REVIEW

Germ Cell Specification in Mice

Katsuhiko Hayashi, Susana M. Chuva de Sousa Lopes, M. Azim Surani*

Specification of germ cells in mice occurs relatively late in embryonic development. It is initiated by signals that induce expression of Blimp1, a key regulator of the germ cell, in a few epiblast cells of early postimplantation embryos. Blimp1 represses the incipient somatic program in these cells and promotes progression toward the germ cell fate. Blimp1 may also have a role in the maintenance of early germ cell characteristics by ensuring their escape from the somatic fate as well as possible reversion to pluripotent stem cells.

Primordial germ cells (PGCs), the founder cells of the germ cell lineage, are usually established early during embryonic development. Specification of PGCs can occur either through the inheritance of germ cell determinants already present in the egg, as in *Caenorhabditis elegans* and *Drosophila*, or in response to inductive signals, as in mice and probably all mammals. In all instances however, germ cells are maintained by mechanisms that prevent them from differentiating into somatic cells.

The Stem Cell Model for PGC Specification in Mice

In *C. elegans* and *Drosophila,* founder PGCs are set aside at the outset from a totipotent zygote and prevented from differentiating into somatic cells by repression of the global transcriptional machinery (*1*). However, in mice, specification of PGCs is deferred until after implantation of blastocysts. The extraembryonic ectoderm (ExE) and visceral endoderm (VE), which surround the epiblast cells of the postimplantation egg cylinder, are the sources of signals that instruct a small number of epiblast cells to become PGCs; the rest of the cells commence differentiation into somatic tissues.

The rapidly dividing mouse epiblast cells are developmentally equivalent to the *Drosophila* egg (*2*). However, whereas in *Drosophila*, the determinants of somatic and germ cells are already segregated in specific regions of the oocyte, no such determinants exist in the mouse oocyte. Furthermore, PGCs originate from the proximal pluripotent epiblast cells that are already transcriptionally active and to some extent have embarked upon a somatic fate. Furthermore, pluripotent embryonic stem cells, which can be propagated indefinitely in vitro, can generate an infinite number of PGCs when returned to the blastocyst or when they otherwise receive specific signals to induce germ cell fate. We could therefore call this the stem cell model for PGC specification.

An elaborate transcriptional program that regulates PGC specification in mice prevents them from a continuing drift toward a somatic fate, and this is coupled with a chromatin-based mechanism that erases this trend, thereby resulting in reexpression of some key pluripotency-associated genes. At the same time, PGCs must acquire and maintain their lineage-specific characteristics. Recent advances are beginning to piece together the key steps that lead to PGC specification.

Origin of PGC Precursors from the Pluripotent Proximal Epiblast Cells

The pluripotent proximal epiblast cells respond to signals from the extraembryonic tissues and begin to express *fragilis/Ifitm3* as they acquire the ability to form PGCs, although only a small minority of them become germ cells in the end (*3*, *4*) (Fig. 1). Within these *fragilis*-positive cells, at embryonic day 6.25 (E6.25), about six cells in the prospective posterior proximal site of the embryo and directly in contact with the overlying ExE begin to show expression of *Blimp1/Prdm1*. Experiments tracing genetic lineage demonstrate that all of the *Blimp1*-expressing cells that originate in the proximal-posterior epiblast are the lineage-restricted PGC precursor cells (*5*).

There is further accretion of *Blimp1*-positive cells after this time. Lineage-tracing experiments had previously shown that some single cells in the proximal epiblast at E6.5 could give rise to both PGCs and extraembryonic mesoderm but never exclusively to PGCs (*6*). Furthermore, distal epiblast cells from E6.5 embryos when transplanted to the proximal-posterior region can contribute to the germ cell lineage, whereas proximal-posterior cells transplanted

