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INTERVIEW

The people behind the papers – Douglas Houston and Marko Horb

Over the years, conflicting data have meant that the role of Wnt in symmetry breaking and axis formation was unclear. Now, a new paper published in Development finds that maternal Wnt11b is required for robust cortical rotation in *Xenopus laevis*, which, in turn, is required for axis induction. To find out more about this story, we caught up with two of the authors, Douglas Houston, Professor at The University of Iowa, and Marko Horb, Director of the National *Xenopus* Resource at the Marine Biological Laboratory in Chicago.

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

DH: I started off my science career (in college at Florida Tech) doing undergraduate research in molecular cytogenetics on a project funded by the then nascent Human Genome Project. The work was challenging, and I learned a lot of molecular biology, but I decided to take a break from academics for a while. I parleyed my cytogenetics experience into a job in Miami doing prenatal genetic testing (mostly amniocentesis karyotypes for Down syndrome diagnosis). The connection between chromosomal/genetic defects and developmental abnormalities sparked my long-term interest in developmental biology and renewed my interest in pursuing my PhD.

I joined Mary Lou King's lab at the University of Miami, studying the roles of localized RNAs in early *Xenopus* development. How these localized RNAs control the fate and activities of the cells that inherit them has remained a central focus of my work ever since. For my thesis, on the role of the germ plasm RNA *dazl*, I needed to learn the esoteric 'host-transfer' method for loss of function in *Xenopus*. This method was pioneered by Chris Wylie and Janet Heasman, and involves using antisense DNA oligos to deplete mRNAs in oocytes and then transfer of these oocytes to host females to facilitate their fertilization and analyses of subsequent events in development.

At the time, our lab was collaborating with Chris and Janet's lab on the depletion of another localized mRNA, *vegt*, which was being worked on separately by Marko, coincidently! After this work was published, I travelled to the Wylie-Heasman lab in Minneapolis to trade expertise; with me learning the host-transfer method while also sharing our lab's RT-PCR methods used in the *vegt* paper. Up until then, my efforts at trying to learn oocyte transfer on my own were less than stellar, but in Chris and Janet's lab everything clicked, and I was able to make the first *dazl*-depleted embryos. A lesson I took away from this experience was that even the best written protocols will miss the critical nuances of a complicated procedure. I have since become committed to helping share the oocyte host-transfer methods whenever possible [including courses at the National *Xenopus* Resource (NXR) and Cold Spring Harbor Laboratory].

D.H.: Department of Biology, The University of Iowa, 257 BB, Iowa City, IA 52242-1324, USA.
M.H.: National Xenopus Resource and Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA 02543, USA.
E-mail: douglas-houston@uiowa.edu

Marko (left) and Douglas (right) at The Captain Kidd in Woods Hole.

The Minneapolis visit led to a postdoc with Chris and Janet (although the lab moved to Cincinnati) and solidified my interest in the early development of *Xenopus*. After working on different projects related to germ layer formation and patterning, I started my lab at the University of Iowa. I've spent my career since then characterizing the identity of localized RNAs (there are about 400), as well as their functions in development. Another major question of my work centres on determining the overall organization of localized RNAs and how extensively co- or similarly localized RNAs can regulate similar processes.

Marko, how did you come to collaborate with Douglas and what drives your research today?

MH: Doug and I started discussing this project in Fall 2015 at the *Xenopus* PI meeting that was held at the Marine Biological Laboratory in Chicago. That was the year I first got funded to make germline mutants in *Xenopus* for the community. Since we knew it would take several years for this maternal effect mutant, we needed to start as quickly as possible. After that, we kept in regular contact when each new generation was sexually mature.

