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## Reprogramming Stars #19: Upgrading Cell Fate Conversions with Engineered Reprogramming Factors—An Interview with Dr. Ralf Jauch

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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 am 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. Ralf Jauch, an Associate Professor at the School of Biomedical Sciences of HKUMed, where he leads a multidisciplinary research program at the interface of protein engineering and stem cell biology. He completed his undergraduate studies at the Universities of Jena, Germany, and Manchester, UK, and obtained his PhD at the International Max Planck Research School and The University in Göttingen. In 2006, he moved to Asia to join the Genome Institute of Singapore, where he developed a passion for stem cells and protein design. In 2013, he joined the Guangzhou Institute of Biomedicine and Health as Principal Investigator, where he established the GIBH-Max Planck Center for Regenerative Medicine. Dr. Jauch's research is inspired by enhancing any biomolecule-driven cell fate conversion with synthetic factors, improving their quality, utility, and efficiency. Dr. Jauch, thank you so much for joining me today. It is a pleasure to have you featured as a reprogramming star.

**Dr. Jauch:** Thank you so much Filipe. It is a great honor to be featured in your interview series.

**Dr. Pereira:** Your lab explores the enhancement of cell fate conversions by engineering fate-instructing factors to apply in regenerative medicine. It would be very



**Dr. Ralf Jauch**

**REPROGRAMMING STAR: Professor Ralf Jauch** leads the protein and cell engineering laboratory at the School of Biomedical Sciences in the Faculty of Medicine of the University of Hong Kong (HKUMed). His group aims to boost cellular reprogramming with the help of unconventional factors from exotic species and re-engineered biomolecules. To achieve this, they look at the natural evolution of pioneer transcription factors, perform direct molecular evolution in mammalian cells, and use structural information for protein design. With this toolkit, they aim to make new types of stem cells that can help to model and revert age-linked disease, enhance the developmental potential of stem cells, and direct stem cell differentiation. The team aims to translate their technologies within the Centre for Translational Stem Cell Biology (CTSCB).

interesting for the audience of *Cellular Reprogramming* to know a little bit more about how you started your path in the field.

**Dr. Jauch:** I finished my PhD at the Max Planck Institute for Biophysical Chemistry, in Göttingen, Germany, co-supervised by Herbert Jäckle, a developmental biologist, and the structural biologist Markus Wahl. Back then, I was focusing on solving crystal structures of kinases and transcription factors. After finishing my PhD, I decided to explore the world and joined the laboratory of the structural biologist Prasanna Kolatkar at the Genome Institute of

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Singapore. This was in 2006—the year when Shinya Yamanaka published the groundbreaking discovery of the induced pluripotent stem cells (iPSC) technology. At the time, the mission of the lab was to study the structural basis of the function of stem cell transcription factors, such as Sox2, Oct4, and Nanog. We wanted to find out what makes them special and how they work together to maintain and induce pluripotency. We were hoping to elucidate the structure of the stem cell enhanceosome, which we thought is a large molecular complex regulating pluripotency. To this day, nobody in the field has ever succeeded in elucidating the structure of such a big complex because it probably does not exist as these transcription factors are floppy and very promiscuous. They engage in different types of transient interaction on different genes. However, we managed to decode a critical difference between Sox2 and Sox17—factors responsible for one of the first cell fate decisions in the developing embryo. We uncovered that a single amino acid distinguishes how Sox2 and Sox17 regulate gene expression programs. Exchanging this amino acid between Sox2 and Sox17 changes how they partner up with Oct4 to bind and turn on genes. We decided to test how this mutation affects iPSC reprogramming. To our amazement, we found that the switch of a single amino acid converts Sox17 into a very powerful inducer of pluripotency (Jauch et al., 2011). Through the re-engineering of Sox17 into an iPSC factor, I was myself gradually reprogrammed from a biochemist and structural biologist to a stem cell researcher.

**Dr. Pereira: Thank you so much for that insight. Just to provide our readers with more details on your story in structural biology, have you started by analyzing the structures of Oct4 and Sox2 using cryogenic electron microscopy (cryo-EM), or were you looking for the structures of all the Yamanaka factors?**

**Dr. Jauch:** In 2006, cryo-EM was not at the level that we could analyze protein structures at high resolution. Back then, we used X-ray crystallography. One of the structures that we successfully solved in Singapore was the structure of DNA-bound Sox17. Then, we started to compare it with the structure of Sox2 bound to DNA. We tried to understand the differences and why they bind and regulate different sets of genes. We were initially puzzled as to the way these two factors bind and deform DNA is almost identical. However, we did observe differences at a molecular interface which determines how they team up with Oct4. Modifying this interface with mutations dramatically changes how Sox2, Sox17, and Oct4 regulate cell fate decisions.