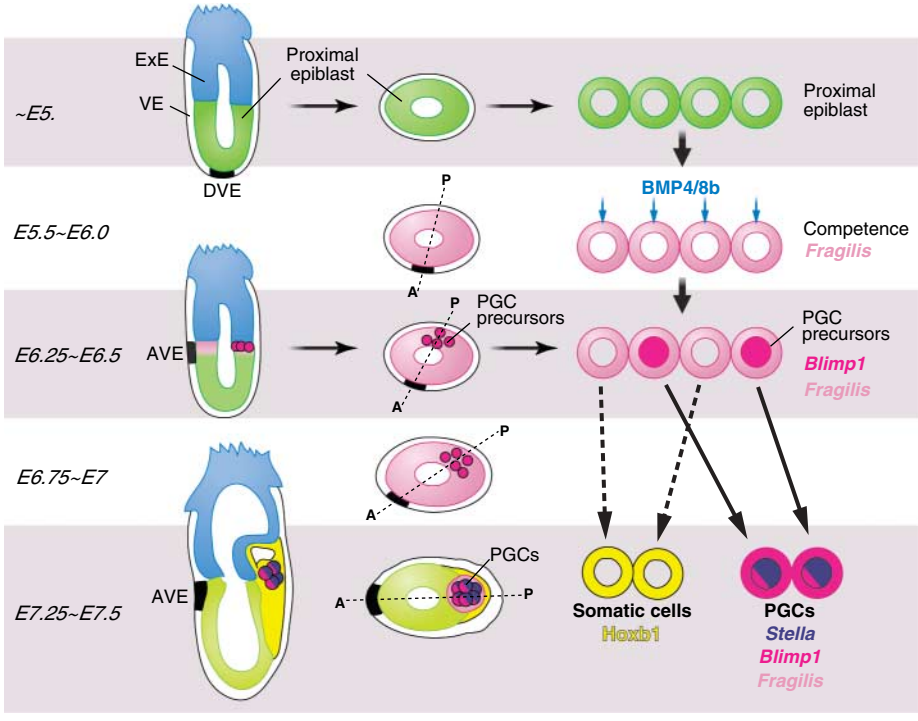

Fig. 1. Development of early postimplantation embryo from E5.0 to E7.5, depicting the formation of PGCs. The proximal epiblast cells respond to signals from the extraembryonic tissues, which induce expression of *fragilis* in the epiblast, and of *Blimp1* in the PGC precursor cells at one end of the short axis before gastrulation. After gastrulation, the PGC precursors locate to the posterior proximal region, where they undergo specification to form the founder population of *Stella*-positive PGCs.

Wellcome Trust Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK.

*To whom correspondence should be addressed. E-mail: as10021@mole.bio.cam.ac.uk

to the distal region of the epiblast can only give rise to somatic cells (*7*). These studies indicate the persistence of signals that can continually induce proximal-posterior epiblast cells at least up to E6.5 to commit to the PGC fate. Consistent with these findings, the number of *Blimp1*-expressing cells increases from about 6 to 16 between E6.25 and E6.5 (*5*). Taking into account the possibility that there is lengthening of the cell cycle time in the PGC precursors from 7 hours to about 16 hours, recruitment of additional precursors is necessary to account for about 40 Stella-positive founder PGCs that are finally observed at the posterior end of the primitive streak at E7.25 (*3*, *5*, *8*).

Signals for PGC Specification

Both the ExE and VE are essential for the acquisition of competence and PGC precursors but not for PGC specification itself (*9*, *10*). Bone morphogenetic protein 4 (BMP4), which is produced by ExE, is capable of inducing *fragilis/Ifitm3* expression (*3*). ExE and VE are also the sources of BMP8b and BMP2, respectively. Loss of any of these signaling molecules abrogates the competence to give rise to all or most of the PGCs (*11*–*13*). BMPs trigger serine phosphorylation of the transducer Smad1/5/8, which translocates into the nucleus with the common mediator, Smad4. Loss of Smad1 and Smad5 (but not Smad8) causes severe reduction in the numbers of PGCs (*14*–*16*), as does the conditional loss of Smad4 (*17*).

