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Of mice and men: *in vivo* and *in vitro* studies of primordial germ cell specification

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Abstract

Specification of mouse primordial germ cells (PGCs), the precursors of sperm and eggs, involves three major molecular events: repression of the somatic program; re-acquisition of pluripotency; and reprogramming to a unique epigenetic ground state. Gene knockout studies in mouse models, along with global transcriptome analyses, have revealed the key signaling pathways and transcription factors essential for PGC specification. Knowledge obtained from these studies has been utilized extensively to develop robust *in vitro* PGC induction models not only in mice but also in humans. These models have, in turn, formed the basis for a detailed understanding of the signaling pathways and epigenetic dynamics during *in vivo* PGC specification and development. Recapitulation of human PGC specification in culture is of tremendous significance for understanding the mechanisms of human germ cell development in normal and disease states and has implications for addressing germ-cell-based causes of infertility.

Keywords

primordial germ cells; germ cell specification; pluripotent stem cells; epigenetic reprogramming; *in vitro* germ cell production

Introduction

Germ cells are the only cell type responsible for passing on genetic information from one generation to the next, thereby providing a continuous link between all generations of an organism. Induction of germ cell fate in metazoans occurs either by preformation or epigenesis¹. In *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Xenopus laevis*, the founder primordial germ cells (PGCs) (or the germ plasm) are set aside exclusively to form the germ cells as soon as a totipotent zygote is formed following fertilization. This process is called preformation and involves repression of a transcriptional network in order to prevent the differentiation of the founder PGCs into a somatic fate. However, in mice and other

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mammals, specification of PGCs occurs much later, after implantation of the blastocyst, and a subset of cells are induced to become germ cells by external signals in a process termed epigenesis²⁻⁴. Interestingly, repression of the somatic program is a key event in both these modes of germ cell specification; however, some of the factors involved in this process can vary among different species. Despite some differences in underlying molecular pathways, many aspects of PGC development are highly conserved across many diverse species¹.

In this review, we provide a summary of the recent advances in the field of germ cell specification in mice. We briefly discuss the signals, transcription factors, and epigenetic reprogramming involved in mouse PGC specification. In addition, we describe *in vitro* PGC induction in mouse and humans using pluripotent stem cells. Understanding the process of germline specification *in vivo* is critical to providing a strong foundation for successful reconstitution of PGCs *in vitro*. Robust *in vitro* PGC induction in mice and humans could potentially aid our understanding of the germline specification process *in vivo* and lead to advances in reproductive and regenerative medicine.

Mammalian germline induction by extrinsic signals

In mice, primordial germ cells (PGCs) originate from a subset of cells located in the proximal epiblast of the developing embryo. These cells can be identified by their alkaline phosphatase (AP) activity during early gastrulation. By 7.25 days post coitum (dpc) these AP-positive cells increase in number to approximately 40 cells and are visible as a cluster at the base of the incipient allantois^{5,6}. Subsequently, these cells start to move from the posterior primitive streak to the embryonic endoderm, where they become motile and migrate en masse through the hindgut mesentery towards the future genital ridge to colonize the gonad by around 10.0 dpc. Following their arrival into the genital ridge, the germ cells undergo morphological changes, such as the loss of polarized morphology and motility, and are thereafter referred to as gonocytes. Subsequently, gonocytes proliferate and start their sex-specific development⁷.

