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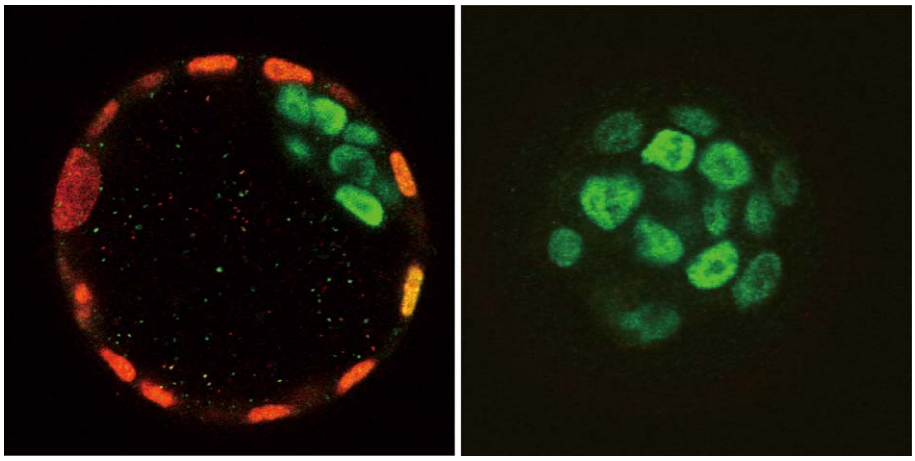
Tead4 triggers trophectoderm

January 28, 2008 – The first few steps of mammalian development see a single fertilized egg divides several times to form a compact sphere of cells known as a morula, which subsequently develops into a hollow ball called a blastocyst, which fills with fluid. It is in the period surrounding the emergence of the blastocyst that the first cellular differentiation processes take place, notably the segregation of the inner cell mass (ICM), which gives rise to the embryo proper, from the trophectoderm, which contributes to extraembryonic tissues, such as placenta. Recent studies have indicated an important role for the mutual inhibition between a pair of genes, *Oct4* and *Cdx2*, which regulate the ICM and trophectoderm, respectively. The question of potential upstream regulation of these genes, however, remains open.



Tead4 is essential for trophectoderm development. (Left) Wildtype blastocyst showing trophectoderm (Cdx2, red) and inner cell mass (Oct3/4, green), respectively. (Right) A Tead4 homozygous mutant embryo at the comparable developmental stage. The blastocoel fails to form, and all cells assume inner cell mass fate, as revealed by the expression of Oct3/4 (green).

Noriyuki Nishioka, Shinji Yamamoto and others in the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) have now shown that the transcription factor Tead4 is required for trophectoderm development, in a manner that suggests it functions upstream of Cdx2. Their findings, which were published in the journal *Mechanisms of Development*, shed new light on the genetic regulation of the earliest cell specialization event in mammalian embryogenesis.

The Sasaki lab's study began with the generation of mice with a homozygous deletion of the *Tead4* gene, following on their previous work that showed roles for Tead-family genes in the regulation of *Foxa2*, a transcription factor known to regulate the development of the midline signaling centers controlling post-implantation development. They were surprised to find that the knockout embryos died at extremely early stages in development, prior to the implantation of the blastocyst into the uterine wall.

This unexpected early lethality prompted Nishioka and Yamamoto to re-examine the expression of *Tead4*, and the related genes, *Tead1*, *-2*, and *-3*, in pre-implantation mouse embryos. Using RT-PCR to detect *Tead* transcripts, they found that *Tead4* expression switched on by the 4-cell stage; two other Tead genes, *Tead1* and *Tead2*, were also expressed. They next used immunohistochemistry to attempt to detect the various Tead proteins, and found both Tead1 and Tead4 in all blastomeres (as the individual cells of the early embryo are called), as well as in the cells of trophectoderm and the inner cell mass slightly later in development.

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Using time-lapse videos of developing embryos, the team determined that, in *Tead4*$^{-/-}$ mutants, although the embryos developed apparently normally up to the morula stage, they failed to form a blastocoel (the cavity of the blastocyst) even as cell proliferation proceeded apace. The blastocoel forms when fluid seeps between gaps in the surface of the embryo and fills the interior, and it is known that this process can be disturbed by defects in cell adhesion. But on studying pathways involved in the formation of adherens and tight junctions, cell polarity and various forms of signaling, they found no abnormalities that could account for the blastocoel failure.

They turned next to the trophectoderm, as this tissue is also a critical requirement for normal blastocyst development. Testing for *Cdx2* expression, which is upregulated in the trophectoderm in wildtype embryos and required for TE lineage specification, they found that *Cdx2* is only faintly expressed in the *Tead4* mutant embryo after the first several rounds of cell division, and not at all in subsequent stages. This was true of other TE-specific genes, such as *Eomes* and *Fgfr2*, as well. *Cdx2* functions in the pre-implantation embryo by repressing the expression of *Oct4*. When Nishioka and Yamamoto checked *Oct4* expression, they found that it was expressed throughout the late blastocyst, indicating that the entire embryo had adopted an inner cell mass fate.

Given that the ICM can serve as a source for embryonic stem (ES) cells, the team tried to establish an ES cell line using *Tead4* mutants, and succeeded in establishing three ES-like cell lines, one of which was capable of forming colonies in culture and differentiating into all three germ layer lineages in vitro, suggesting that Tead4 is not required for development of embryo proper.

“The finding that trophectoderm completely fails to form in *Tead4* mutants provides an important clue for us to better understand early development in mammals,” says Sasaki. “But the important question of how this gene, which is expressed in every cell in the embryo, can prompt trophectodermal differentiation in only a subset of those cells still needs to be addressed.”

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