The *Bmp-Smad* gene dosage is critical for PGC specification. For example, in the *Bmp4*-heterozygous mutants, the number of PGCs is almost halved, which is also the case in the double heterozygous *Smad1* and *Smad5* (*18*). Indeed, the PGC precursors emerge from the most proximal layer of the epiblast, where the BMP-Smad signaling is strongly activated (Fig. 1).

The detection of *Blimp1*-positive PGC precursors at the posterior side of the early embryo indicates that the anterior-posterior (A-P) axis formation may play a role in determining their numbers and location. The anterior visceral endoderm (AVE) (*19*) produces Nodal and Wnt antagonists, thus restricting Nodal and Wnt3 signaling to the posterior side of the embryo, the site where the *Blimp1*-positive PGC precursors are detected. Notably, *Smad2*-mutant embryos, which disorder A-P axis formation and result in the expression of "posterior" genes, including *Nodal* and *Fgf8* in the entire epiblast, show many ectopic clusters of PGCs (*16*, *20*). It appears that an orchestration of growth factors, which may include Nodal and Fgf8, creates an environment for PGC precursors to be segregated from somatic cell lineages.

Blimp1: The Key Regulator of PGC Specification

A crucial part of PGC specification in many model organisms includes repression of the somatic program. In mice, a unique germ cell–specific transcriptional network seems to regulate PGC specification. Extensive analysis of gene expression profile in single cells shows the involvement of a molecular program during germ cell specification (*3*, *5*, *21*, *22*) (Fig. 2).

Among the genes identified so far, Blimp1 protein is a key transcriptional regulator that is partly responsible for repressing the somatic program in PGCs while allowing establishment of germ cell character in these cells (*5*, *23*). Blimp1 protein has a PR/SET domain, a proline-rich region, five C_2H_2 zinc fingers, and a C-terminal acidic domain.

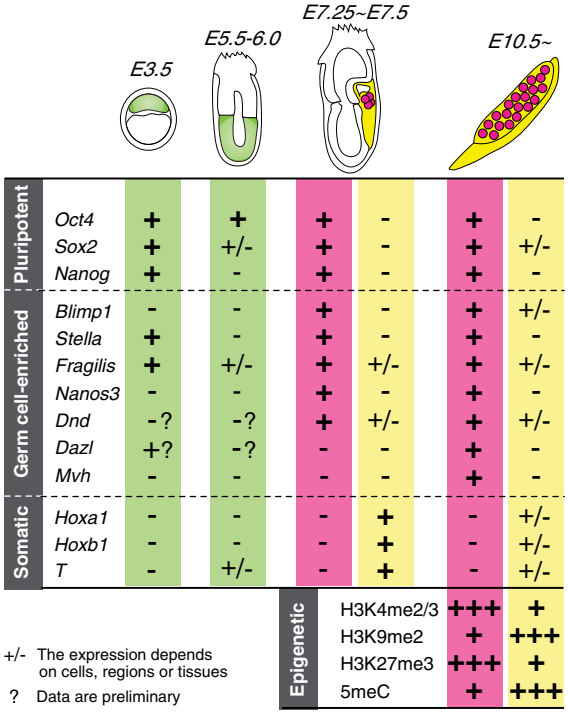

Fig. 2. A summary of PGC specification. Progressive changes in gene expression from the epiblast in early embryos (green) to PGCs (red) are indicated. Epigenetic differences between PGCs and somatic cells (yellow) are shown at E10.5. *Nanos3*, *Mvh*, *Dnd*, and *Dazl* are also germ cell–enriched genes. DNA methylation (5meC) is erased in imprinting control elements and gene-encoding regions after E10.5 (*33*).

Detailed analysis suggests that the PGC-competent proximal epiblast cells expressing *fragilis/Ifitm3* are initially destined for a somatic fate. Accordingly, early *Blimp1*-expressing cells at E6.75 originating in the proximal epiblast cells exhibit expression of *Hoxb1* as well as other mesodermal genes, including *T*, *Fgf8*, and *Snail* (*21*). These genes continue to be up-regulated in the neighboring mesodermal somatic tissues. However, they become repressed in the *Blimp1*-positive cells in an orderly manner along with the progression of PGC specification (*22*). The repression of somatic genes in PGCs is consistent with the phenomenon of repression of the somatic program observed in *C. elegans* and in *Drosophila*.