My research program changed after I became Director of the NXR in 2011. Prior to that, I had a lab and focused on pancreas development in *Xenopus*. Once I started running the NXR, I realized it was difficult to run such a stock centre and have a specific research

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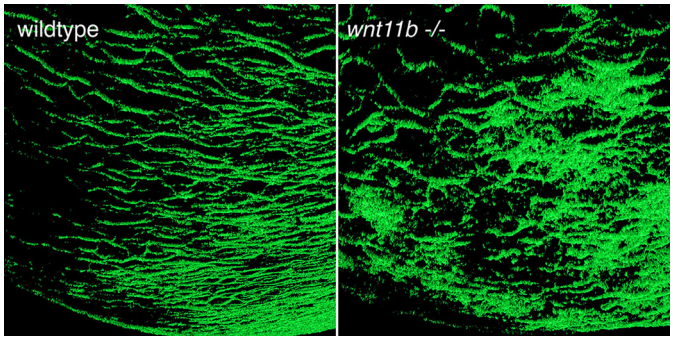

3D reconstructions of anti-β-tubulin (mAb E7) immunostaining of the vegetal surface of wild-type (left) and *wnt11b* mutant (right) eggs fixed at 60 min post-fertilization (images courtesy of Karen L. Elliott).

program, especially as we had limited access to graduate students. So, I changed to focus on generating resources for the community. When genome editing techniques came about, it was obvious that the NXR would be perfectly situated to make these mutants because we specialized in raising *Xenopus* and had the space for these new strains. So now I work closely with anyone who wants a mutant *Xenopus* strain, and we do our best to accommodate both *Xenopus* and non-*Xenopus* researchers.

What was known about the role of Wnt11 in dorsal axis formation prior to your work?

DH & MH: A lot! When Wnt11(b) was first described as a vegetally localized RNA (in a Development paper from Doug Melton's lab), it was shown to rescue UV light-induced ventralization and was thus an ideal candidate for an axis inducer. In those experiments, Wnt11 was overexpressed at the four-cell stage, so the rescue had nothing to do with cortical rotation but likely was through β-catenin activation. Later experiments showed that Wnt11 (and other Wnts) also regulated morphogenesis. This set up the polemic 'canonical' versus 'non-canonical' Wnt signalling debate, even though we knew back then that, for many Wnts, the response is cell context-dependent. Then, there were other studies showing that secreted Wnt inhibitors and dominant-negatives failed to block the endogenous body axis and that intracellular Wnt activators were transported dorsally during cortical rotation. This meant that the role of Wnt in axis induction was questioned. The pendulum swung back, however, and Janet Heasman's group showed that depletion of maternal *wnt11* RNA in oocytes led to ventralized embryos after oocyte host-transfer. However, it was still problematic to assemble all this information into a convincing model of axis specification. The largely coincident establishment of the NXR, *X. laevis* genome resources and CRISPR allowed some of these old problems to begin to be addressed genetically.

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

DH & MH: Using CRISPR, we generated a maternal-effect mutation in *X. laevis wnt11b* – the first such engineered maternal mutant in this organism. We found some predicted results based on published work, such as gastrulation and left-right abnormalities. We show that *wnt11* is required maternally for gastrulation (probably in a complex relationship with zygotic *wnt11* and other Wnts), but zygotically for left-right patterning. There were also unexpected phenotypes, such as relatively normal axis development in many of the maternal mutant embryos. The main exciting results were that *wnt11* was not required for hallmarks of Wnt/β-catenin signalling in the egg or early embryo. In fact, these purported hallmarks (phospho-Lrp6 and Dvl puncta) turned out not to match the predicted activity pattern of β-catenin stabilization. To account for the variable instance of axis defects, we examined microtubule assembly and cortical rotation. We found that the extent and directionality of microtubule growth was reduced but not eliminated in eggs from homozygous mutant females. Overall, we conclude that maternal Wnt11b activity is required for robust cortical rotation and for timely gastrulation movements.

Why do you think there is such variability in the defects observed during embryogenesis in the absence of maternal *wnt11b*?

DH & MH: One reason for the variability is that the cortical rotation process itself is variable. Older studies by John Gerhart showed that some eggs normally 'over-rotate', whereas others barely make the threshold to establish the axis. If the role of Wnt11 is to enhance or facilitate microtubule assembly, then some eggs should still be able to achieve sufficient cortical rotation without Wnt11. Other amphibians like axolotl (as well as primitive fish) undergo microtubule-mediated cortical rotation but don't have localized Wnt11, so the role of Wnt11 isn't fundamental to cortical rotation *per se*. *wnt11* RNA is vegetally localized in *Xenopus*, but not in other animals. It's interesting to speculate that this localization might have allowed *Xenopus* to dispense with other ways of controlling cortical rotation and thus became somewhat dependent on Wnt11.