Induction of mouse PGC specification in a subset of epiblast cells is dependent on signals from the surrounding extraembryonic ectoderm (ExE) and visceral endoderm (VE) of the developing embryo^{3,8,9} (Figure 1). Bone morphogenetic protein (BMP) signals from the ExE and VE are absolutely essential for induction of PGC specification in the posterior proximal epiblast at 6.25 dpc. Targeted deletion of *Bmp2*, *Bmp4*, *Bmp8b*¹⁰⁻¹² or the downstream signaling components *Smad1*, *Smad4*, and *Smad5*¹³⁻¹⁵ in mice resulted in embryos with a severely reduced number of PGCs. BMP4 and BMP8b produced by the ExE and BMP2 produced by the VE induce the formation of PGC precursors that are identified by expression of the PGC marker PR domain zinc-finger protein 1 (PRDM1), also known as BLIMP1 (B-lymphocyte-induced maturation protein 1)^{3,9}. Approximately six PRDM1-positive PGCs are visible at 6.25 dpc in the posterior proximal epiblast of the post-implantation embryo. Lineage tracing experiments have demonstrated that these PRDM1-positive cells are lineage-restricted PGC precursors¹⁶. Dosage of BMP4 and BMP2 signal is a critical factor for PGC specification, as *Bmp4* heterozygous mutants have half the number of PGCs compared to wildtype embryos¹⁶. Similarly, *Smad1* and *Smad5* double

heterozygous mutants also have half the number of PGCs compared to wildtype embryos, indicating that PGC specification mediated via BMP signaling is dosage-dependent¹⁷.

In addition to BMP, WNT signaling is also essential for PGC specification. Targeted deletion of *Wnt3* and *Ctnnb1* (encodes for β -catenin, the downstream signal transducer of WNT) results in a complete absence of PRDM1-positive PGCs by 7.0 dpc in mutant embryos^{3,9}. WNT3 is expressed in both the posterior VE and the epiblast of the developing embryo at 6.25 dpc¹⁸. Later, WNT3 expression becomes restricted to the posterior proximal epiblast and posterior VE. Expression of WNT3 is important for inducing expression of primitive streak genes required for gastrulation at around 5.5–6.5 dpc¹⁸. These mesodermal genes are expressed in PGC precursors but their expression is repressed in PGCs by 7.5 dpc in order to maintain the germ cell fate. However, some mesodermal factors activated by WNT3 signaling such as mesodermal factor T (encoded by the *brachyury* gene) have been shown to be required for PGC specification⁹.

How WNT3 and BMP signaling coordinate the induction of germ cells is not clear. BMP signaling components were expressed in the ExE of WNT3-null embryos; however, active BMP signaling, as indicated by the presence of phosphorylated SMAD1/5/8, was absent in mutant embryos³. Thus, one possibility is that WNT signaling might be important to make the epiblast competent to respond to BMP signaling for PGC specification. The role of Nodal signaling in the induction of germ cell fate has not been investigated; however, *Bmp4* and *Wnt3* expression is absent or reduced in *Nodal*-null embryos, suggesting that Nodal signaling might play a crucial role in PGC specification¹⁹.

The specified PGCs are restricted to the posterior epiblast by inhibitory signals emitted from the anterior visceral endoderm (AVE). These signals include: CER1 (a BMP antagonist encoded by the *cerberus 1* gene) that inhibits BMP signaling; LEFTY1 that inhibits Nodal signaling; and DKK1 (a WNT3 antagonist encoded by the *dickkopf 1* gene) that inhibits WNT signaling in the anterior epiblast³. Hence, BMP, Nodal, and WNT signals are restricted to the posterior side of the embryo, thereby creating an environment for the PGC cluster to remain distinct from the somatic cell lineages³.

Gene regulatory network during PGC induction: Role of *Prdm1*, *Prdm14*, and *Tcfap2c*

PRDM1 (also known as BLIMP1) is one of the key regulators of PGC specification^{16,20}. Loss of PRDM1 activity results in fewer PGC precursors that form aberrant aggregates and fail to migrate towards the hindgut endoderm^{16,20}. Detailed single-cell cDNA microarray analysis of PRDM1-deficient PGCs revealed that they fail to repress the expression of somatic genes, indicating that PRDM1 is essential for repressing the somatic program in PGCs²¹. Following expression of PRDM1, two other transcription factors, namely PRDM14 and TCFAP2C (also known as AP2 γ), are expressed in PGCs at 6.5 dpc and 6.75 dpc, respectively^{22,23}. PGC-competent proximal epiblast cells are initially destined for a somatic fate and, hence, express mesodermal genes such as *Hoxa1*, *Hoxb1*, and mesodermal factor *T/brachyury*^{21,24}. However, PRDM1, PRDM14, and TCFAP2C form a tripartite transcription factor network that represses the expression of these somatic genes and