Coupled with the repression of mesodermal-specific genes, there is up-regulation of other genes, including *Sox2* (*5*, *22*). Another gene, *Nanog*, is also reexpressed in PGCs (*24*). Thus, among all the lineages that develop from the epiblast cells, only germ cells regain expression of pluripotency-associated genes during the course of their specification. There is also ex-

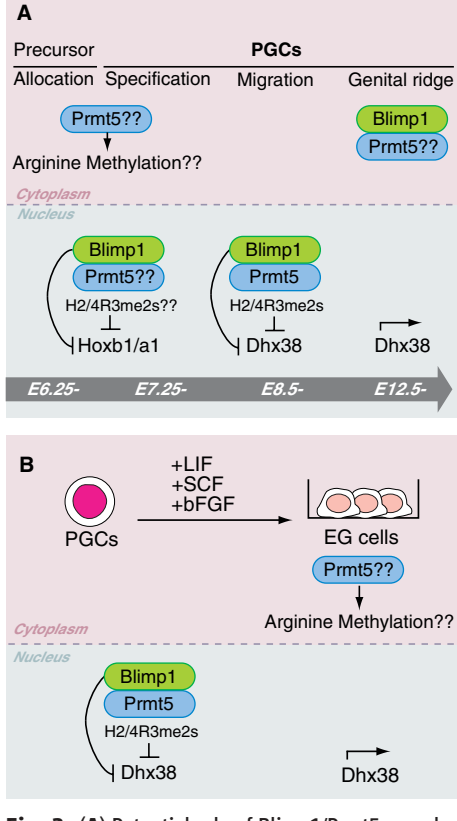

Fig. 3. (**A**) Potential role of Blimp1/Prmt5 complex in PGC specification until the migration of PGCs into the genital ridges, when the complex translocates from the nucleus to the cytoplasm. (**B**) The loss of Blimp1 during dedifferentiation of PGCs into pluripotent EG cells. Dhx38 is a target of Blimp1/Prmt5 complex.

pression of other unique genes in PGCs, including *Prdm14*, a gene that is closely related to *Blimp1/Prdm1*, which may also have a role in PGC specification (*22*).

The functional importance of Blimp1 in PGC specification became evident during the analysis of the *Blimp1*-mutant mouse embryos, which results in aberrant development of founder PGCs. In the absence of Blimp1, the mutant cells form a tight PGC-like cluster, but they cease to proliferate and they show little evidence for migration out of the cluster. They also show inconsistent repression of *Hoxb1*, which is a hallmark of PGC specification, while failing to show consistent upregulation of *stella* and *Sox2*, as observed in normal PGC. Thus, Blimp1 plays a critical role in the establishment of the founder PGCs.

The Role of Prmt5 Arginine Methylase in PGC Specification

Recent studies have shown a previously unrecognized Blimp1/Prmt5 complex in germ cells. Prmt5 is an arginine-specific histone methyltransferase, which mediates symmetrical dimethylation of arginine-3 on histone H2A and/or H4 tails (H2Ame2s/H4R3me2s), which is detected in germ cells (*21*). A few targets of the Blimp1/Prmt5 complex have been identified in germ cells, including *Dhx38*. In PGCs, *Dhx38* is repressed and shows an H4R3me2s epigenetic mark until E12.5. Its expression at this time coincides with the translocation of Blimp1/Prmt5 from the nucleus to the cytoplasm at E11.5, after which the expression of pluripotency-associated genes also begins to be down-regulated. Thus, Blimp1/Prmt5 complex may play an essential role in maintaining the PGC lineage during the migration of the cells into the gonads (Fig. 3A).

