions to sharpen subtype identity. I think the reprogramming field accepted quite well that some of the early protocols produced cells with very ambiguous subtype identity. That’s also the case in the stem cell differentiation field, where it had been previously hard to make arterial and venous subtypes of endothelial cells. How can we sharpen the subtype identity of these cells?

I can make an analogy with reprogramming and the work of researchers like Barbara Treutlein, who has shown that

NGN2-induced neurons express some central nervous system markers and peripheral nervous system markers (Lin et al., 2021). In a recent paper, the team of Marius Wernig discovered that overexpression of *NGN2* together with *EMX1*, sharpens the identity of these cells to look much more like cortical forebrain neurons (Ang et al., 2024). I thought that sharpening of identity was really nice.

Dr. Pereira: Do you see any opportunity for therapeutic intervention?

Dr. Loh: Yes. One question is whether we can use these endothelial cells to vascularize synthetic tissues. Our lab and many others are trying to mix these endothelial cells with engineered tissues in the hopes of vascularizing them. That has proven to be a difficult task. I find that surprising because, in the body, tissues are densely vascularized, so organs naturally like having endothelial cells around. There’s probably an interesting basic biology mechanism underlying this, which will take a lot of work to figure out.

Dr. Pereira: All things considered, what is your vision for the future of the reprogramming field?

Dr. Loh: I would look to other colleagues like yourself who know a lot more than I do! Something that I’ve found very interesting is that, while many labs are focused on synthetically reprogramming cells by TF expression, there are many reports that cells can reprogram or differentiate in the healthy body or upon injury. For instance, intestinal stem cells differentiate to form secretory progenitors and absorptive progenitors. In the steady state, both of these progenitors are committed to differentiate. They generate short-lived, lineage-restricted clones, as shown by lineage tracing. However, upon tissue injury, these progenitors can seemingly revert their fate and become intestinal stem cells (Post and Clevers, 2019). I really wonder how widespread this phenomenon is. Reprogramming could happen quite often in parts of the body we don’t expect. We just don’t have a living tissue microscope to witness it.

Dr. Pereira: Exactly. Particularly in situations like cancer or inflammation, there’s more and more evidence that can happen. Sometimes, it’s just difficult to trace which was the cell of origin.

Dr. Loh: Lineage tracing of Th17 cells has shown, in some contexts, their transdifferentiation to anti-inflammatory, immunosuppressive T cells (Gagliani et al., 2015). Such transdifferentiation might happen more often than we think.

Dr. Pereira: CD4⁺ T cells usually show a lot of plasticity. During our talk, you mentioned several collaborators. Are they essential for your activity as a principal investigator (PI)? Since you started your career as an independent researcher, you have had so many projects—I assume these were only possible precisely because of collaborations.

Dr. Loh: It may sound obvious, but collaboration is absolutely essential. So many people have had important intellectual influences on me, helping to introduce me to different fields. Thomas Graf, Irving Weissman, and Bing Lim had an enormous intellectual influence on me, getting me to think about

mutually exclusive lineage decisions and stem cell differentiation. My collaborators Lay Teng Ang, Joe Prescott, and Matthew Porteus helped me think about vascular differentiation, BSL-4 virology, and the safety of cell therapies and genetic engineering. I'm really grateful to all these people and many other people as well. Almost every paper from our lab features a shared co-corresponding authorship with multiple people—we really try to work with others.

Dr. Pereira: Do you start the collaborations at the ideation phase or later? Some of my collaborations start once we have some data, but others start earlier by bouncing ideas.

Dr. Loh: For me, it's also a mix of both.

Dr. Pereira: Do you have any advice for reprogramming scientists who are thinking about doing a PhD or postdoc in the field?

Dr. Loh: I don't have much advice to share, given that I'm so junior! The only thing I'd encourage is more optimism. Understandably, there's concern for the future of science around the world, especially among trainees. For instance, people in the US worry about funding and the broader perspective on science, but I am relentlessly optimistic. Like every other aspect of the world, there are going to be cycles of ups and downs. There's no point in being discouraged. We all have to do our best, and I'm personally still very excited about science every day. Moreover, we must be positive for the next generation; there are so many people who can achieve things beyond what we did.

