© 2022. Published by The Company of Biologists Ltd | Development (2022) 149, dev201082. doi:10.1242/dev.201082

INTERVIEW

The people behind the papers – Jennifer Watts and Amy Ralston

Zika virus infection during pregnancy can have severe consequences for fetal development and survival. A new paper in Development investigates whether sexually transmitted Zika virus could infect preimplantation embryos and dissects the impact of infection at this stage. We caught up with first author, Jennifer Watts, and corresponding author, Amy Ralston, a professor at Michigan State University, to find out more about their research.

Amy Ralston (L) and Jennifer Watts (R)

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

AR: For as long as I can remember, I have been a huge fan of embryos. As a high school student, I washed dishes in a sea urchin lab at the University of Kansas, in Lawrence, USA. Although the urchin lab only used the eggs for studies of microtubules, I was lucky that a rogue graduate student whipped up some urchin sperm and eggs one day so that we could marvel at the embryonic and pluteus stage urchins. Shortly thereafter, I attended Oberlin College, in Ohio, where Dr Yolanda Cruz ignited my love of the molecular regulation of developmental biology. I then went on to graduate school at the University of Wisconsin-Madison, where I worked with Dr Seth S. Blair, to understand morphogen gradients and pattern formation in the *Drosophila* wing. I loved fruit flies, but decided to challenge myself by switching to mice for my postdoctoral studies. As a postdoc with Dr Janet Rossant in Toronto, Canada, I learned about the unique features of mammalian development, which continue to fascinate me. I am a now professor of Biochemistry and Molecular Biology at Michigan State University, in East Lansing, which is a very nice place to live and work. Our lab wants to understand the origins and regulation of stem cell progenitors in the early mouse embryo.

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

JW: I conducted research, mainly in cancer biology, at the University of Texas at San Antonio for a few years. This was mainly fuelled by my passion for helping my mother who was afflicted with this disease. However, I wanted to shift my research focus towards developmental biology as a graduate student, because I believe that the field of development is essential to understanding disease progression. I learnt about Amy's work before starting graduate school, specifically the lab's discovery of iXEN cells, and wanted to be a part of her team. I did a rotation in Amy's lab as a graduate student in 2016, where she expressed her interest in studying the impact of Zika virus (ZIKV) on development. With Amy's newfound interest and my translational science background, it was the perfect opportunity for us to both try something new and highly relevant at the time. I officially joined her lab in 2017 and the rest is history. While I am studying something new in my postdoctoral position, development and birth defects are still at the forefront of my research today and will continue to be later in my career.

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

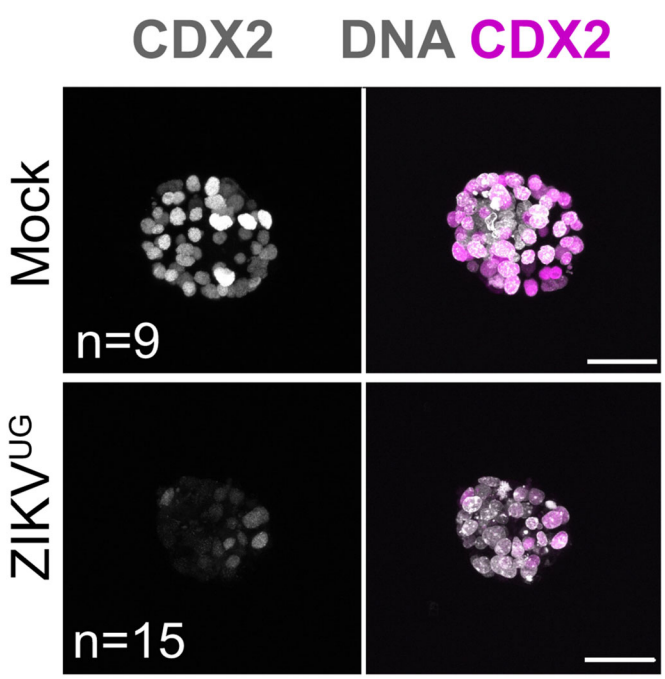
AR & JW: Our key findings are that ZIKV infection disrupts embryo growth and cell fate specification throughout preimplantation development, which could be the cause of more severe ZIKV syndrome outcomes, such as miscarriage. ZIKV can be sexually transmitted and can interact with embryos in the fallopian tube/uterus, so we decided to investigate its impact on embryos at this stage. We utilized a mouse embryo culture system to study the effects of ZIKV on embryo development from the two-cell stage (E1.5) to the blastocyst stage (E4.5). We found that ZIKV infected the future placenta cells of the embryo, which had also been shown in a previous study. More concerningly, however, we found that ZIKV infected other cell types, including those that will become the fetus, which was a new observation. In addition, we recognized that the embryo's glycoprotein coat, the zona pellucida, could impede ZIKV infection, which had never been investigated. Surprisingly, ZIKV infection at the two-cell stage led to embryo arrest, even in the presence of the zona pellucida.