***Xenopus laevis* is not generally used for genetic studies due to its long generation time. Why did you decide to use *X. laevis* for your research on the maternal effect of Wnt11b?**

DH: I was primarily interested in using *X. laevis* so that I could directly compare with previous work. Marko convinced me that the generation time for *X. laevis* at the NXR was not significantly longer than for 'trops' (*X. tropicalis*), so we went for *X. laevis*. We knew it would take a long time, but a project can never be finished if it's never started. Having the NXR as a community resource was critical for this work; it would have taken much longer if I had to breed these frogs myself.

MH: The traditional barriers for genetic studies in *Xenopus* were the long generation times and the allopolyploid genome. At the NXR, we've optimized much of the husbandry for rearing frogs to where we can get sexually mature *X. laevis* within 1 year. Also, in 2015, the NXR hosted a PI workshop at MBL and a lot of the talk surrounded the pending publication of the *X. laevis* genome (published in 2016; the *X. tropicalis* genome was published in 2010). One of the main conclusions of that work was the confirmation of allotetraploidy; but, despite this condition, many genes were lost from one 'subgenome'. One such gene was *wnt11b*, so we felt confident that we could use a simple breeding scheme to get maternal mutants. We also started making 'trop' mutants as a backup, but it turned out that the *X. tropicalis* genome has a duplicated *wnt11b* gene! So, it was good we kept our options open.

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

DH & MH: In this case, it was actually kind of a non-eureka moment! When we saw that the mutant eggs weren't totally ventralized, it meant something else was going on other than Wnt11 being strictly an essential dorsal determinant. Some of my earlier work on *trim36*, which has a mRNA localization pattern similar to *wnt11b*, prompted us to think about microtubules. It wasn't until we had ruled out other possibilities that looking at microtubule dynamics became imperative.

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

DH & MH: The main frustration was that the F2 mutant females were finally mature enough to begin egg-laying in the summer of 2020, right in the middle of pandemic restrictions. Marko did the first fertilizations and sent samples, but I did a lot of the experiments in my lab myself. We were also a bit concerned for a while that the indel reported in our paper might alter splicing, as it was near the end of the exon (and might result in a normal or hypomorphic allele). As predicted, however, the indel did create the correct premature stop codon. Otherwise, the paper kind of wrote itself.

Where will this story take your labs next?

DH: We have some more maternal mutants in mind, some related to this work, others unrelated. Clearly it will be important to figure out the connection between Wnt11 signalling and microtubule activity. I have also become interested in spatial transcriptomics as the technology has advanced, so it may be possible to make an unbiased spatial atlas of all localized RNAs in the vegetal cortex. This would identify potential sub-patterns of RNA localization, RNA:RNA interactions and possible roles for novel RNAs.

MH: Two years ago, I obtained a new R24 resource grant to create the *Xenopus* Mutant Resource, which focuses on producing new mutants and working with others on those already made. To date, we have over 200 different *Xenopus* mutants at various stages of development. The new grant provides housing for visiting researchers to come and work on their mutant here at the NXR. I see the NXR as a resource that can facilitate researchers in generating *Xenopus* mutants, where we make the mutant for a lab and they then analyse the phenotype, either by us sending them the samples or them coming here. The future is to continue to provide support for the *Xenopus* community so that their research can progress.

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

DH: In addition to helping to parent two teenagers, I've been a long-time enthusiast of various endurance sports and would like to eventually qualify for the Boston Marathon (nowhere close though). I also enjoy travelling and cooking, and I usually have a stack of books in various states of perusal.

Reference

Houston, D. W., Elliott, K. L., Coppenrath, K., Wlizla, M. and Horb, M. E. (2022). Maternal Wnt11b regulates cortical rotation during *Xenopus* axis formation: analysis of maternal-effect *wnt11b* mutants. *Development* **149**, dev200552. doi:10.1242/dev.200552