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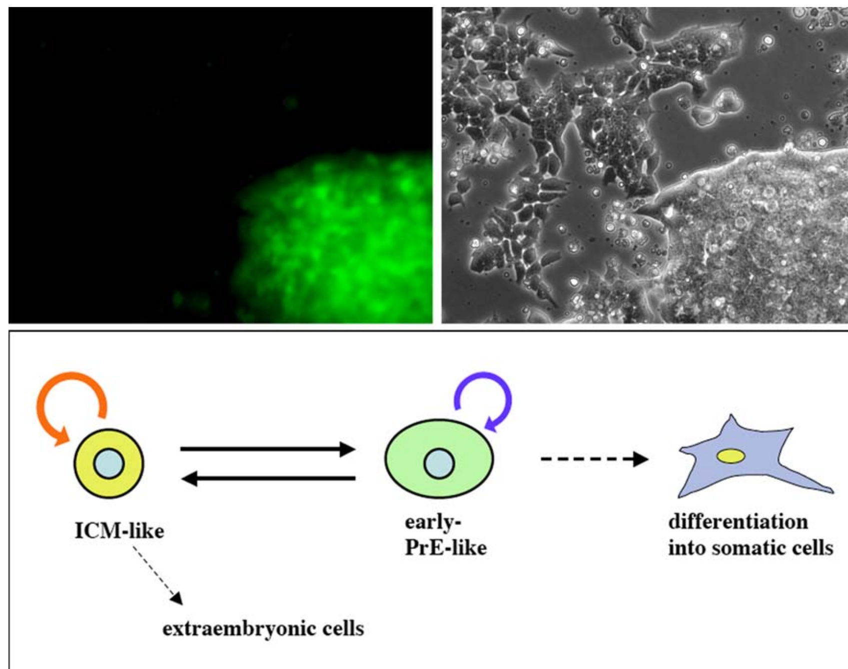
ES cells show diversity

February 20, 2008 – The first few days of embryonic development in the mouse witness the zygote undergo a transformation from a single cell, to a raspberry-like cluster (morula), to a hollow ball (blastocyst) before implanting in the uterine wall. Cell differentiation begins by about the third day of embryogenesis, with exterior cells entering the trophectodermal lineage, which forms the placenta and other extraembryonic tissues, and interior cells, which take the first step of the path leading to various somatic fates. The inner cell mass (ICM) of the blastocyst is famous as the source of embryonic stem cells, although it has been shown that ES-like pluripotent cells can also be obtained from the epiblast, a transient structure that arises slightly later in development, after implantation. The epiblast in turn gives rise to primitive ectoderm, which is considered to be the source of the somatic lineages that yield the many differentiated cell types in the adult body. ES cells are of great interest because they show similar ability to give rise to all somatic lineages in culture, but it remains unclear whether all ES cells are truly created equal, or whether they show diversity among themselves.



Upper panels show Rex1-positive cells labeled by GFP. Both Rex1^{+} and Rex1^{-} cells can be seen.
The lower panel shows a cartoon of the two ES cell subpopulations.

A new study by Yayoi Toyooka and colleagues in the Laboratory for Pluripotent Cell Studies (Hitoshi Niwa; Team Leader) comes down clearly in favor of the heterogeneity theory, showing that subsets of ES cells exhibit gene expression variations corresponding to the ICM, epiblast and primitive ectoderm stages of development. In a report published in *Development*, Toyooka, now at the University of Oxford, reveals that while all ES cells express the pluripotency marker *Oct3/4*, they show fluctuations in the expression of another gene previously taken as an ES hallmark, *Rex1*.

The study began with the establishment of a system for visualizing the expression of these two genes using a pair of fluorescent proteins. When they cultured the cells using a method to select for *Oct3/4*-expressing cells, they found that some of these expressed *Rex1* while others did not, with predominantly *Rex1*$^{+}$ colonies showing compacted morphologies, and *Rex1*$^{-}$ colonies tending to be flatter.

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They used quantitative PCR to examine gene expression in the two subpopulations in more detail, and found that the overall expression patterns in *Rex1*$^{+}$cells was similar to that seen in inner mass cells, while *Rex1*$^{-}$cells expressed genes in patterns reminiscent of primitive ectoderm. Interestingly, when they separated these fractions into purified populations, they found that each was able to give rise to the other spontaneously; that is, *Rex1*$^{+}$ cells showed up in *Rex1*$^{-}$ colonies, and vice versa. This worked even in colonies derived from a single *Rex1*-positive or negative cell.

It has long been known that cells from the inner cell mass or epiblast can contribute to chimera formation when injected into blastocyst-stage embryos, but cells taken from primitive ectoderm cannot. Investigating whether this might be true of ES cells in which *Rex1* expression is switched on or off, Toyooka et al. injected either *Rex1*$^{+}$ or *Rex1*$^{-}$ cells into mouse blastocysts and found that, similar to ICM and epiblast cells, the *Rex1*$^{+}$ cells contributed strongly to chimeric embryonic tissues, but that, as is the case for primitive ectoderm, the *Rex1*$^{-}$cells did not.

Given this apparent correspondence of *Rex1*$^{+}$ and *Rex1*$^{-}$ cells respectively to the inner cell mass and primitive ectoderm stage of development, the Niwa team next checked whether their differentiation would follow a similar pattern. When differentiation was induced in colonies enriched for *Rex1*$^{-}$ cells by the withdrawal of LIF (a factor that keeps mouse ES cells in an undifferentiated state) they showed a lower tendency to give rise to extraembryonic tissue than did a control group, indicating another similarity with primitive ectoderm cells in vivo. Conversely, *Rex1*$^{-}$ cells were comparatively easier to steer down somatic pathways, such as mesoderm or neuroectoderm, than were their *Rex1*$^{+}$ counterparts.

“I suppose that many people who have worked with ES cells already suspected that they were not entirely homogeneous,” says Toyooka, “but now, by looking at Rex1 expression, we’ve been able to show that there are definite subpopulations in terms of both gene expression and differentiative potential. Perhaps this work will serve as a model for studying the control of fluctuations in gene expression.”

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