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Review

Gametogenesis from Pluripotent Stem Cells

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The germ cell lineage originates early in development and undergoes a series of complex developmental processes that culminate in the generation of fully matured gametes, the spermatozoa and the oocytes. Remarkably, researchers have been recapitulating these developmental pathways using mouse and human pluripotent stem cells (PSCs). With further studies, including those involving non-human primate models, human gametogenesis may be fully reconstituted from PSCs, which would profoundly facilitate our understanding of human germ cell development and infertility. Here we discuss groundbreaking studies that lay the foundation for this achievement, the current state of the field, and challenges for deriving gametes from hPSCs.

In multicellular systems, including humans, germ cells are the origin of new individuals and the driving force for genetic diversity and evolution. This remarkable capacity of germ cells has inspired tremendous efforts by many researchers to clarify the mechanisms that underlie germ cell development using a wide range of model organisms. In parallel and primarily in mice and humans, researchers have been working to reconstitute germ cell development in vitro using pluripotent stem cells (PSCs) (i.e., embryonic stem cells [ESCs] [Evans and Kaufman, 1981; Thomson et al., 1998] and induced pluripotent stem cells [iPSCs] [Takahashi et al., 2007; Takahashi and Yamanaka, 2006]) as starting materials. Accordingly, significant progress has recently been made for this research area. Mouse (m)ESCs/iPSCs are induced into primordial germ cell (PGC)-like cells (mPGCLCs), which contribute to spermatogenesis and oogenesis and to healthy offspring (Hayashi et al., 2011, 2012a). Furthermore, several key questions in germ cell biology have been resolved using this in vitro system (Aramaki et al., 2013; Kurimoto et al., 2015; Murakami et al., 2016; Nakaki et al., 2013). More recently, human (h)ESCs/iPSCs have been induced into hPGCLCs using similar methodologies (Irie et al., 2015; Sasaki et al., 2015), heightening the prospect that fully functional human gametes will eventually be induced from PSCs in vitro. If successful, this will have a profound effect on our knowledge of the basic biology of human heredity and development and on reproductive medicine. However, the appropriate use of such cells, including whether we would allow zygotes from hPSC-derived gametes to be used for experimental or medical purposes, should be considered very carefully from both a scientific and ethical viewpoint (briefly discussed below and reviewed in Ishii et al., 2013).

In this review, we will discuss seminal advances in the history of in vitro gametogenesis, the current state of the field, and key future challenges driving this exciting and rapidly evolving research area forward.

Mouse Germ Cell Development: A Mammalian Paradigm

The mouse is widely used as a paradigm for mammalian germ cell development (Figure 1). The mouse germ cell lineage is induced in the most proximal posterior epiblasts at the outset of gastrulation at around embryonic day (E) 6.0 and establishes as a cluster of ~30–40 mPGCs within the extra-embryonic mesoderm (ExM) at around E7.0 (Ginsburg et al., 1990; Saitou et al., 2002). The mPGCs then individually migrate through the hindgut endoderm and mesentery and colonize the embryonic gonadal primordia from around E10.5 (~1,000 in number) (Molyneaux et al., 2001; Seki et al., 2007; Tam and Snow, 1981). In males, mPGCs continue to proliferate in embryonic testes until they enter into mitotic arrest and differentiate into pro-spermatogonia (also referred to as gonocytes) (around E13.5, ~25,000 in number), whereas, in females, mPGCs undergo substantial proliferation as cysts in embryonic ovaries prior to entering the first meiotic prophase to differentiate into oocytes (around E13.5, ~25,000 in number) (Hilscher et al., 1974; Speed, 1982; Tam and Snow, 1981). Epigenetic reprogramming in mPGCs, which includes genome-wide DNA demethylation and histone modification remodeling, erases sex-specific imprints (Lee et al., 2014; Saitou et al., 2012). Consequently, mPGCs in embryonic gonads at around E13.5 acquire paternal and maternal genomes with an essentially identical epigenetic state.

In males, the pro-spermatogonia located within the luminal compartment of seminiferous tubules remain arrested during the fetal period and acquire an androgenic epigenome, including paternal imprints (Kato et al., 2007; Kobayashi et al., 2013; Kubo et al., 2015; Seisenberger et al., 2012). After birth, the pro-spermatogonia translocate to the basal compartment of seminiferous tubules to differentiate into spermatogonia. Around postnatal day (P) 10, the vast majority of spermatogonia proceed into the first wave of spermatogenesis, resulting in the formation of the first population of haploid spermatozoa at around 3 weeks (Bellvé et al., 1977; Yoshida et al., 2006), whereas a small population of spermatogonia generate spermatogonial stem cells (SSCs), which sustain spermatogenesis throughout adulthood (Kanatsu-Shinohara and Shinohara, 2013; Spradling et al., 2011).

