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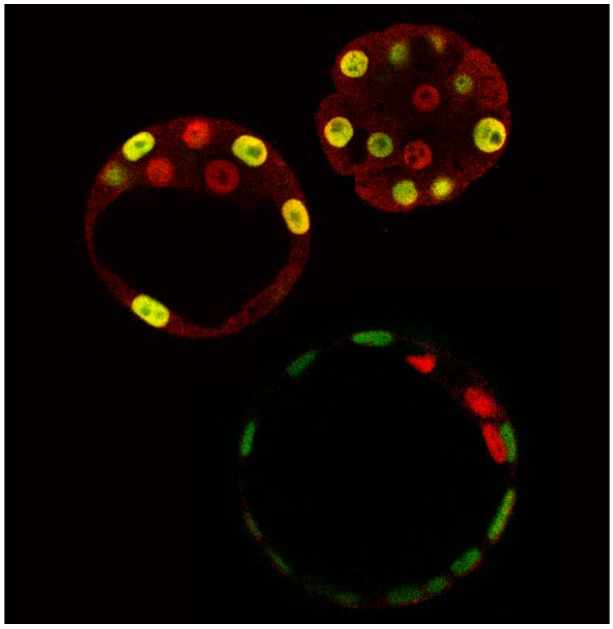
Whence pluripotency? Interaction between Cdx2 and Oct3/4 sorts out the early embryo

December 1, 2005 – Differentiation, the process by which cells assume sets of properties and specialized functions, is one of the most fundamental mechanisms of development. In the embryo, cells progress from a less differentiated to a more specifically differentiated state, a maturation that generally results in the loss of the differentiated cell's ability to take on other roles. This trade-off between potential and lineage commitment begins very early in the life of the mammalian embryo, as shown by the loss of totipotency (the autonomous ability of a cell to develop into an organism entire) of cells after only a few rounds of division. This first differentiation event results in the segregation of the early, ball-shaped embryo (called a blastocyst) into two distinct tissue types: an outer layer of trophectoderm, which goes on to form placenta, and the inner cell mass (ICM), a population of cells that both serves as the wellspring for every one of the cells in the embryo proper and contributes additional extraembryonic tissue as well. Despite the primary importance of this pivotal event, its molecular underpinnings have remained a tantalizing puzzle to researchers for nearly two decades.



Photomicrographs of early morula (top) later morula (middle) and blastocyst (bottom) stained for localization of Cdx2 (green) and Oct3/4 (red)

A significant portion of that puzzle has now been fit into place by researchers from the Laboratory for Pluripotent Cell Studies (Hitoshi Niwa; Team Leader), who describe the interaction between a pair of factors, Oct3/4 and Cdx2, that sets up the trophectoderm/ICM divide. Working with colleagues from Kobe University, the Japan Science and Technology Agency and Mount Sinai Hospital (Canada), Niwa puts forth a new model for the genetic basis of the earliest instance of cellular differentiation in the December issue of the journal *Cell*.

In previous work with embryonic stem (ES) cells, which share many of the properties of cells from the blastocyst interior, Niwa discovered that he could induce the ES cells to differentiate into trophectoderm (something they do not normally do) by repressing the function of the transcription factor, Oct3/4. Other work has further

recently identified a second molecule, Cdx2, as intimately involved in trophectoderm formation in the mouse blastocyst. With that finding as a cue, the Niwa team tested the effects of overexpressing *Cdx2* in ES cells in vitro and found that this also could induce trophoblast stem (TS) cells under certain culture conditions. On injection back into a blastocyst, Cdx2-induced TS cells that had been labeled with a fluorescent transgene to allow them to be visualized were seen to give rise to placental cell lineages in the chimeric embryos, as evidenced by the green glow from the placenta when exposed to UV light.

Prompted by their discovery to investigate the possibility of a relationship between Oct3/4 and Cdx2 (specifically, that they might act in a mutually inhibitory fashion), the team transfected ES cells with both molecules and observed the effects on genes normally activated by endogenous Oct3/4. They found that Cdx2 represses Oct3/4's transcriptional activating effects and, interestingly, that this repression depended on Oct3/4; in the absence of Oct3/4, no Cdx2-mediated inhibitory effect was seen on its downstream target genes. Intriguingly, Oct3/4 seems to exert a suppressive influence on Cdx2 as well. Cdx2 is thought to positively regulate its own expression in ES cells, but when these same cells are cultured with an Oct3/4 expression vector, this autoregulation is significantly damped.

This set of findings hinted that Cdx2 and Oct3/4 are locked in a mutually inhibitory relationship. Curious about the mechanics of this interaction, they first labeled the two molecules to study their localization within cells and found that when both were expressed together, they relocated to inactive regions of the nucleus. Next, they performed immunoprecipitation analysis (a means of determining whether two molecules bind to each other) and found that Oct3/4 and Cdx2 do indeed co-precipitate, indicating a direct interaction on the molecular level.

The story took on a new twist when Niwa et al looked at ES cells lacking both Cdx2 and Oct3/4 function. Although Cdx2 was thought to be an induce of trophectoderm, they discovered that, when Oct3/4 function is inhibited, even ES cells with homozygous deletions of Cdx2 can give rise to trophectoderm. The unexpected dispensability of Cdx2 in trophectodermal differentiation led them to inquire just what does Cdx2 actually do. Creating a line of cells in which Cdx2 expression could be controlled conditionally, they compared TS cells derived solely as a result of Oct3/4 donwregulation with those actively induced by Cdx2 expression, and found that the TS cells lacking Cdx2 were deficient in their ability to self-renew, which is one of the hallmark properties of all stem cells. As it turns out, a second factor, Eomesodermin (Eomeso), is capable of inducing trophectodermal differentiation even in the absence of Cdx2; Eomeso, however, could not entirely compensate for Cdx2 function, and had no repressive effect on Oct3/4.

The results of these in vitro experiments in hand, the Niwa team next observed how cells immunostained for Cdx2 and Oct3/4 behave in morula and blastocyst-stage embryos. In early morula, both factors were detected in all cells' nuclei, but by the later 10-16 cell morula stage Cdx2 could only be found in some of the outermost cells (Oct3/4 expression remained pervasive). By the blastocyst stage, the segregation was complete with Cdx2 limited to the outer layer and Oct3/4 restricted to the interior. These findings present the strong possibility that the loss of Cdx2 expression in the inner cells of the morula may be the trigger for the territorial sorting of the Cdx2-expressing trophectoderm from the Oct3/4-expressing inner cell mass. Niwa suggests that this process may be the result of the mutual repression of the two molecules, but whether the dynamics of this sorting out are simply

stochastic, or actively determined by cell polarity or size is yet to be determined. Other questions, such as whether this reciprocal inhibition is both necessary and sufficient to drive Cdx2 out to the morula periphery and the mechanism by which Oct3/4 maintains pluripotency in ES cells, also await answers. Answers that no doubt will stand on the solid foundations laid by this landmark series of experiments.

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