Dr. Janet Rossant

Dr. Rossant PhD, FRS, FRSC is the former Chief of Research at the Hospital for Sick Children and is currently a Senior Scientist in the Developmental & Stem Cell Biology program at SickKids. She is also a Professor in the Department of Molecular Genetics and the Department of Obstetrics and Gynaecology at the University of Toronto.

Dr. Rossant's research interests centre on understanding the genetic control of normal and abnormal development in the early mouse embryo using both cellular and genetic manipulation techniques. Her work in the early embryo have led to the discovery of a novel placental stem cell type, the trophoblast stem cell. Dr. Rossant is also the Director of the newly formed Ontario Institute for Regenerative Medicine, and was the recipient of the Canada Gairdner Wightman Award for 2015.

Interview conducted by JULS

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JULS: Every JULS' interview starts off with this question – why the life sciences?

JR: My interest in the life sciences really goes back to high school. I was always interested in science, just the discovery aspect and the fascination of trying to understand the world around us. I was interested in chemistry, not physics, biology, but I really got turned on to biology because of a very inspirational high school teacher. She recognized that I had potential and she gave me lots of books to read, she took us all on field trips into nature and various places that really engaged us in the fascination of life.

JULS: So how did you get into developmental biology?

JR: She encouraged me to apply to Oxford and Cambridge because nobody in my family had ever been to university – I was certainly going to go to university – but I wasn't really thinking of the elite universities because at that stage you have to do special exams. So she encouraged me to apply and I did and I got into both of them. I went to the University of Oxford and read zoology, the study of animal life. One of the courses was developmental biology and it was taught by John Gurdon, who won the Nobel Prize in 2012 for cloning frogs. He was doing that work, published his first paper, and was still doing additional papers showing that you could take adult cells from a frog and put them back into an egg [to] have them reprogram development, which at the time was used to show that the DNA code has to be the same in all cells. What makes cells different is how the [genetic] code is read out. Since then, it also is the underpinning of reprogrammed cells – to make stem cells – so that was why he won the Nobel Prize with Shinya Yamanaka. But, he was really the person that turned me onto developmental biology. He also had a whole group of people working in the lab at Oxford, and the way that the training works in Oxford is that you get 'tutorials', where you have one-on-one meetings, where you go in depth in particular areas. I had tutorials from members of his group and so that really enhanced my interest in the developmental biology field. It is still a fascinating and simply stated problem – how do you get from a fertilized egg to us? Whether it is a frog, a mouse, or a human, it is a fascinating and amazing problem. Here, at SickKids, we deal with some of the problems when it goes wrong; but generally, most of us are just fine, yet it is just a complicated process that it is amazing that it doesn't go wrong.

JULS: Speaking of that quite important question, if someone were to ask, how does a single cell become life, what would the developmental biologist say?

JR: Well, the developmental biologist, I guess, really would start with that fundamental finding that you start with a single cell – it has, within the nucleus, a genome that encodes all the information that can be used to be read out and turned into an organism, and the challenge is, how do you read it out in the right order, at the right time, and the right place? And that is the fundamental question of development – how do you read out of a single cell as it grows and turn on the right genes at the right time and place. We understand more and more about that and we could probably sit down and design an embryo. You could work out that if we turned on this gene – we know, for example, that this is the gene that is absolutely critical to make muscle cells – so if we turn it on, all downstream genes are going to turn on the muscle. It is not quite that simple, but we are beginning to understand the hierarchy of gene control. But that is what we have to understand – how do you read out that information in time and space – that is the fundamental difference between studying developmental biology as opposed to studying cells in culture, where you are looking at the fundamental aspects of how a cell looks. It is that time and space aspect. We are asking how do cells and tissues develop and bringing those two additional components, which make it that much more complicated.

JULS: Just on the issue of gene control, in simple layman's terms, what is gene control?

JR: So, here we have the DNA, it encodes proteins but there is a whole lot of DNA there that doesn't encode proteins and does other things, and among the other things it does, it has DNA sequences that can bind other proteins and be modified and changed such that if you bind proteins to this area, you will turn on or off genes. So, the particular coding sequence will be read out in one cell and not another. In a blood cell, the genes that make globin are turned on, and in a nerve cell, there are other proteins that bind upstream in the DNA and turn off that gene. It is that complicated dance of making sure that you have these networks of transcription factors that can regulate gene expression, again in time and space.

JULS: You are famous for discovering the trophoblast. Would you be able to tell me a little about it?