Notably, recent studies in *Drosophila* indicate that a mutation in the Prmt5 homolog, *Capsuleen/dart5*, affects germ cell specification in females and development of spermatocytes in males (*25*, *26*). Both the formation of nuage in nurse cells and pole plasm integrity are affected in *Capsuleen/dart5* mutant flies. *Capsuleen/dart5* has the potential to methylate protein substrates, which have a role in the integrity of P granules in the germ cells of *C. elegans* (*27*). Both P granules and nuage are RNA rich and contain several proteins. In *Capsuleen/dart5* mutant flies, the localization of Tudor, an essential component of the pole plasm and nuage, is abolished.

It will be important to determine whether Prmt5 has a role earlier in the PGC precursors in mice, either through any cytoplasmic substrates or through its association with Blimp1 (Fig. 3A). It remains to be seen whether Tudor domain proteins, some of which are detected at the time of PGC specification (*28*), contribute to PGC specification in conjunction with Prmt5 or Blimp1/Prmt5 complex.

Postspecification Establishment of PGC Epigenetic Signature

An integral part of the PGC specification process includes substantial epigenetic modifications, which occur in the *stella*-positive PGCs. At E8.0, the level of H3K9me2 (an epigenetic mark associated with transcriptional repression) diminishes, whereas H3K27me3 (another repressive epigenetic mark associated with high levels of Ehz2) becomes prominent (*29*) (Fig. 2). These changes are followed by upregulation of H3K4me2/3 (Fig. 2). The epigenetic marks H3K27me3 and H3K4me2/3 are notable as the facultative marks of gene loci that are repressed in pluripotent embryonic stem cells.

Germ Cells and Pluripotent Stem Cells: A Reversible Phenotype

PGCs undergo dedifferentiation into pluripotent embryonic germ (EG) cells when they are cultured with basic fibroblast growth factor (bFGF), leukemia inhibitory factor (LIF), and stem cell factor (SCF) (*30*–*32*). EG cells can only be derived from PGCs between E8.5 and E11.5 when H3K27me3 and H3K4me2/3 marks become prominent and when H3K9me2 is absent (*29*, *30*). At the same time, PGCs show expression of pluripotency-associated genes *Oct4*, *Sox2*, and *Nanog*. Blimp1 is down-regulated during the derivation of EG cells, whereas Prmt5 is detected not only in EG cells but also in all other pluripotent cells. The loss of Blimp1 may result in derepression of certain genes that maintain the germ cell lineage, such as *Dhx38* (Fig. 3B), a target of Blimp1/Prmt5 complex in PGCs until E11.5 (*21*). Reciprocally, ectopic expression of Blimp1 in pluripotent embryonic carcinoma (EC) cells leads to the repression of *Dhx38*. It will be of interest to determine whether the EC cells and indeed all pluripotent stem cells acquire aspects of the PGC character upon expression of Blimp1.

Outlook

Analysis of PGC specification in different organisms demonstrates both the differences and some common features of the mechanisms involved in their specification. An essential necessity for the germ line cycle is to prevent a loss of pluripotency and totipotency, which are lost from somatic cells as they begin to undergo differentiation.

An emerging theme in PGC specification is the potential role of the arginine methylase, Prmt5. In flies, it seems to have a role as a protein arginine methylase that acts on components of the germ plasm and helps to maintain its integrity through the involvement of Tudor. The role of Prmt5 in the mouse PGCs remains to be fully elucidated. It also seems that Blimp1 probably helps to direct Prmt5 to its targets, such as *Dhx38*, but a more comprehensive search for other targets is needed to unravel its role more fully in early PGCs. Further investigations will deepen our insights on the specification of germ cells, the most critical lineage in all species.

