Researchers discover how the first two groups of cells form in a developing organism

Soumen Paul, Pratik Home, Proceedings of the National Academy of Sciences, Institute for Reproductive Health and Regenerative Medicine, TEAD4

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Last year, Soumen Paul, Ph.D., published an important discovery: how to grow pluripotent stem cells in the laboratory while keeping them from spontaneously differentiating into random types of cells. By suppressing protein kinase C signaling, scientists can purposefully direct pluripotent stem cells toward becoming specific cell types — a necessary move if they are to be useful in regenerative medicine.

Paul, an assistant professor in the department of pathology and laboratory medicine at the University of Kansas Medical Center, has continued carving out a niche in studying the early events of mammalian development. A member of KU Medical Center's Institute for Reproductive Health and Regenerative Medicine (IRHRM), he's particularly interested in the biological complexities that dictate how a single fertilized egg turns into a full-blown organism.

He and his postdoctoral fellow Pratik Home, Ph.D., recently uncovered the mechanism by which the first two groups of cells form in a developing organism.

As the cells of a fertilized egg rapidly divide — two becoming four, four burgeoning into eight, and so forth — the growing organism assumes a ball-like structure, Paul and Home explain. Because it has not attached to the uterus, it's called a pre-implantation embryo. As it increases in diameter, a second and smaller sphere of cells develops inside, akin to a ball within a ball, while a cavity simultaneously emerges. The smaller group of cells is known as the inner cell mass and will become the embryo itself; the ball-like structure's outer layer of cells, called the trophectoderm, eventually turns into the placenta that connects embryo and mother.

Understanding these early details is crucial, Paul says, because if things go wrong and one or both cell types aren't properly formed, a pregnancy won't happen. "The cavity within the pre-implantation embryo is the signature that both groups of cells — the inner cell mass and the trophectoderm — have separated," Paul says. "We've figured out how, at the molecular level, these two lineages are specified."

The "how" involves a protein called TEAD4. By binding to and turning on the expression of certain genes in the pre-implantation embryo's outer cell layer, TEAD4 ensures that the stage is set for the trophectoderm to form.

Location matters, though. TEAD4 can only do its job if it's in the nucleus, which is the cell's control center and contains the bulk of its genetic information. While TEAD4 is also found in the inner cell mass that forms the embryo, Paul and Home have shown that its cellular location there is *not* the nucleus, so the genes that would otherwise dictate formation of the trophectoderm are essentially silenced.

"It's known that TEAD4 exists in both cell types," Home says, "and we've helped to explain how the specific lineages form anyway — it's where this protein is that's important." He and Paul published their findings in the May 2012 issue of the *Proceedings of the National Academy of Sciences* .

In drilling down into the mechanics of early development, the researchers learned that nature has its own strategy for making sure these first two crucial groups of cells consistently take shape. Evolution has seen to it that TEAD4 exists in a variety of mammals, including Rhesus monkeys, rodents, cows and humans.

"Without TEAD4, these early cells don't know who they are," Paul says. "Gene expression is very plastic at the start of development, but TEAD4 provides some rigidity in giving trophectoderm cells their final identity. Unlike previous models that only explained *how* these two groups of cells are situated, ours details *why* an inside cell — or an outside cell — is what it is."

At first, Home says, he and Paul pursued a different protein, GATA3, which they observed was important in distinguishing between both cell types: the gene responsible for producing this protein is expressed only in cells of the trophectoderm. "We really wanted to learn more about how GATA3 is regulated," Home says. "That led us to TEAD4, which we've now discovered is actually the master regulator involved in specifying cell lineages."

It took approximately two years of hard work at the laboratory bench for the researchers to finalize their conclusions. "Our biggest problem was that the existing model indicated that TEAD4 was not just found in the trophectoderm and inner cell mass but, in both cases, fully present in the *nucleus*," Paul says. "Scientists figured some other protein was ultimately responsible for driving the separation process, not TEAD4. Our work contradicted two big research publications from a Japanese group."

"We got different results, leading us to question whether our findings were true or false," Home says, "and we needed to make sure we were on the right track." Which they did, by repeating experiments multiple times.

It may be basic biology, but the researchers see a potential application for their research in at least one avenue: the screening of embryos for *in vitro* fertilization (IVF). "If TEAD4 isn't in its proper location to specify these first cell lineages, the embryo won't be viable," Paul says. "This knowledge could be useful in grading the quality of and selecting IVF embryos for implantation."

Whether it's studying reproductive events at the front end or further along the timeline — at levels like placenta formation and fetal health — as others within the IRHRM are doing, Paul and Home believe that understanding early mammalian development will eventually result in more successful pregnancies with fewer medical complications all around.

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