Scientists

Developing answers to fundamental questions

Janet Rossant

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Janet Rossant's pioneering research into blastocyst cell lineages caught our attention. Curious to know more, *BioTechniques* contacted her to find out about the ambition, character, and motivation that led to her success.

What first interested you in developmental biology?

When I was an undergraduate at the University of Oxford, I attended a few courses taught by John Gurdon, a developmental biologist famous for early studies on nuclear transfer, and became fascinated with the fundamental question of development: how does a single cell become a complex organism? This curiosity led me to pursue graduate work at Cambridge with Richard Gardner.

Although these were the very early days of experimental work on mouse embryos, by the time I arrived in the lab, Richard had demonstrated that it was possible to inject cells into blastocysts, a technique used today to make chimeric mice with embryonic stem (ES) cells. Those experiments highlighted the need to study different cell types in the embryo to find out when they become committed to a given lineage, and define the molecules involved in the process. At the time, this was all a complete mystery since the cell types first formed when creating a blastocyst are those that compose the placenta and various membranes specific to mammals that don't have homologs in flies, worms, or other available models. We had no obvious entry point into the gene pathways, so we started mapping at the cellular level and only later were able to approach the genes controlling the processes. Since then, we have contributed significantly to the understanding of how the first three cell types

form at the molecular and cellular levels, identifying the genes that specify cell types and basic signaling pathways that direct the separation of the lineages.

What are you working on at the moment?

Our current goal is to understand the genetic and cellular networks involved in blastocyst formation. We can do single-cell analysis and monitor gene expression in real time using fluorescent markers and live confocal imaging while the embryo is forming. For example, if we image antibodies with fluorescent markers, we are able to see a complex mosaic of gene expression within the inner cell mass. We can then watch this mosaic gradually resolve into two cell types, one of which is pluripotent while the other is primitive endoderm, an extraembryonic cell type. We can monitor those changes over time and even manipulate the system to see what pathways shift cells from one type to another. These techniques are very powerful, especially when combined with single cell RNA Seq or qPCR analysis. We believe single cells interpret the levels of signals they receive to determine their cell fate, and we would like to understand how they do that.

Of course, the mouse blastocyst is an interesting and important model system, but this is also the stage of development where pluripotent and ES cells are formed. We believe our findings on normal development are highly relevant to understanding control of pluripotency in ES and induced pluripotent stem (iPS) cells, which will be necessary for developing therapeutic applications.

Do you have any "pet projects" outside of your main focus?

We recently began working with human ES cells and iPS cells to understand cystic fibrosis. Specifically, we are working to develop iPS cell lines from patients here at Sick Kids, and drive the cells toward differentiation into lung cells to be used in drug screens. This is a much more clinically focused application than our main research, but the opportunities that exist at the moment to use iPS cells for human disease studies make it an attractive time to step in.

During the course of your research career, what has been your greatest surprise?

Several years ago, we didn't know how development was controlled or if the function of any of the main gene players was evolutionarily conserved. *Drosophila* researchers were publishing a lot of studies on the roles of *box* genes in pattern formation during development. Staining for one of these genes, *orthodenticle*, showed a discrete domain with a very strict boundary at the anterior in flies. Siew-Lan Ang, when she was a postdoctoral fellow in my lab, decided to clone the mouse homolog of *orthodenticle* through sequence homology. I think the biggest surprise I ever had was when she took me to the microscope to see an early mouse embryo stained for the mammalian *orthodenticle* ortholog, *Otx2*. There it was, absolutely defining an anterior domain with a very strict boundary, just as in *Drosophila*; it was amazing. That was the moment I really believed in a true conservation of some of these major patterning genes.

What is the most important open question in your discipline today?

We now know a lot now about how to make a mouse embryo, but the real question is how much of that is directly transferable to the human system. Are the general pathways the same? Is it just a matter of timing? Or are there other significant differences that we need to understand? Human ES cells are not identical to mouse ES cells, but we still don't know enough about normal human development to really make direct comparisons. Going forward, it will be a challenge to look at the fundamental similarities and temporal differences between the species.

Interviewed by Kristie Nybo, Ph.D. Image courtesy of Janet Rossant, Ph.D.

BioTechniques 53:129 (September 2012)
doi 10.2144/000113918

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