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INTERVIEW

The people behind the papers – Dennis de Bakker, Mara Bouwman and Jeroen Bakkers

Unlike mammals, adult zebrafish are capable of regenerating their hearts without scarring after injury – a process that has great therapeutic potential. A new paper in Development investigates the role of Prxx1b, a transcription factor that is expressed in epicardial heart tissue after injury, to understand its role in the scar-free regeneration of the adult zebrafish heart. To hear more about the study, we caught up with joint first authors, Dennis De Bakker and Mara Bouwman, and the corresponding author, Jeroen Bakkers, the group leader at the Hubrecht Institute and professor of Molecular Cardiogenetics at the University Medical Center in Utrecht, The Netherlands.

Dennis (left), Mara (centre) and Jeroen (right)

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

JB: I was trained as a molecular biologist and did my PhD project in a lab studying plant-microbe interactions and root nodule development in what is now the Institute of Biology Leiden in The Netherlands. Two years into my PhD project, I visited the Massachusetts Institute of Technology (MIT) for a 3-month collaborative project. Here, I was exposed for the first time to zebrafish embryo development when I met members from the lab of Prof. Nancy Hopkins. I joined them to attend the 1996 Meeting on Zebrafish Development and Genetics at the Cold Spring Harbor Laboratory, which was a very inspiring meeting since many mutants from the forward genetic screens were presented here. This event was a turning point in my scientific career, as I established a small zebrafish colony in Leiden upon my return and have continued to work in the field of developmental biology using the zebrafish model since then. After I obtained my PhD in 2000, I moved to Germany to work on BMP signalling during zebrafish gastrulation as a postdoc in the lab of Matthias Hammerschmidt at the Max Planck Institute in Freiburg. Matthias is a great mentor who inspired and stimulated me to establish my own research group, which I did at the Hubrecht Institute for Development and Stem Cell Research in Utrecht, The Netherlands in 2003. Here, at the Hubrecht Institute, we started several projects with a strong focus on cardiac development. We performed forward genetic screens to identify mechanisms that regulate how the heart transforms from a linear tube into an asymmetric S-shaped structure as perturbations in this process result in severe congenital heart defects. When CRISPR/Cas9 became available, we started to use zebrafish to model rare genetic cardiac diseases in order to better understand the mechanisms leading to impaired cardiac development or function. In the most recent years, we have studied mechanisms of cardiac regeneration, as zebrafish have the amazing ability to regenerate their hearts after injury, which is the topic of this paper.

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Dennis, how did you come to work in Jeroen's lab and what drives your research today?

DdB: As a Masters student, I did a research internship in the Schulte-Merker lab at the Hubrecht institute. There, I worked on vascular development in zebrafish, which was an amazing experience for me. Therefore, I looked for a zebrafish lab for my PhD project. I eventually joined Jeroen's lab for the opportunity to study heart regeneration by comparing the zebrafish and mammalian heart injury response, which sounded like a really exciting project! In addition, the friendly and enthusiastic atmosphere in Jeroen's lab was also a big factor in my choice. Driving my research today is the hypothesis that, for many complex diseases we suffer from as humans, other species may have already acquired the molecular mechanism necessary to circumvent these diseases through natural selection. Asking the right question in the right species might, therefore, help to discover novel strategies to treat human disease.

And Mara, what brought you to Jeroen's lab and what are you currently researching?

MB: I learned about Jeroen's work on zebrafish through one of his lectures during my Bachelor's degree and was amazed to hear that these fish can completely regenerate their heart following injury. After doing my Master's internship in Jeroen's lab, under the supervision of Dennis, I am now pursuing my research as a PhD student in the group, studying the involvement of several genes in zebrafish heart regeneration.

Before your work, what was known about Prrx1?

DdB, MB & JB: *Prrx1* encodes a paired-related homeobox transcription factor and its function has been studied mainly in the

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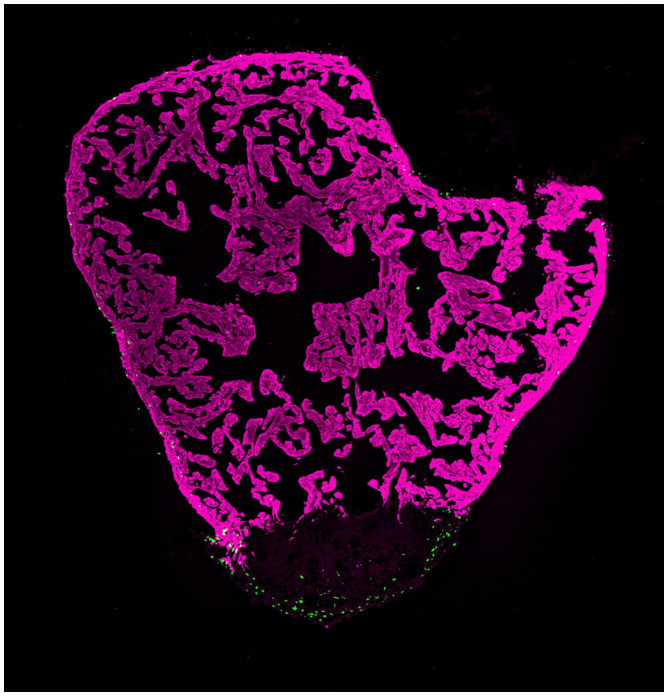

Regenerating zebrafish heart with Prrx1 expression (green)

context of cancer and craniofacial development. In addition, expression of *Prrx1* has been correlated to scar-free wound healing and limb regeneration in axolotl and *Xenopus*, but little was known about its function during these processes.