References and Notes

1. S. Strome, R. Lehmann, *Science* **316**, 392 (2007).
2. P. H. O'Farrell, J. Stumpff, T. T. Su, *Curr. Biol.* **14**, R35 (2004).
3. M. Saitou, S. C. Barton, M. A. Surani, *Nature* **418**, 293 (2002).
4. S. S. Tanaka, Y. Matsui, *Mech. Dev.* **119** (suppl. 1), S261 (2002).
5. Y. Ohinata *et al.*, *Nature* **436**, 207 (2005).
6. K. A. Lawson, W. J. Hage, *Ciba Found. Symp.* **182**, 68 (1994).
7. P. P. Tam, S. X. Zhou, *Dev. Biol.* **178**, 124 (1996).
8. A. McLaren, K. A. Lawson, *Differentiation* **73**, 435 (2005).
9. S. M. de Sousa Lopes *et al.*, *Genes Dev.* **18**, 1838 (2004).
10. T. Yoshimizu, M. Obinata, Y. Matsui, *Development* **128**, 481 (2001).
11. K. A. Lawson *et al.*, *Genes Dev.* **13**, 424 (1999).
12. Y. Ying, X. M. Liu, A. Marble, K. A. Lawson, G. Q. Zhao, *Mol. Endocrinol.* **14**, 1053 (2000).
13. Y. Ying, G. Q. Zhao, *Dev. Biol.* **232**, 484 (2001).
14. H. Chang, M. M. Matzuk, *Mech. Dev.* **104**, 61 (2001).
15. K. Hayashi *et al.*, *Mech. Dev.* **118**, 99 (2002).
16. K. D. Tremblay, N. R. Dunn, E. J. Robertson, *Development* **128**, 3609 (2001).
17. G. C. Chu, N. R. Dunn, D. C. Anderson, L. Oxburgh, E. J. Robertson, *Development* **131**, 3501 (2004).
18. S. J. Arnold, S. Maretto, A. Islam, E. K. Bikoff, E. J. Robertson, *Dev. Biol.* **296**, 104 (2006).
19. P. Q. Thomas, A. Brown, R. S. Beddington, *Development* **125**, 85 (1998).
20. W. R. Waldrip, E. K. Bikoff, P. A. Hoodless, J. L. Wrana, E. J. Robertson, *Cell* **92**, 797 (1998).
21. K. Ancelin *et al.*, *Nat. Cell Biol.* **8**, 623 (2006).
22. Y. Yabuta, K. Kurimoto, Y. Ohinata, Y. Seki, M. Saitou, *Biol. Reprod.* **75**, 705 (2006).
23. S. D. Vincent *et al.*, *Development* **132**, 1315 (2005).
24. S. Yamaguchi, H. Kimura, M. Tada, N. Nakatsuji, T. Tada, *Gene Expr. Patterns* **5**, 639 (2005).
25. J. Anne, R. Ollo, A. Ephrussi, B. M. Mechler, *Development* **134**, 137 (2007).
26. G. B. Gonsalvez, T. K. Rajendra, L. Tian, A. G. Matera, *Curr. Biol.* **16**, 1077 (2006).
27. S. A. Barbee, T. C. Evans, *Dev. Biol.* **291**, 132 (2006).
28. M. A. Surani, K. Hayashi, P. Hajkova, *Cell* **128**, 747 (2007).
29. Y. Seki *et al.*, *Dev. Biol.* **278**, 440 (2005).
30. G. Durcova-Hills, I. R. Adams, S. C. Barton, M. A. Surani, A. McLaren, *Stem Cells* **24**, 1441 (2006).
31. Y. Matsui, K. Zsebo, B. L. Hogan, *Cell* **70**, 841 (1992).
32. J. L. Resnick, L. S. Bixler, L. Cheng, P. J. Donovan, *Nature* **359**, 550 (1992).
33. P. Hajkova *et al.*, *Mech. Dev.* **117**, 15 (2002).

34. We thank M. Saitou for critical comments on the manuscript and the Wellcome Trust for a Programme Grant.

10.1126/science.1137545

Germ Cell Specification in Mice

Katsuhiko Hayashi, Susana M. Chuva de Sousa Lopes and M. Azim Surani

Science **316** (5823), 394-396.
DOI: 10.1126/science.1137545