**Dr. Pereira: Interesting. So, it is very important to look at what works and what does not work to be able to correlate the observed differences with successful reprogramming. Before we go more into details on how you are harnessing your findings, can you elaborate on what we can learn by mapping the structures of the reprogramming factors in fine detail?**

**Dr. Jauch:** We are just starting to understand what makes a particular reprogramming factor unique. We have almost

2000 transcription factors in the human genome and many different structural classes of transcription factors can direct cell fate conversions. It is hard to pinpoint unifying features. It appears that factors that can reprogram need to have the activity of a pioneer transcription factor, meaning that they need to be able to engage epigenetically silenced chromatin. However, the structural determinants of this activity remain to be understood. That is one of the reasons a part of my lab is now returning to structural biology and applying cryo-EM to elucidate how transcription factors bind and open up compacted chromatin.

**Dr. Pereira: From what you told us, I understand that we have learned quite a lot about the interactions between transcription factors and their correlation with the final outcome of the cellular reprogramming process; however, the other functions of reprogramming factors, like pioneer-ing for example, need to be further explored. Is that right?**

**Dr. Jauch:** Yes, indeed. Our first successful approach to engineering and enhancing a transcription factor was based on a rational analysis: we looked at the structure of Sox factors and redesigned how Sox17 and Oct4 work together. Later on, after recognizing the limitations of this rational approach as we do not have a clear idea of how pioneer factors work, we developed a technique called DERBY-seq, which stands for directed evolution of reprogramming factors by cell selection and sequencing (Tan et al., 2021; Veerapandian et al., 2018). We used mutant libraries, where we randomized amino acid positions to identify variants that improve a certain cell fate decision. In our screens, we select a set of amino acids, shuffle domains, or even randomize all of the amino acids in a whole domain one by one.

**Dr. Pereira: So, you can mutate a specific amino acid or change the entire sequence of transcription factors. Then, you analyze how those changes favor or abolish the reprogramming-inducing activity of the factor. Is it a clear-cut analysis?**

**Dr. Jauch:** We can do this in different ways. We can follow a completely agnostic approach. For example, Sox transcription factors have a DNA-binding domain composed of 79 amino acids that we can mutate and evaluate in a pooled library screen. However, you miss combinatorial interactions and pleiotropic effects. Therefore, it is better to use a focused approach, where we select individual positions that, based on structural or evolutionary analyses, seem to be very important for function. Then, we make a library focused on these amino acids. We did this in several studies for the Sox family, Oct4, and more recently for Klf4.

**Dr. Pereira: You show that three Sox17 mutations are critical for the function of the transcription factor. You reached this conclusion with a candidate-based and rational approach. Given the number of interactions, a completely unbiased screening approach would be significantly more challenging, right?**

**Dr. Jauch:** A combinatorial assessment is indeed challenging. If the target transcription factor is not well understood

structurally, I would recommend starting with a deep mutational scanning approach and examine all amino acids one by one. After that, we can apply the knowledge obtained from the screening effort to do combinatorial analysis as the next step.

**Dr. Pereira:** And this is how you currently code the function of artificially evolved transcription factors or direct their function in the reprogramming toward pluripotency. I am also wondering: do the same rules apply for lineage reprogramming?

**Dr. Jauch:** Yes, they do. For every screening effort, you need a very good readout to select the desired phenotypes. This can be a reporter gene, a surface marker, or a single-cell phenotyping approach to fish out the desired cells. Lineage reprogramming and forward programming would follow the same ideas. We also have a very strong interest in lineage reprogramming, especially reprogramming toward neural stem cells (NSCs).

**Dr. Pereira:** I think it is a good idea to use single-cell analysis to increase the throughput in a single experiment. Let us go a little bit further on your lineage reprogramming studies toward iNSCs. You published an article on the role of the engineered Sox17 in the generation of NSCs (Weng et al., 2023). If you could give us a summary of those results, it would be fantastic.