JR: So, I have always been interested in the very early stages of development in the mammalian system, but we focused a lot of our attention on the mouse. The first stage of development where cells make any special decisions in the mouse is called the blastocyst and it is a nice model system to address the problem of how cells read out gene expression because in about four cell divisions, they go from an egg to three different cell types that are expressing different genes. I've been working on this for about 30-40 years and we are just beginning to really understand the molecular pathways. One of the first cell types that forms is called a trophoblast, and it is the outer cells that the embryo uses in a mammal to interact with the uterus, so it is what makes mammals special – we don't have egg shells, we have a placenta and the first thing an embryo does in a mammal is make the cells that are going to make the placenta. It is quite special and very specific to mammals, which meant that we couldn't look to flies or worms to find analogous systems, and so we had to study it in a mouse. We have been studying trophoblasts for a long time and one of the things that helped us study it was when we were able to take that trophoblast from the blastocyst and culture them into stem cells (cells which have grown and would divide indefinitely). Now we have lots and lots of cells, [so] we can begin to look at the genetic pathways to make them become trophoblast cells and make them continue to grow as stem cells. So they are a nice model system to understand how a placenta works. Interestingly enough, we have not yet been able to make the same cells from humans, which is telling us that things are not quite the same in a mouse and human. We think what is going on there is that in the mouse, the early trophoblast is very proliferative so you can capture it and grow it in a petri dish. In humans, the first trophoblast actually invades right into the uterus and only later do you get a proliferative cell type, so we have to find other ways, which we are trying to do.

JULS: Regarding your work on the mouse embryo, if one speaks to a person on the street and says that we are spending one million dollars on embryo studies, it may not get the best response, so how would you justify the expense and effort we are putting into mouse studies versus human studies?

"Why the mouse? There are many reasons, but perhaps most important is that it has been a system in which we share about 95% of our genes."

JR: Why the mouse? There are many reasons, but perhaps most importantly is that it has been a system in which we share about 95% of our genes. We don't look like a mouse, it's true, but many of the functions we should have are shared with the mouse, so in many ways, you can study disease and general processes in the mouse and use it as the first path to understand how the human develops and grows. Importantly, you can make mouse models of disease. The other important thing about the mouse is that we have incredibly powerful tools to manipulate the genome. So first of all, we were able to use gene targeting in embryonic stem cells to make all sorts of alterations and make them in mouse. And more recently, with the whole CRISPR-Cas gene editing that has been applied to mouse zygotes, we can model very subtle alterations in humans and ask if it causes the same disease in the mouse. And, of course, in the mouse, we can do experiments that you can't do in humans. So if the mouse models the human disease, we can really dig into mechanisms. Having said that, there are differences. In many cases, you do the mutations in a mouse and it is very similar to what would happen in a human. But not all cases. We are not mice and there are differences. So, we have to also be able to complement the in-depth, whole-organism things we can do in mouse with the increasingly power to study human disease directly in a petri dish using induced pluripotent stem cells. Because we have complimentary tools now, we can tweak the genome of a mouse and model human disease in a whole mouse to study the physiology. We can now even take human cells from people with disease and turn them into stem cells in culture, and then make them differentiate into cell types that can

be used to help understand the disease as well. It is a dance. We can go from mouse to human, but we shouldn't forget the other model organisms – the fly, worm, zebrafish, xenopus. Because genetics pathways that control development, like organ formation, how brains work, how nerve cells work are highly conserved across evolution, we can get at the fundamental underpinning and find new pathways by going into flies and then take them up into humans. Or, you can go the other way around and find a human disease gene and start to study it in zebrafish because you can do more imaging analysis. Every system has its uses, so we never say the mouse is going to be absolutely the same as a human, because that would be foolish, but we can learn an awful lot from the mouse and many other model organisms.

JULS: Where do you see the field of developmental biology, specifically your work, being applied in the future?

JR: The fundamental issues of developmental biology is to try and understand how organisms develop in time and space, so that in itself satisfies our curiosity of where we came from. It also provides direct information that is relevant to birth defects and many of the kind of developmental syndromes that we see here at the Hospital for Sick Children, for example. Understanding normal development helps us understand what goes wrong in genetic diseases and developmental syndromes, so that is the most obvious direct applications. Also, we should remember that exploring development is how people identified important signalling pathways that can also be disrupted in disease. Perhaps one of the most famous signalling pathways in development from flies to man – and it's not my work in this case – is the Hedgehog signalling pathway, which was first identified in the Drosophila. [Drosophila researchers] like silly names, and mutations in this gene made the embryo look prickly. Hedgehog is a signalling molecule that is absolutely critical for patterning many different parts of the developing body, including limbs, skin, and nervous system, and it turns out that alterations in this signalling pathway is involved in a number of different diseases, including cancer. Some forms of skin cancer are caused by mutations in Hedgehog pathway, and some forms of brain cancer. This is a pathway that was first identified in Drosophila developmental biology and now is identified as a key pathway in cancer so, of course, researchers are directing drugs to block this pathway. From my own work – we worked years ago and still do work on how the blood vessels develop in the embryo. It is an early pathway because the embryo needs to a have circulatory system early on, so the heart, blood vessels, and blood develops as early progenitors. We identified a pathway involving a molecular called VEGF (vascular endothelial growth factor) and its receptor, and showed that when knocked out in embryos, no blood vessels could form. That was fundamental information that suggested that this is a really key pathway, and over time, it has become clear that VEGF is very important for normal development. If you have too much or too little VEGF, you will get abnormal vessels. Cancer cells actually produce this signal and attract more blood vessels to a tumour. So people have developed drugs to block the development of blood vessels and the VEGF receptor pathway. We didn't develop the drugs, but some of our most highly cited work is on this pathway because it demonstrated that this was a key signalling pathway. And if you disrupted it, you could block blood vessels – not just in the embryo but in the adult as well. It is a fundamental discovery and you never quite know where it is going to go, but developmental pathways, in general, are often re-activated in adult systems and in disease, so this is how you find new pathways and explore in disease.