facilitates the PGC specification process²⁵ (Figure 1). Targeted deletion of *Prdm1*, *Prdm14*, and *Tcfap2c* results in failure of proper PGC specification in mutant embryos, highlighting the importance of these transcription factors in germ cell specification and maintenance^{16,22,23}. In addition to suppressing the somatic program, PRDM1 and PRDM14 are important for upregulating the expression of germ-cell-specific genes and pluripotency genes in PGC precursors²⁵. Furthermore, studies have shown that they are also required for initiating the process of epigenetic reprogramming, such as repressing the expression of Euchromatic histone methyltransferase 1 (EHMT1), an enzyme that catalyzes the addition of methyl groups to H3K9 (H3K9me1 and H3K9me2), and DNMT3A and DNMT3B enzymes that perform *de novo* DNA methylation^{16,21,22}. Overexpression of *Prdm14* alone or any two of the three transcription factors (PRDM1, PRDM14 and TCFAP2C) induced mouse PGC-like cells (mPGCLCs) from mouse epiblast-like cells (mEpiLCs) in culture^{26,27}.

It has been shown that PRDM1 mainly binds to promoter regions of genes^{26,28}, PRDM14 binds to their distal regulatory elements^{26,29}, and TCFAP2C is enriched at targets of both PRDM1 and PRDM14 to regulate gene activation or repression^{26,30}. Studies have demonstrated that PRDM14 interacts with SUZ12, a component of Polycomb repressive complex 2 (PRC2), to add the H3K27me3 repressive mark in mouse embryonic stem cells³¹. However, in addition to interacting with SUZ12, PRDM14 interacts with a co-repressor, CBFA2T2 (also known as MTGR1), which recruits histone deacetylases (HDACs) to induce gene silencing^{32,33}. Targeted deletion of *Cbfa2t2* or disruption of the CBFA2T2 and PRDM14 interaction resulted in severe defects in PGC specification and epigenetic reprogramming³². PRDM1 interacts with histone modifiers such as HDAC2, EHMT1, protein arginine N-methyltransferase PRMT5, and histone lysine demethylase 1A (KDM1A)³⁴. In all, PRDM1, PRDM14, and TCFAP2C form a transcription factor network that influences epigenetic modifiers and complexes to regulate the process of PGC specification.

Pluripotency factors and germ cell induction

In addition to suppression of the somatic program and expression of germ-cell-specific genes, PGCs express pluripotency genes, such as *Pou5f1* (also known as *Oct4*), *Sox2*, *Klf2*, and *Dppa3* (also known as *Stella*). Gene knockout/knockdown studies have revealed that *Dppa3* and *Klf2* are dispensable for PGC development, but *Oct4*, *Nanog*, and *Sox2* are required for PGC development^{35–38}. POU5F1 is expressed throughout the post-implantation epiblast and in PGCs during specification^{39,40}. Its expression continues at high levels in germ cells until sexual differentiation ensues in the developing gonad. Expression of NANOG is seen in the posterior proximal epiblast, the region where PGCs are specified in 6.5 dpc and 7.5 dpc embryos. Short-hairpin-RNA-mediated conditional knockdown of *Nanog* in PGCs results in apoptosis of migrating PGCs and loss of PGCs by 12.5 dpc, indicating that *Nanog* is dispensable for PGC specification but is absolutely essential for PGC maintenance³⁶. SOX2 expression in PGCs is detected at 7.5 dpc embryos onwards. Conditional deletion of *Sox2* in the PGCs shortly after specification at 7.5 dpc leads to significant decrease in germ cell number; by 13.5 dpc germ cells are not detected³⁸. SOX2 regulates expression of *Kit*, the latter of which regulates PGC survival and proliferation, providing a link between *Sox2* function and PGC maintenance³⁸. Recently, it was reported

that *in vitro* overexpression of *Nanog* alone in mEpiLCs, but not *Pou5f1* or *Sox2*, was able to induce germ cell fate by directly activating the *Prdm1* and *Prdm14* enhancers independent of BMP4⁴¹. Loss of *Nanog*, on the other hand, impaired the induction of mPGCLCs by BMP4⁴¹. These studies indicate that NANOG and BMP4 might cooperate to induce germ cell fate *in vivo*.