In the blastocyst, do you think that infection alone is sufficient for the disruption in cell fate specification, or is this caused by viral replication?

JW: We did observe disruption of cell fate specification after ZIKV exposure. And we detected ZIKV envelope protein (ZIKV-E) in all cell types of the blastocyst, which could be indicative of the synthesis of new virus particles after replication. However, this does not demonstrate that replication is causal for cell fate disruption. It is a hard question to tease apart experimentally.

J.W.: Molecular, Cellular and Integrative Physiology Graduate Program, Michigan State University, East Lansing, MI 48824, USA.
J.W. & A.R.: Reproductive and Developmental Biology Training Program, Michigan State University, East Lansing, MI 48824, USA and Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA.
E-mail: aralston@msu.edu

Development (2022) 149, dev201082. doi:10.1242/dev.201082



Maximum projection of control and ZIKV infected embryos labelled with the trophoblast marker CDX2.

Do you have a hypothesis as to why the two-cell stage is so uniquely sensitive to ZIKV infection with an intact zona pellucida?

JW: Yes, we have several! First, the zona pellucida could be different or more porous at the two-cell stage than at later stages. Second, ZIKV entry receptors could be more highly expressed at the two-cell stage, allowing ZIKV particles to easily enter the cells. Lastly, early embryos begin to produce their own cellular machinery at the two-cell stage, as the zygotic genome is activated, and perhaps the added stress of infection could cause greater lethality at this stage.

What implications will your work have for human studies of ZIKV infection?

JW: Our study could inform epidemiologists of the consequences of ZIKV infection during very early stages – before the mother knows that she is pregnant.

AR: This means that, in addition to recommending use of mosquito repellent in areas of the world where ZIKV has been detected, contraception should also be considered.

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

JW: There were actually a few! Seeing that the embryos arrested shortly after infection was fascinating because it showed me that ZIKV has an undeniable effect on embryo development. Observing that ZIKV infects all layers of the preimplantation embryo was also really surprising and new!

Seeing that the embryos arrested shortly after infection was fascinating because it showed me that ZIKV has an undeniable effect on embryo development

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

JW: Oh, for sure! A lot of researchers have the usual technical issues and exhaustive troubleshooting, but our situation was a bit unique in that this research was brand new to everyone in the lab. We did have the expertise in embryology, but we did not know as much about virology and the essential viral techniques. We just had to figure it out carefully, because this project was important to us. Luckily, at Michigan State University, the Zhiyong Xi lab are experts in ZIKV, and provided guidance in experimental techniques and guided me throughout my training. Amy and I learnt together from beginning to end, and that's what makes this paper so special.

What is next for you after this paper?

JW: I recently started a postdoctoral scientist position at Nationwide Children's Hospital, Ohio, in October of 2021 working on the genetic basis of craniofacial birth defects. I am excited to embark on this new endeavour! I cannot thank Amy enough for the great mentorship throughout my formative research years and for continued support.

Where will this story take your lab next?

AR: I was so lucky to recruit a student like Jenn to work on this project. I love training graduate students, and I love to follow where they lead our work. My latest group of recruits are really excited about the Hippo signalling pathway and its role in regulation of pluripotency in the early embryo, a discovery that my lab made in the last decade.

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

JW: I recently took up rowing classes and, while exhausting, I look forward to it every time. I also love walking in wooded areas and playing board games.

AR: When I am not doing science, I enjoy doing arts and crafts, and making food and music with my spouse and our two kids.

Reference

Watts, J. L. and Ralston, A. (2022). The fetal lineage is susceptible to Zika virus infection within days of fertilization. *Development* **149**, dev200501. doi:10.1242/dev.200501