In females, the oocytes arrest at the diplotene stage of the first meiotic prophase, and, at around birth, a layer of granulosa cells

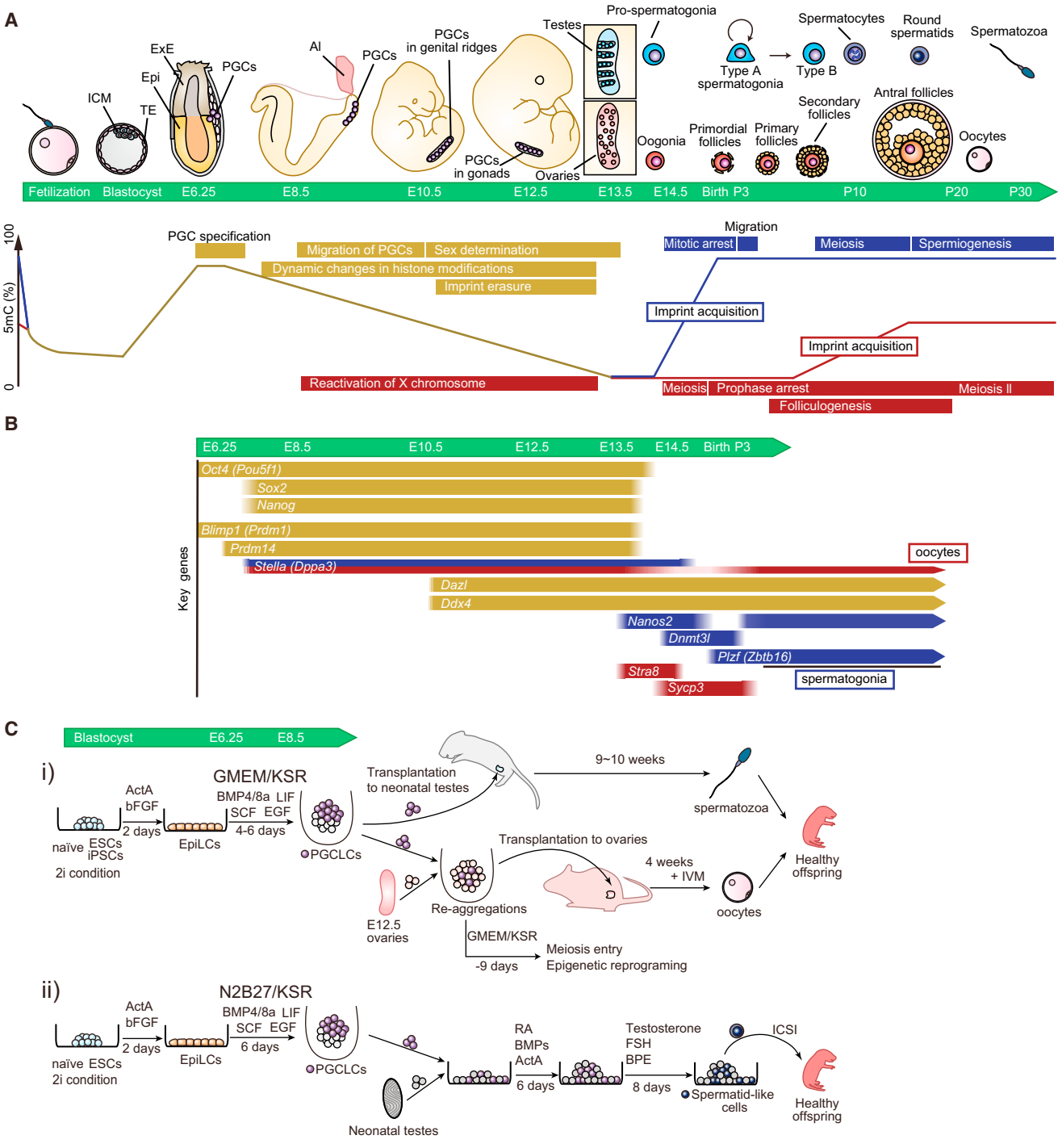


Figure 1. Mouse Germ Cell Development and Its Reconstitution from mPSCs

(A) Top: schematic of the development of the mouse germ cell lineage. ICM, inner cell mass; TE, trophectoderm; Epi, epiblast; Al, allantois. Bottom: key developmental events associated with mouse germ cell development with the dynamics of the 5mC levels.