**Dr. Jauch:** The motivation behind this article was that we wanted to find ways to make adult stem cells of the central nervous, which cannot be accessed by biopsies. iPSCs are fantastic to study early-onset diseases or neurodevelopmental disorders. However, if we want to study late-onset diseases or age-linked diseases, iPSCs have shortcomings because they represent rejuvenated embryonic cells. Therefore, it is desirable to bypass this fully rejuvenated pluripotent state and directly go toward a specific lineage. There were several studies in the field reporting lineage reprogramming into induced NSCs. None of these methods had become very popular because efficiency was low and it was not entirely clear whether the cells indeed bypassed a pluripotent state. We decided to pick this up and ask whether our engineered factors could help. After a lot of tests, we ended up using engineered Sox17 to turn human blood cells and mouse skin cells directly into new NSCs. Now, we want to develop this platform further to model age-linked and adult-onset diseases.

**Dr. Pereira:** I just want to follow up on the rejuvenation aspects. When we reprogram a somatic cell into an iPSC, we erase the epigenetic clocks. However, in the case of lineage reprogramming directly to neurons, this does not happen. It is still a bit unclear to me how this works for NSCs or any other somatic stem cells. How does achieving multipotency affect the clocks? Do you think that the clocks are maintained? Are they partially erased? Given that you can induce self-renewal, I think induced NSCs can be an interesting system to compare levels of potency and separate the process of reprogramming and the rejuvenation aspects. What did you find in terms of DNA methylation, for example?

**Dr. Jauch:** This is a very interesting question for which we do not have the final answer yet. We did look at DNA methylation in mouse lines and found that cells are not fully rejuvenated. We have not done the full analysis with human cells, but it is certainly a high priority on our to-do list. Using time-resolved expression analysis, we find that reprogramming cells at no point activate pluripotency genes. We are therefore hopeful that some age features of the donor cells are retained. After all, endogenous stem cells age as well. It is possible to detect aging features in primary NSCs and other tissue-resident stem cells. We hope it is possible to take the best of two commonly used cell models: the stem cell character of iPSCs and the maturity of directly induced postmitotic neurons (iN) that are mature and “aged.” Yet, iN cells no longer divide. With iNSC, we could produce useful cells and construct multicellular systems to capture the pathologies of age-linked disorders.

**Dr. Pereira:** Very interesting. Compared with the pluripotency system, you only use two factors to induce NSCs. Do you think that the NSC outcome is explained by different cooperation between factors present on the cell or are media different as well?

**Dr. Jauch:** Indeed the factor cocktails to get iNSCs is less complex. In this study, we looked for lineage-exclusive systems that can only make iNSCs but fail to make iPSCs. In our screens, we found a range of Sox17 variants but the one that worked best was the Sox17 with enhanced cooperation with Pit-Oct-Unc (POU) proteins. Reprogramming human blood cells into NSCs required C-Myc as an additional factor, while reprogramming mouse skin cells required Klf4. So yes, the starting cells do make a difference as to which factors we have to use. I would say that the medium is very important to mature into desired cell types and to maintain the cells but it is less important to initiate reprogramming.

**Dr. Pereira:** In one of your articles, you describe the composite transcription factor binding sites and how it is important to look at how shortened or more extensive the composite sites are. I have a more general question. How do you think that the cooperation between the two reprogramming factors (and combining of composite sites into functional complexes) initiates the cascade of reprogramming? Does it relate to the transient recruitment of other chromatin modifiers?

**Dr. Jauch:** My way of thinking about gene regulation is that the assembly of transcription factors on regulatory DNA does not necessarily require a “lock and key” type of molecular interaction. However, the Sox and POU transcription factors are somehow an exception to this rule. They have a very defined composite DNA motif that needs to be positioned with a certain spacing, allowing them to work together and regulate the gene expression program. In early mammalian development, we have a switch composed by two different types of DNA signatures: a canonical DNA element targeted by Sox2 and Oct4 to induce pluripotency and a compressed DNA element targeted by Sox17 and Oct4 to induce the primitive endoderm or the hypoblast. Here, we have the prototype of an enhancer code: distinct composite

DNA motifs, which recruit distinct combinations of transcription factors. I would love to apply this kind of engineering of transcription factor partnerships to other systems. Unfortunately, I am not aware of many other structurally characterized examples whereby transcription factors read a defined motif grammar and assemble in such an accurately crafted molecular architecture.

**Dr. Pereira:** So, we still do not know how generic these features are. This leads me to another question. You mentioned that you have been trying to evolve Oct4 and Klf4. Can you tell us a little bit more about that and the importance of factor conservation between species?