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JULS: Speaking of VEGF, JULS spoke to Professor Titia de Lange last year, who discovered the telomere abnormalities in cancer cells. When JULS asked her how she made the discovery, she said she just looked and happened upon it. How did you discover VEGF?

JR: The way we got at the VEGF pathway was because we were interested in early patterning. This was early days, going back to the 1990s, and we were interested in trying to find genes that might be involved in gastrulation. After the blastocyst, the next important phase is when you make the ectoderm, endoderm and mesoderm. Lewis Wolpert famously said that the most important time in your life is not birth, marriage, or death – it's gastrulation. So we were trying to find how to get into the genetic pathways, and one of the ways we could find them was to look for particular gene families, by PCR, so we looked for kinase receptors, receptors that had a particular domain that related to their signalling capacity, and we did a screen. One of the receptors was this VEGF receptor, and when we looked to see where it was expressed, we found it in all blood vessels. At the same time, Napoleone Ferrara at Genentech discovered VEGF, and had also identified the receptor from cells in culture. We had seen its embryo expression and he had seen how important it was in cells in culture. We made a mutation and it was clear that it was very important. It was because we looking for developmental patterning genes, and because blood vessels develop early, it came up as in gastrulation, where VEGF is expressed. It marks precursors which go on to form blood vessels, smooth muscle cells, and heart muscle cells.

JULS: You were the Chief of Research at SickKids. If an undergraduate wants to go beyond the principal investigator level, and go to something like the Chief of Research, how would they work out for them?

JR: As scientists, when you enter the field and you decide that you want to go down the academic pathway, that is a big enough decision in the first place. It is a tough road, but you have to have passion, and commitment. As you go down the pathway and you are starting to enjoy the science, there comes a time when you want to give back. First of all, anyone who is going into research should focus their first few years entirely on their research because that is what is most important. But as you go forward, we do need people who can help direct and support research, and mentor young scientists, and to go down that path one step at a time is what I would say. Absolutely always get involved; you always want to be engaged with your fellow investigators, graduate students, and university department. Be

useful, be on committees – but don't over commit – and see what you like. Not everybody, by any means, is destined or should end up as an administrator. It is another set of skills that scientists are not generally trained to do, but maybe more of them should be trained. I never had any formal training, but maybe these days, at some point it is worth having an MBA along the way, if that is really the path you want to take. I would say, take it a step at a time and it is not a necessary step. Most Nobel Prize winners have never run anything in their lives and just stuck with their science, so it really is a matter of what you like to do and what you find your skills are appropriate for.

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JULS: What advice would you have for any undergraduate in life science?

JR: My advice for any undergraduate in life science is just to remember that we are in an amazing time for life science research. The tools and the technologies to explore nature, the applications of life science and medicine in new and exciting ways are amazing. Explore and take the opportunity to explore as many different areas as you can. Find out what you have a passion for and follow your passion. Your passion doesn't necessarily have to take you in the academic route or the medicine route for that matter. Just exploring a scientific education is really important. We are in a technological age and we need an educated public who understands the issues of science and technology. We need an educated public service, we need educated health politicians, and we need educated journalists. We need to have an environment in which we, as a country, are educated about these strengths, challenges, and the applications of technology. Too often we hear, even apparently educated people, not understanding some of the implications of science. My favourite hobby is vaccinations. In Canada and elsewhere, there are many people who are highly educated, who do not want to vaccinate their children, despite the fact that the science is extremely strong, and the idea that this is dangerous has come from completely discredited work, and yet the highly educated people still are not able to evaluate the scientific evidence properly. Science is training in being critical and being able to evaluate evidence. I'm very excited about the Liberal government's mandate letters to their ministers, many of which talk about science, innovation, evidence, and that I think is what we want to see our politicians do, and we need to be making sure that the public, also is as far as possible, able to weigh the evidence and make the right choices.

JULS: So, the undergraduate must learn the sciences, essentially?

JR: Yes. If you leave as an undergraduate and never directly use your science in whatever you decide to do, your training in being critical and evaluating evidence is really important. Use it. Talk to your neighbours, talk to whomever. When we look at global warming issues, stem cell tourism issues, vaccination issues, [there is] so much misinformation [that] you have to be able to sort out what is valid and what is not, and that is probably the training of being a critical scientist.