(B) Expression of key genes during mouse development. Among key regulators for pluripotency, *Pou5f1* shows continuous expression, whereas *Sox2* and *Nanog* are reactivated during PGC specification. *Blimp1* (*Prdm1*) and *Prdm14* are activated upon mPGC specification, whereas *Stella* serves as a key marker for mPGCs and oocytes. *Dazl* and *Ddx4* initiate their expression in mPGCs from around E10.5 and continue to be expressed subsequently. In male pathways, *Nanos2* is a key determinant for differentiation into pro-spermatogonia (Suzuki and Saga, 2008; Tsuda et al., 2003), which express genes such as *Dnmt3l* (Bourc'his et al., 2001), *Plzf* (Buaas et al., 2004; Costoya et al., 2004), and *Gfrα1* (Meng et al., 2000). *Plzf* and *Gfrα1* are markers for spermatogonia. In female pathways, *Stra8* is essential for the initiation of meiosis (Baltus et al., 2006), which accompanies expression of meiotic genes such as *Spo11* and *Sycp3* (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000; Yuan et al., 2000).

(C) Schematic of the methodologies for in vitro gametogenesis from mPSCs as demonstrated by Hayashi et al. (2011, 2012a) (top) and Zhou et al. (2016) (bottom). Top: mESCs/iPSCs in 2i are induced into EpiLCs for 2 days by ActA and bFGF. EpiLCs are then induced into mPGCLCs in floating aggregates by cytokines,

(legend continued on next page)

surrounds the oocytes to form primordial follicles (Edson et al., 2009; McGee and Hsueh, 2000). The follicles develop into primary, secondary, and antral follicles (Edson et al., 2009; McGee and Hsueh, 2000), and, in association with follicle development, the oocytes grow in size and gradually acquire a gynogenetic epigenome, including maternal imprints (Kobayashi et al., 2012; Lucifero et al., 2002). The development into antral follicles from a pool of primordial follicles (the majority of which are maintained in a dormant state after birth) continues thereafter, and, when estrus cycles commence at around 6 weeks after birth in response to hormonal stimulation, fully grown oocytes resume the first meiotic division, extrude first polar bodies, and form secondary oocytes. The secondary oocytes ovulate, are fertilized with spermatozoa, and complete the second meiotic division to form zygotes.

Gametogenesis from PSCs in Mice

Over 10 years ago, several studies began to lay the groundwork for generating gametes from mESCs, but these early efforts lacked the robustness required to determine whether bona fide germ cells/gametes were created. One study reported generating oocyte-like cells in a two-dimensional long-term culture (Hübner et al., 2003), whereas other studies showed the induction of PGCs or round spermatids within embryoid bodies (Geijsen et al., 2004; Toyooka et al., 2003). These approaches employed random differentiation followed by selection of rare cells expressing germ cell markers (~less than 1%). Consequently, the low differentiation efficiency was unsuitable for further mechanistic studies of germ cell development. The PGC- or gamete-like cells also did not contribute to offspring. A subsequent study reported the generation of live but abnormal offspring from gamete-like cells induced from mESCs (Nayernia et al., 2006), but this study has not been reproduced.

Key Advances Leading to the Induction of mPGCLCs from mPSCs

Because gametogenesis involves complex, multi-step processes, successful in vitro gametogenesis requires a precise, step-by-step reconstitution of these events. In turn, such precise reconstitution may serve as a robust foundation for the analysis of gametogenesis mechanisms. Early studies were hindered by the lack of an induction strategy based on sufficient knowledge of germ cell development and an insufficient evaluation of the induced cells. However, key advances in recent years have now enabled precise reconstitution and evaluation of the first step of in vitro gametogenesis: the induction of PGCLCs.

Assays for Germ Cell Function. The claim that the cells induced from PSCs are bona fide germ cells requires functional support, shown most robustly by the generation of offspring derived from the induced cells. Artificial reproductive technologies have been the major driving forces of the progress of reproductive biology, medicine, and many relevant fields (Table 1 and references therein). In addition to well established technologies that involve germ cells at relatively late developmental stages, including terminally differentiated gametes, newer technologies such as germ cell transplantation now allow functional evaluation of germ cells at relatively immature developmental stages (Table 1 and references therein).