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

DdB, MB & JB: The zebrafish genome contains two Prrx1 genes (*prrx1a* and *prrx1b*) and we find that *prrx1b* is required for heart regeneration, while *prrx1a* is dispensable. Upon cardiac injury, Prrx1b expression is induced in the epicardium. Performing single-cell RNA-sequencing on epicardial and epicardial-derived cells (EPDCs) from wild-type and *prrx1b* mutant hearts, we then find that loss of *prrx1b* leads to an excess of pro-fibrotic fibroblasts and fibrosis. Furthermore, through *in vitro* experiments in human fetal EPDCs and *in vivo* experiments in zebrafish, we find that Prrx1b regulates the expression of Nrg1 and thereby promotes cardiomyocyte proliferation. Altogether, our study reveals that Prrx1b is a key transcription factor balancing the fibrotic response and regeneration post-injury in the zebrafish heart.

What is NRG1 and why is it important in cardiac regeneration?

DdB, MB & JB: Neuregulin 1 (NRG1) is part of the EGF family of ligands and can be produced as a secreted or as a transmembrane protein. Upon binding with receptor tyrosine kinases of the ERBB family, it plays various indispensable roles during organ development, such as the nervous system and the heart. Importantly, NRG1-mediated ERBB2 signalling also plays a key role during heart regeneration. In the regenerating zebrafish heart, NRG1 is expressed in epicardial-derived cells and induces cardiomyocyte proliferation. In addition, stimulating the NRG1/ERBB2 pathway in the adult mouse heart induces cardiomyocyte proliferation and restores cardiac function after a myocardial infarction. We and others have recently shown that NRG1/ERBB2 signalling changes energy metabolism in cardiomyocytes from oxidative phosphorylation to glycolysis, which is essential for cardiomyocyte proliferation during heart regeneration.

What implications do these results have for understanding cardiac injury in non-regenerative species, such as mammals?

DdB, MB & JB: The epicardium is essential for zebrafish heart regeneration, as epicardial-derived cells secrete growth factors, such as NRG1, to stimulate cardiomyocyte proliferation. Very little was known about the regulation of NRG1; in our paper, we show that Prrx1b regulates the expression of NRG1 in epicardial-derived cells. While Prrx1 and NRG1 are also expressed in mammalian embryonic epicardial-derived cells, their expression is nearly absent from the injured adult mammalian ventricle. Therefore, the lack of Prrx1 and NRG1 expression could help explain the limited regenerative capacity of the mammalian heart. However, it should be noted that artificial induction of NRG1 by Prrx1 in the mammalian heart would likely not result in increased cardiomyocyte proliferation, as adult cardiomyocytes also lack the ERBB2 receptor required for the mitogenic effect of NRG1.

When you were carrying out the research, did you have any particular result or 'eureka' moment that has stuck with you?

DdB: When we started the project, we were mainly interested in *prrx1a*, not *prrx1b*. When it became clear that *prrx1a* was not required for zebrafish heart regeneration, we nearly dropped the project. Luckily, we decided to test *prrx1b* before that happened – I still remember the 'eureka' feeling when Mara showed me the first graphs of the *prrx1b* mutant showing a reduction in cardiomyocyte proliferation!

MB: I remember when we observed a strong reduction in *nrg1* expression in *prrx1b* mutant hearts, which was the initiation for us to look further into the connection between *nrg1* and *prrx1b* – this was a really exciting moment!

Sometimes, you just have to follow the evidence no matter where it leads you!

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

DdB: It all started with a transcriptomic screen, where we tried to identify factors that are strongly expressed in the regenerating zebrafish heart with a focus on the border zone. As we expected expression in the myocardium of the border zone, it was quite a shock to find that Prrx1 showed strong expression in the epicardium instead! Only through the excitement of epicardium enthusiasts, such as Esther Dronkers, did we decide to continue the project. Sometimes, you just have to follow the evidence no matter where it leads you!

MB: I think I share this moment with Dennis, we were very focussed on factors that are expressed in the border zone cardiomyocytes and that promote regeneration, so it took some time to switch gears and look into the role of Prrx1b in the epicardium. But it was worth it: an epicardial expressed protein that affects the proliferation of border zone cardiomyocytes and functions to balance the fibrotic response – how cool is that?

What is next for you after this paper?

DdB: I'm currently preparing for my PhD defence ceremony later this year. Also, I'm very excited to share that I have recently

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started my first postdoctoral project in the lab of Dario Valenzano! Here, I will delve into evolutionary-focused methodology to investigate the genetic basis of killifish neurodegeneration.

MB: I'm continuing my work in Jeroen's lab by looking into other processes and genes that we think are crucial for zebrafish heart regeneration.

Where will this story take the Bakkers lab?

JB: During heart regeneration, Nrg1 is important to stimulate cardiomyocyte proliferation but very little is known about its regulation. Here, we show that Nrg1 expression in epicardial-derived cells is regulated by Prrx1 and as a follow-up we want to better understand the mechanism of this regulation. Furthermore, we want to understand how cardiomyocyte proliferation is stimulated, as this may help to develop new strategies to induce cardiomyocyte proliferation in the non-regenerating mammalian heart.

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

DdB: Playing board games and video games with my friends! Also, I love after-work drinks, meditation, hiking and barbeques.

MB: I love hosting dinner parties for friends and baking cakes. I'm also always up for a board game night or karaoke.

JB: I like to take long bike rides on the road or off-road. Especially biking off-road through the forest; it helps me to clear my head and to get inspired.

Reference

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