Epigenetic reprogramming during mouse PGC specification

During mammalian development, epigenetic reprogramming occurs in two phases: the first one occurs after fertilization in the zygote, and the second one occurs during early germline development^{42,43}. Reprogramming during the pre-implantation phase serves to reset the epigenome of the zygote by erasing the epigenetic marks acquired from the gametes (except the marks on the imprinted loci), so as to allow for totipotency to be acquired.

Reprogramming in the early germline serves to erase the parental epigenetic memory, thereby facilitating the process of germ cell development^{42,43}. In this section we discuss the major epigenetic reprogramming events that occur during PGC specification.

Global DNA demethylation in the early germline

Following specification and as they start to migrate and colonize the genital ridges, PGCs undergo extensive genome-wide epigenetic reprogramming, such as DNA demethylation⁴⁴, erasure of paternal imprints⁴⁴, and re-activation of the inactive X chromosome^{45–48}. Global DNA methylation levels drop from 70% in PGC precursors in the posterior epiblast to 4% in 13.5 dpc PGCs^{49,50}. The entire genome, including imprinted regions, is hypomethylated at this stage. In males, sex-specific methylation patterns are re-established immediately after 13.5 dpc; however, re-establishment of methylation patterns occurs after birth in female PGCs⁵¹. The mechanism of global demethylation in the early germline is thought to be a passive process. PRDM1 and PRDM14 directly or indirectly repress the expression of DNMT3A and DNMT3B (enzymes which mediate *de novo* DNA methylation), and UHRF1, a recruitment factor for DNMT1 (an enzyme that mediates maintenance DNA methylation)^{21,22}. Thus, both *de novo* methylation and maintenance of methylation are repressed, leading to passive loss of DNA methylation following rounds of DNA replication as the PGCs proliferate^{49,52–54}.

Recently, it has been shown that loss of DNA methylation in early PGCs could partly occur via active DNA demethylation, especially at imprinted loci that are protected from DNA demethylation, by the time the PGCs settle in the genital ridge at around 9.5–10.5 dpc⁵⁵. The Tet methylcytosine dioxygenases TET1 and TET2 (enzymes responsible for conversion of 5-methylcytosine to 5-hydroxymethylcytosine) are upregulated at 9.5–11.5 dpc, concomitant with increased levels of 5-hydroxymethylcytosine (5hmC) and decreased levels of 5-methylcytosine (5mC) seen in PGCs⁵⁶. Despite these findings, gene knockout studies clearly suggested that global DNA demethylation of the early germline occurs passively, except for the imprinted loci and promoters of genes involved in meiosis during germ cell differentiation^{57–59}.

Reorganization of histone marks during germline specification

In addition to DNA demethylation, PGCs undergo reorganization of chromatin modifications. PRDM1-positive PGC precursors around 6.75 dpc have H3K4me2, H3K4me3, and H3K9ac active marks, along with H3K27me2 and H3K27me3 repressive marks, similar to those found in the neighboring somatic cells⁶⁰. Once PGCs start their migration at 7.75 dpc, PGCs show a reduction in the level of DNA methylation, as well as repressive marks H3K9me1 and H3K9me2 marks^{60,61}. Studies have demonstrated that PRDM1 and PRDM14 directly or indirectly repress *de novo* methylation and maintenance of DNA methylation, and also repress the histone methyltransferase EHMT1, which mediates the methylation of H3K9 (H3K9me1 and H3K9me2) during embryonic development⁶⁰. It has been speculated that both active and passive mechanisms are at play in PGCs that drive this genome-wide epigenetic reprogramming. For example, loss of DNA methylation could occur passively following cell division after 9.0 dpc; another possible contributor could be reduction of H3K9me1/2 due to turnover of the methyl group or replacement of the entire H3 histone protein with an H3.3 variant^{60,62}. In contrast, H3K27me3, another repressive mark, is upregulated as PGCs begin migrating around 8.25 dpc; by 9.5 dpc, nearly all PGCs show high levels of H3K27me3. Components of PRC2 that mediate the trimethylation of H3K27 are expressed at high levels in PGCs and the neighboring somatic cells by 8.25 dpc^{21,60,63}.