The male germ cell transplantation protocol developed by the Brinster group is a powerful technology that assesses the SSC activity within the donor cell population and restores fertility to infertile mice as well as other animals (Brinster and Avarbock, 1994; Brinster and Zimmermann, 1994; Valli et al., 2014). In this assay, cells of interest are transplanted into seminiferous tubules of germ cell-depleted mice (either by busulfan treatment or by genetic mutation of *cKit* [typically, W/W^v mice]), and, consequently, the cells with SSC activity form spermatogenic colonies, providing a basis for constant spermatogenesis. This is a quantitative assay, and although the homing efficiency of cells with SSC activity is difficult to estimate, one can evaluate the relative enrichment of SSC activity within the donor cell population by counting the number of spermatogenesis colonies (Brinster, 2002). Interestingly, the testes of neonatal mice constitute a more hospitable environment than the testes of adult mice for the colonization of cells with SSC activity (Shinohara et al., 2001). Strikingly, newly specified mPGCs can colonize and undergo spermatogenesis in the testes of neonatal mice in a heterochronic fashion, and the resultant spermatozoa contribute to normal offspring (Chuma et al., 2005). The pro-spermatogonia after E14.5, but not mPGCs earlier than E12.5, bear the capacity to colonize adult testes (Ohta et al., 2004). Thus, the male germ cell transplantation assay is a powerful strategy for evaluating the developmental capacity of cells induced from mPSCs.

The spermatogenic capacity of cells of interest, particularly mPGCs, can also be assessed through the creation of a "reconstituted testis" (Matoba and Ogura, 2011; Ohinata et al., 2009). One can purify mPGCs from embryonic testes (typically at E12.5) and re-aggregate them with somatic cells isolated from other embryonic testes (also typically at E12.5) to create reconstituted testes. The reconstituted testes can be transplanted under the adult testicular capsule and can house apparently normal spermatogenesis, with the resultant spermatozoa contributing to offspring (Matoba and Ogura, 2011; Ohinata et al., 2009).

For assessment of the oogenic capacity of the cells of interest, particularly mPGCs, a "reconstituted ovary" can be created in a similar fashion (Hashimoto et al., 1992; Matoba and Ogura, 2011). As with the reconstituted testes, one can purify mPGCs from embryonic ovaries (typically at E12.5) and aggregate them with somatic cells isolated from other embryonic ovaries (also typically at E12.5) to create reconstituted ovaries. The reconstituted ovaries can be transplanted under the ovarian bursa or kidney capsule, where the donor mPGCs develop and mature into fully grown oocytes that contribute to offspring through in vitro maturation (IVM) and in vitro fertilization (IVF) (Hashimoto et al., 1992; Matoba and Ogura, 2011).

In vitro activation (IVA) is also a powerful strategy for examining the activation potential of primordial follicles (Kawamura

including BMP4, for 4–6 days. Male mPGCLCs are sorted by fluorescence-activated cell sorting (FACS) and transplanted into the testes of neonatal W/W^v mice for spermatogenesis. Female mPGCLCs are sorted by FACS and aggregated with somatic cells from embryonic ovaries to form reconstituted ovaries. Reconstituted ovaries are cultured for further germ cell development, including epigenetic reprogramming and progression into meiosis or transplanted under the ovarian bursa for oogenesis. Bottom: male PGCLCs (induced in N2B27-based medium) are cultured with dissociated testicular cells of neonatal W/W^v mice with RA, BMPs, and ActA for 6 days for meiosis initiation, followed by further stimulation with testosterone, FSH, and BPE for 8 days for meiosis completion.

Table 1. Reproductive Technologies for Evaluating Germ Cell Function

Methods	Comments	Key References[a]
Male germ cell transplantation	transplantation of PGCs or spermatogonia into seminiferous tubules; donor germ cells colonize the testes and undergo spermatogenesis	Brinster and Zimmermann, 1994 (Ms); Brinster and Avarbock, 1994 (Ms); Chuma et al., 2005 (Ms); Hermann et al., 2012 (Mn)
Reconstituted testis transplantation	PGCs are re-aggregated with somatic cells of embryonic testes, and the resultant reconstituted testes are transplanted under the testicular capsules of recipient animals, where spermatogenesis takes place, and the resultant spermatozoa contribute to offspring	Ohinata et al., 2009 (Ms); Matoba and Ogura, 2011 (Ms)
Reconstituted ovary transplantation	PGCs are re-aggregated with somatic cells of embryonic ovaries, and the resultant reconstituted ovaries are transplanted under the ovarian bursa or kidney capsules of recipient animals, where oogenesis takes place, and the resultant oocytes contribute to offspring	Hashimoto et al., 1992 (Ms); Matoba and Ogura, 2011 (Ms)
IVA	in vitro activation of dormant follicles (primordial follicles); activated follicles are returned to donors for follicle maturation; the resultant oocytes contribute to offspring	Li et al., 2010 (Ms, Hu); Kawamura et al., 2013 (Ms, Hu)
IVG	in vitro growth of primordial follicles to antral follicles containing oocytes competent for maturation	Eppig and O'Brien, 1996 (Ms)
IVM	maturation of GV (germinal vesicle) oocytes removed from antral follicles to MII oocytes in vitro	Pincus and Enzmann, 1935 (Rb); Edwards, 1965 (Ms, Mn, Hu)
IVF	fertilization of mature oocytes with capacitated spermatozoa in vitro	Chang, 1959 (Rb); Yanagimachi and Chang, 1963 (Ham); Yanagimachi and Chang, 1964 (Ham); Steptoe and Edwards, 1978 (Hu)
ICSI	direct injection of spermatozoa or elongated spermatids into oocytes	Uehara and Yanagimachi, 1976) (Ham); Uehara and Yanagimachi, 1977 (Ham); Kimura and Yanagimachi, 1995a (Ms); Palermo et al., 1992 (Hu)
ROSI (round spermatid injection)	direct injection of round spermatids into oocytes	Ogura et al., 1994 (Ms); Kimura and Yanagimachi, 1995b (Ms); Tesarik and Mendoza, 1996 (Hu); Tesarik et al., 1996 (Hu)