**Dr. Jauch:** We did evolve Oct4, and we got a very potent reprogramming factor which we called ePOU in mice, but it did not perform well in humans. Our engineered Sox17 which we originally found in mice worked across species but that is not the case for Oct4. What we learned is that, if possible, we should screen in the species of interest right away. We have unpublished Klf4 variants which allow for very interesting reprogramming approaches, and we are now testing it in humans. In general, the reprogramming factors are highly conserved but the regulatory DNA information they read and interpret varies widely across species.

**Dr. Pereira:** Relating to this topic, the train of thought in pluripotency reprogramming was really expanded with the article: “Highly cooperative chimeric super-Sox induces naïve pluripotency across species” (MacCarthy et al., 2024). Can you tell us a little bit more about this article and its main findings?

**Dr. Jauch:** Yes. First, I should say the credit for this article goes to the lab of my long-term collaborator Hans Schöler and his colleagues Caitlin MacCarthy and Sergiy Velychko. They started to use one of our re-engineered Sox17 called Sox17EK and gradually designed their own Sox versions and ended up with a hybrid of Sox2 and Sox17. They used Sox factors alongside Klf4 as a genetic switch to turn primed pluripotent cells into naïve iPSCs in several species. With this switch, it could be possible to make stem cells with a higher developmental potential, which can help to differentiate extraembryonic tissues and even develop embryo models in various species.

**Dr. Pereira:** Inducing naïve pluripotency across species was a challenge, right?

**Dr. Jauch:** Yes, it is challenging and needs to be verified in a larger panel of species. We have succeeded in directly reprogramming human somatic cells into pluripotent stem cells with enhanced potential and are interested in testing other species such as dolphins. A lot of people optimize culture conditions for naïve iPSCs. I like the idea of using a cell-autonomous genetic switch to reversibly push cells to a naïve pluripotent state.

**Dr. Pereira:** Can you walk us through the current projects in your lab? What are you excited about?

**Dr. Jauch:** Yes, sure. As you know we have made efforts to artificially engineer and evolve reprogramming factors. More recently, we have begun to study the natural evolution of the Yamanaka factors. The gene families to which Sox2 and Oct4 belong were previously thought to be specific to animals. However, we found that they are much older than previously thought. We discovered that ancestral Ur-Sox factor already existed in our unicellular ancestors (Gao et al., 2024). With the help of Alex de Mendoza, we found Sox genes in choanoflagellates and filastereans—the closest unicellular relatives of animals. These new genes helped us to revise the phylogenetic history of Sox genes. With the help of Georg Hochberg’s lab, we could then perform molecular time travel to predict ancestral Ur-Sox sequences that may have existed in our evolutionary past. Interestingly, Ur-Sox factors share many properties with modern Sox2 in the way they bind DNA and partner up with Oct4. To our amazement, they can even induce pluripotency in mice. We think that understanding the natural evolution of reprogramming factors can help us further boost our efforts in protein engineering.

**Dr. Pereira:** And in quantitative terms, how does reprogramming efficiency compare with the mouse and human?

**Dr. Jauch:** Ur-Sox are not more efficient than modern Sox2, but they work. However, we identified Sox factors from animals that lack a head or tail that seem to perform like naturally occurring super Sox and outperform Sox2.

**Dr. Pereira:** It is fascinating to see that these transcription factors seem to have an ancestral function. They did not evolve with multicellularity. What are your thoughts about this?

**Dr. Jauch:** No, to our surprise, Sox and POU factors evolved before the emergence of the first multicellular animals. However, in our unicellular ancestors, Sox and POU factors may not have partnered up through intimate heterodimer formation as we see in many modern animals. One of our ideas is that perhaps evolutionary changes that allowed Sox and POU factors to team up and regulate genes as dimer are somehow associated with the origin of animal stem cells. It is possible that the first animal, the Ur-metazoan, resembles a pluripotent stem cell. This cell could make identical copies of itself but could also differentiate into different cell types that at some point stayed together. Sox and POU factors may already have played important roles in these ancestral cells.

**Dr. Pereira:** Very interesting, we look forward to reading the article. One of the previously discussed research questions that stayed with me was: can we really separate fate conversions from rejuvenation? Can you further elaborate on your strategy to tackle this challenge? How is your lab advancing on this front?