It has been reported that 60% of migrating PGCs between 8.0 dpc to 9.0 dpc are in the G2 phase of the cell cycle⁶⁰. PRDM1 might play an important role in this G2 arrest, as PRDM1 is known to suppress expression of S-phase-promoting factors such as *Ccne1*, *Ccnd1*, *Cdc25a*, *Cdc6*, *Pold2*, *E2F3* and *Myc*²¹. PGCs during this period also pause RNA-polymerase-II-mediated transcription⁶⁰. Thus, by repression of active epigenetic enzymes, arresting in the G2 phase of cell cycle, and pausing of RNA-polymerase-II-mediated transcription, PGCs acquire a unique cellular state for epigenetic reprogramming during the specification process⁶².

Additionally, there is upregulation of histone H4 arginine 3 dimethylation (H4R3me2) during PGC specification⁶⁴. This methylation is catalyzed by the arginine methyltransferase PRMT5. PRDM1 forms a complex and co-localizes with PRMT5 in the nuclei of migrating PGCs⁶⁴. The co-localization of PRDM1 and PRMT5 in the PGC nuclei was observed between 8.5–10.5 dpc. Subsequently, the two proteins translocate to the cytoplasm coincident with the downregulation of H4R3me2 in PGCs^{64,65}. The functional significance of the PRDM1/PRMT5 association as a complex in PGC specification is not well-understood and needs further study.

In summary, PGC specification in the developing mouse embryo involves at least three key events: suppression of the somatic program; re-acquisition of pluripotency; and reprogramming to a unique epigenetic state⁶⁵.

***In vitro* derivation of germ cells from pluripotent stem cells**

In vitro derivation of germ cells from pluripotent stem cells has served as a powerful tool to understand the molecular mechanisms (signaling, transcriptional control, and epigenetic regulation) of germline specification not only in mice, but also in humans, which ultimately has implications for restoring reproductive capacity. Hence, there is increasing interest in the area of *in vitro* PGC induction. Initial attempts to reconstitute germ cell specification in culture involved spontaneous differentiation of mouse embryonic stem cells (ECs) to germ-cell-like cells^{66–69}. However, this method was met with little success owing to the very low efficiency of germ cell generation.

Later, Ohinata and colleagues³ isolated mouse epiblast cells and efficiently induced them to become PRDM1-positive and PRDM14-positive PGC-like cells under defined conditions following stimulation by cytokines and BMP4. Furthermore, these cells were able to undergo spermatogenesis following transplantation into neonatal testes of germ-cell-deficient (*W/W^y*) mice³. However, the ability of epiblast cells to form PGC-like cells was shown to be transient. In other studies, a subset of pluripotent stem cells derived from the epiblast, also called epiblast stem cells (EpiSCs), was able to express germ cell markers, but with very low efficiency in culture. In light of these findings, it was speculated that since PGCs are specified from 5.75 dpc to 6.25 dpc, EpiSCs derived from post-implantation embryos would be germline-competent⁷⁰. However, only very few (1.5%) of the post-implantation EpiSCs were able to become primordial germ-cell-like cells (PGCLCs), indicating a germ-cell-incompetent pluripotent state of EpiSCs⁷⁰.