Dr. Pereira: Indeed, I completely agree. We should keep focused on scientific questions. I would like to close the interview with some questions that are not strictly related to your research. If you could answer any single scientific question, regardless of your expertise or chosen field, what would it be?

Dr. Loh: It's very hard to say, but one that comes near the top is the evolutionary origin of the first animals. According to our current understanding, the last known predecessors to animals are choanoflagellates that can transiently form multicellular colonies, but don't display distinct long-lived cell types. When animals first emerged, two things happened simultaneously: multiple stable cell-types emerged, and these different cell-types were spatially positioned across the body axis (Loh et al., 2016b). How was symmetry broken in these multicellular colonies to generate the first animals?

Dr. Pereira: That sounds like a great question to ask. If you were not a scientist, what would you be?

Dr. Loh: My two hobbies are writing science fiction and riding my road bike.

Dr. Pereira: But not at the same time!

Dr. Loh: Hopefully not at the same time!

Dr. Pereira: Dr. Loh, thank you so much for taking the time to join me today. It was great to learn more about you and your science.

Dr. Loh: Thank you very much for doing this. I appreciate your efforts to connect people in the field.

References

- Ang CE, Olmos VH, Vodehnal K, et al. Generation of human excitatory forebrain neurons by cooperative binding of pro-neural NGN2 and homeobox factor EMX1. *Proc Natl Acad Sci USA* 2024;121(11):e2308401121.
- Ang LT, Nguyen AT, Liu KJ, et al. Generating human artery and vein cells from pluripotent stem cells highlights the arterial tropism of Nipah and Hendra viruses. *Cell* 2022;185(14):2523–2541.e30.
- Dundes CE, Jokhai RT, Ahsan H, et al. Two parallel lineage-committed progenitors contribute to the developing brain. *bioRxiv* 2025.
- Fowler JL, Zheng SL, Nguyen A, et al. Lineage-tracing hematopoietic stem cell origins in vivo to efficiently make human HLF+ HOXA+ hematopoietic progenitors from pluripotent stem cells. *Dev Cell* 2024;59(9):1110–1131.e22.
- Gagliani N, Vesely MCA, Iseppon A, et al. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature* 2015;523(7559):221–225.
- Graf T. Historical origins of transdifferentiation and reprogramming. *Cell Stem Cell* 2011;9(6):504–516.
- Lin H-C, He Z, Ebert S, et al. NGN2 induces diverse neuron types from human pluripotency. *Stem Cell Reports* 2021;16(9):2118–2127.
- Loh KM, Ang LT. Building human artery and vein endothelial cells from pluripotent stem cells, and enduring mysteries surrounding arteriovenous development. *Semin Cell Dev Biol* 2024;155(Pt C):62–75.
- Loh KM, Ang LT, Zhang J, et al. Efficient endoderm induction from human pluripotent stem cells by logically directing signals controlling lineage bifurcations. *Cell Stem Cell* 2014;14(2):237–252.
- Loh KM, Chen A, Koh PW, et al. Mapping the pairwise choices leading from pluripotency to human bone, heart, and other mesoderm cell types. *Cell* 2016a;166(2):451–467.
- Loh KM, van Amerongen R, Nusse R. Generating cellular diversity and spatial form: Wnt signaling and the evolution of multicellular animals. *Dev Cell* 2016b;38(6):643–655.
- Martin RM, Fowler JL, Cromer MK, et al. Improving the safety of human pluripotent stem cell therapies using genome-edited orthogonal safeguards. *Nat Commun* 2020;11(1):2713.
- Pani AM, Mullarkey EE, Aronowicz J, et al. Ancient deuterostome origins of vertebrate brain signaling centres. *Nature* 2012;483(7389):289–294.
- Post Y, Clevers H. Defining adult stem cell function at its simplest: The ability to replace lost cells through mitosis. *Cell Stem Cell* 2019;25(2):174–183.

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