**Dr. Jauch:** The partial reprogramming paradigm suggests that this is possible. In this approach, the Yamanaka factors are expressed for defined time periods which turns back the

aging clock but stops short of changing cell identity. We are approaching this problem from two sides. First, we are testing if it is possible to reprogram cells and turn them into tissue-resident stem cells without the rejuvenation we see for iPSCs. We also test if we can rejuvenate cells without the accurate timing that is essential when we use the Yamanaka factors. Because if we express them too long, we induce tumors. We seek to find a cocktail of biomolecules that completely lost the ability to change cell identity and only erase features of aging. If we were to succeed, we have a safer way to decouple these two processes. A challenge is to develop the right screening system to independently monitor cell identity and rejuvenation.

**Dr. Pereira:** Indeed, the two processes are connected. That is why I was interested in the induced NSCs—they could be a way of answering this question. However, achieving both your goals can be significant. Can I ask what is your future vision for the field of cellular reprogramming?

**Dr. Jauch:** Well, I definitely think new types of stem cell models will be very useful. We already talked about stem cell models that are better suited to study and reverse age-linked diseases and stem cells with higher developmental potential that allow us to effectively make extraembryonic tissues or embryo models. *In vivo* reprogramming is an exciting area. If we learn to change cell types in a controlled way in the context of an organism to repair damaged tissues that would be a game changer.

**Dr. Pereira:** Very good. I think those are three very important lines of research right now. You are located in one of the most densely populated regions on the planet. The audience of *Cellular Reprogramming* would be interested in understanding how you collaborate within and outside your region. Do you want to share your experience with us?

**Dr. Jauch:** I enjoy cross-disciplinary collaborations to a level that I sometimes worry that we are getting too diverse. For example, we collaborate with structural biologists and experts in cryo-EM to better understand how reprogramming factors work. We are also collaborating with phylogeneticists and molecular time travelers to understand their natural evolution. For our work with NSCs, I need the expertise of neuroscientists and bioengineers. Our collaborations are defined by topics and “chemistry” and not by geographical location.

**Dr. Pereira:** Great. Can you name a couple of benefits of being located in Hong Kong?

**Dr. Jauch:** Hong Kong remains a hub and connector between the East and the West with English as a working language. When it comes to travel regulations or shipment of biological reagents, we are very open and easily accessible. At the same time, we are very close to mainland China and can easily cross over and interact with people there—The University of Hong Kong, for example, has an excellent hospital in Shenzhen, and we are starting to work with the

doctors there. Being at this intersection is a huge advantage. As you might know, in 2025, the international society for stem cell research (ISSCR) conference will take place in Hong Kong, and the whole world can visit us. We are looking forward to welcoming the global stem cell community.

**Dr. Pereira:** I think it will be a fantastic opportunity for every reprogramming scientist to know a little bit more about the environment in Hong Kong and visit the region. Since we are mentioning our research community, do you have any advice to spare for reprogramming scientists at early career stages?

**Dr. Jauch:** Do not get into research to collect degrees but to have fun and follow your passion. Take intellectual ownership and lead your project rather than waiting for your mentors’ instructions. Attend seminars and conferences, make friends, build networks and collaborate. Do not be afraid to take 180-degree turns if you are up to a great discovery. Honestly, I probably would never have gone into the stem cell field and would have stayed a structural biologist if the finding that a single point mutation can completely change how reprogramming factors work would not have me excited so much.

**Dr. Pereira:** So, be brave and follow what the data tell you; yes, this is critical! I would like to close the interview with some questions that are not strictly related to science or your research. If you were not a scientist, what would you be?

**Dr. Jauch:** As a young boy and teenager, I wanted to be a footballer, but unfortunately, I did not have enough talent and also suffered a pretty bad injury when I was 17. Maybe it saved me from investing too much time and energy into this. And, if I had not become a biomedical scientist, I would probably be an archaeologist. At the University, I started off studying archaeology. It is a fun fact that I am now digging out ancestral transcription factors, which could be viewed as molecular archaeology.

**Dr. Pereira:** You can combine both interests! What is the best piece of advice that you have ever been given?

**Dr. Jauch:** I give you a contrarian answer. When I told my PhD adviser about 20 years ago about my idea to move to Singapore, he told me: do not do this and if you do it, just go for one or two years to have fun and then return to a famous lab at a more established place. I didn’t listen and went for the adventurous option in a tropical city state at a brand-new institute few had heard of, and I am still in this part of the world to this day.

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

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