In order to achieve robust PGCLC induction, a germline-competent pluripotent epiblast-like-state was recently established *in vitro*⁷¹. This reconstitution of mouse PGC specification involves a two-step model (Figure 2A). Naive ESCs from the 3.5–4.5 dpc preimplantation embryo (under basal culture conditions consisting of Leukemia inhibitory factor (LIF), Glycogen synthase kinase β inhibitor (GSK3 β i), and inhibitors of extracellular signal-regulated kinases ERK1 and ERK2), are able to give rise to self-renewing ESCs⁷¹. These ESCs are then primed to become epiblast-like cells (EpiLCs) following addition of Activin A and fibroblast growth factor 2 (FGF2) for 2 days. These cells are transcriptionally similar to 5.75 dpc pre-gastrulation epiblast cells. These EpiLCs are germline-competent, like their *in vivo* counterparts, and robustly give rise to PGCLCs following addition of BMP4, BMP8b, LIF, Stem cell factor (SCF, also known as Kit ligand/KITL) and epidermal growth factors (EGF)⁷¹. Day 6 PGCLCs and *in vivo* migratory PGCs have virtually identical transcriptomic and epigenetic profiles⁷¹. Furthermore, it was shown that XY PGCLCs were able to mature into functional sperm following transplantation into the seminiferous tubules of neonatal germ cell deficient mice (*W/W^y*)⁷¹. Intracytoplasmic sperm injection of PGCLC-derived sperm into oocytes yielded healthy fertile offspring, while XX PGCLCs were able to mature into functional oocytes following transplantation into the ovarian bursa of adult mice⁷¹.

In vitro induction of PGCs in humans has been challenging mainly because human pluripotent stem cells (hPSCs) are in a primed pluripotency state rather than a naive pluripotency state like mouse PSCs. Hence, hPSCs are similar to mEpiSCs with little germ

cell competence. Secondly, knowledge regarding human PGC specification is limited due to difficulty and ethical challenges involved in analyzing the early human embryo. Despite these challenges, various groups have attempted to induce human PGCs in culture (Figure 2B). Initial attempts included spontaneous differentiation of human ESCs into PGCLCs, but at very low frequency (5%), indicating low germline competence^{72–75}. Subsequently, several studies have reported the establishment of culture conditions conducive to establishment of naive human ESCs^{76,77}. It was shown that human ESCs grown in a four inhibitor (4i) media (composed of transforming growth factor β 1 (TGF β 1), FGF2, and LIF, along with inhibitors of ERK1/ERK2, GSK3 β and JUN amino-terminal kinase (JNK) and mitogen-activated kinase p38) were able to respond directly to BMP2 and BMP4 and give rise to human PGCLCs with up to 50% efficiency after 4–5 days⁷⁸. These cells have similar transcriptional profiles to human PGCs *in vivo*. Human ESCs grown in 4i media have a more similar transcriptome profile to primed human ESCs than to the naive pluripotent stem cells from the pre-implantation epiblast^{79–81}. The reason for this difference is thought to be upregulation of mesodermal genes such as *T*, *Mix paired-like homeobox (MIXL1)*, *runx related transcription factor 1 (RUNX1)*, and *platelet derived growth factor receptor alpha (PDGFRA)* in hESCs grown in 4i media, possibly resulting in higher germ cell competence⁷⁵. Subsequently, two independent reports demonstrated that cells with early mesodermal character have higher propensity towards human PGC specification^{82,83}. In one of these reports, it was demonstrated that when human induced pluripotent cells (hiPSCs) were differentiated into human incipient mesoderm-like cells (hiMeLCs), they were able to give rise to PRDM1- and TCFAP2C-positive cells with an efficiency of 40% following exposure to BMP2 or BMP4⁸². These hiMeLCs expressed early mesoderm genes like human ESCs grown in 4i culture media. These studies indicate that perhaps human PGCs originate from mesoderm precursors and not from the pre-implantation epiblast as in mice. PGC specification from the mesoderm occurs in other organisms such as crickets and axolotls, indicating that this phenomenon is potentially well-conserved^{84,85}. In mice, mesodermal factors such as T also play an essential role in regulating the expression of important PGC specification factors⁹.