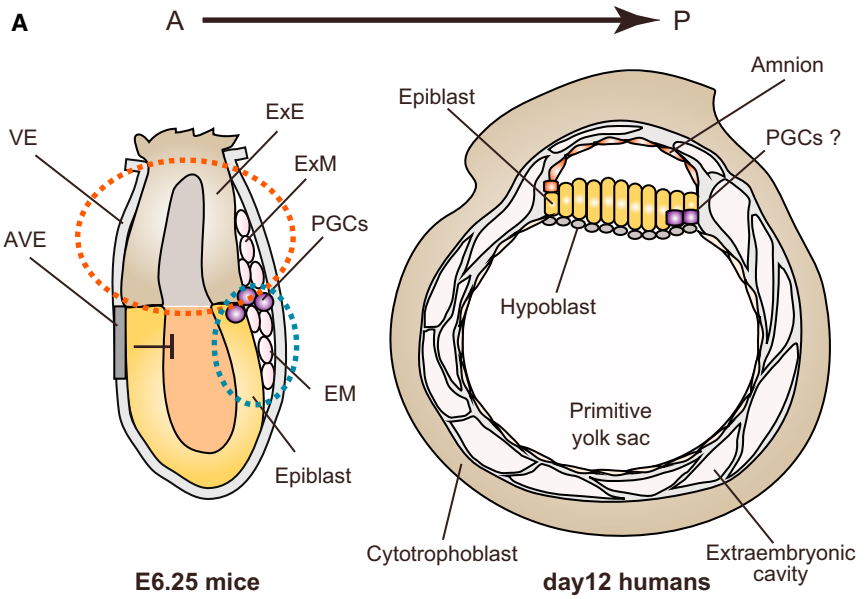
[a]In parentheses, studied species are shown. Ms, Ham, Rb, Mn, and Hu denote mice, hamsters, rabbits, monkeys, and humans, respectively. Edwards (1965) also described data about sheep, cows, and pigs besides mice, monkeys, and humans.

et al., 2013; Li et al., 2010; Suzuki et al., 2015). One can isolate fragments of ovarian cortexes where dormant primordial follicles are enriched, activate the growth of the dormant follicles with an inhibitor for phosphatase and tensin homolog (PTEN) and an activator for phosphatidylinositol 3-kinase (PI3K) in culture, and transplant the ovarian fragments under kidney capsules. After around 3 weeks, the activated primordial follicles develop into antral follicles, and the resultant oocytes contribute to healthy offspring through IVF (Li et al., 2010). Remarkably, this strategy has been applied to human patients with primary ovarian insufficiency (POIs) and has resulted in the birth of healthy babies (Kawamura et al., 2013; Suzuki et al., 2015).

It is also important to note that immature oocytes can be matured into fully grown oocytes by in vitro growth (IVG) (Eppig and O'Brien, 1996; Hirao, 2011). Eppig's group reported the IVG of primordial follicles into antral follicles and succeeded in obtaining offspring using such oocytes (Eppig and O'Brien, 1996). By culturing whole embryonic ovaries at E12.5 and then maturing the resulting secondary follicles, Obata et al. (2002) succeeded in maturing PGCs at E12.5 into oocytes bearing fully programmed nuclei that can support full-term development after nuclear transfer and IVF. Further improvements of these methodologies may realize the generation of fully functional oocytes from PGCs.

Mechanism for PGC Specification. