STEM CELLS

Gametes from stem cells

In vitro reconstitution of mouse germ cell development makes it possible to convert mouse pluripotent stem cells into primordial germ cells, which go on to generate functional sperm _in vivo_.

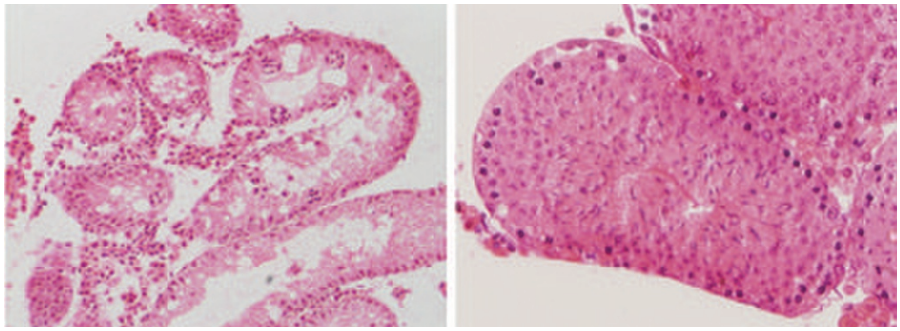
Gametes—oocytes and sperm—are arguably one of the more interesting cell types in the body. To pass on the genome to the next generation, these very specialized cells must successfully participate in the intricate dance of fertilization and then give rise to pluripotent cells that go on to generate all body tissues, including more germ cells.

The ability to make germ cells from pluripotent stem cells would aid basic studies of germline development, provide a source of germ cells to study and could in principle also be useful for assisted reproduction. In a recently published paper, Mitinori Saitou and colleagues at Kyoto University describe methods for the directed differentiation of pluripotent stem cells to primordial germ cells (PGCs) in the mouse.

PGCs go on to give rise to both male and female gametes. In the mouse, they are derived from the embryonic epiblast early in development. In previous work, researchers in Saitou's group had derived PGCs *ex vivo* from the isolated embryonic epiblast 5–6 days after fertilization. They reasoned that lessons learned from these previous studies could be harnessed to define a method that let them go all the way from pluripotent stem cells to PGC-like cells *in vitro*.

Although the so-called epiblast stem cells are in principle good candidates for generating PGC-like cells, based on their biological origin in the embryonic epiblast, this is known to occur at a very low efficiency. Could this efficiency be increased, Saitou and colleagues asked, by starting with epiblast-like cells that have not been cultured *in vitro* over time? The researchers set out to generate epiblast-like cells afresh from embryonic stem cells (ESCs).

They derived ESCs from mice bearing transgenes for expression of fluorescently tagged markers of PGC fate. Then, they established culture conditions (notably, this must be done serum-free) to convert these ESCs into epiblast-like cells. Finally, the researchers applied the floating three-dimensional culture conditions they had previously developed for converting isolated epiblast into PGCs and monitored germ cell fate specification from the ESC-derived cells using the fluorescent reporter transgenes. Under the right conditions, the researchers observed rapid (a few days) and efficient (about 40%) conversion of the epiblast-like cells to PGC-like cells *in vitro*. As they had seen previously, BMP4 signaling is key in this process.



Spermatogenesis by ESC-derived primordial germ cells. Hematoxylin and eosin–stained tissue sections of germ cell–deficient mouse testis without (left) and with (right) transplant of *in vitro*–differentiated PGCs. Images courtesy of K. Hayashi and M. Saitou.

An analysis of whole-genome expression profiles indicated that the epiblast-like stem cells are closest to *in vivo* embryonic day (E)5.75 epiblast and that PGC-like cells are closest to *in vivo* E9.5 PGCs. From this and other analyses, Saitou and colleagues conclude that they essentially recapitulated germ-cell development *in vitro*. Perhaps most importantly, the PGC-like cells they generate undergo functional spermatogenesis when transferred to the gonads of germ cell–deficient mice. Sperm derived from these transplanted PGCs yield viable and grossly normal offspring when used to fertilize oocytes *in vitro*.

The researchers generated PGC-like cells from several ESC lines, but notably, also from mouse induced pluripotent stem cells (iPSCs). What is more, they screened their cultures for cell-surface markers that can be used to enrich the *in vitro*–generated PGC-like cells by FACS, without the need for genetically encoded reporters.

This work has immediate value for generating large numbers of mouse PGC-like cells for basic research (several orders of magnitude more cells can be obtained this way than from mouse embryos). But in addition, one of the exciting distant vistas opened up by robust and functional *in vitro* germ cell differentiation is that such methods could be used to generate gametes from stem cells of other species. Indeed, in this issue of *Nature Methods*, Jeanne Loring and colleagues report the derivation of iPSCs from two endangered species, the silver-maned drill and the white rhinoceros. Although translating knowledge about mouse germline specification to deriving germ cells from different, nonrodent species will be a huge challenge, it is tempting to speculate that the findings of Saitou and colleagues could eventually have some bearing on efforts in other species as well.

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RESEARCH PAPERS

Hayashi, K. *et al.* Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. *Cell* **146**, 519–532 (2011).