Significance and conclusions

In recent years, extensive research into mouse germline specification has led to the identification of key molecular pathways and transcription factors involved in PGC specification. These discoveries include identification of: the key signaling pathways BMP and WNT; PRDM1 as a key regulator of PGC specification; and the tripartite transcription factor network (formed by PRDM1, PRDM14, and TCFAP2) required for PGC specification. These studies have formed the basis for reconstitution of the germline lineage *in vitro*. Despite recent advances in the field, there are still unanswered questions that need further study. For example, the mechanism by which BMP signaling induces PRDM1 expression in the early PGCs is not clear. Additionally, the precise mechanism by which PRDM1 and PRDM14 suppress the somatic program and how epigenetic reprogramming is triggered following PGC specification require further investigation. An *in vitro* PGC induction model could serve as a valuable tool to answer some of these outstanding questions. Regarding human PGC specification, limited information is available due to

difficulty in procuring human embryos and the associated ethical issues. Thus, knowledge gained from *in vitro* human PGC induction and PGC specification in mice will be essential to understand fully the mechanisms of human PGC specification. Insights gained from research in these fields will have significant implications for understanding reproductive disorders and will help elucidate the origins of germ-cell-related infertility.

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Abbreviations

PGC	primordial germ cell
AP	alkaline phosphatase
dpc	days post coitum
ExE	extraembryonic ectoderm
VE	visceral endoderm
BMP	bone morphogenetic protein
AVE	anterior visceral endoderm
mPGCLC	mouse PGC-like cell
mEpiLC	mouse epiblast-like cell
hPSC	human pluripotent stem cell
EpiSC	epiblast stem cell
PGCLC	primordial germ cell like cell
ESC	embryonic stem cell
hiPSC	human induced pluripotent stem cell
hiMeLC	human incipient mesoderm-like cell

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Figure 1. Embryonic development and PGC specification in mice

At the time of primordial germ cell (PGC) specification (6.5 dpc), BMP4 and BMP8b signaling from the extraembryonic ectoderm, along with BMP2 and WNT3 from the posterior visceral endoderm, induce the formation of PGC precursors (red circles) at the proximal posterior epiblast (the region of maximum BMP and WNT signaling). Antagonistic signals (CER1, LEFTY1, and DKK1) from the anterior visceral endoderm prevent the induction of PGCs in the anterior epiblast. The inset (right) depicts the transcriptional regulation mediated by PRDM1, PRDM14, and TCFAP2C that is essential for suppression of somatic genes within PGCs and for proper PGC specification.

A: Mouse **B: Human** **Figure 2. In vitro reconstitution of mouse and human germ cell lineage**

A) Under basal culture conditions, mouse embryonic stem cells (mESCs) derived from the preimplantation epiblast are able to self-renew and are primed to become mouse epiblast-like cells (mEpiLCs) in the presence of Activin A and fibroblast growth factor 2 (FGF2). These mEpiLCs are germ-cell competent and can give rise to mouse primordial germ-cell-like cells (mPGCLCs) following induction of BMP signaling in culture. These mPGCLCs are able to give rise to functional sperm and eggs and produce healthy offspring following intracytoplasmic sperm injection (ICSI). B) In humans, naive embryonic stem cells (hESCs) derived from the preimplantation epiblast are in a primed pluripotent state with low germ-cell competence. These cells, when grown under 4 inhibitor (4i) culture media (4i hESCs), express early mesodermal genes and are able to give rise to human primordial germ-cell-like cells (hPGCLCs) in the presence of BMP and other cytokines. GSK3 β i, glycogen synthase kinase 3 β inhibitor; ERK1, extracellular signal-regulated kinase 1; ERK2, extracellular signal-regulated kinase 2; ROCKi, Rho-associated protein kinase1 inhibitor.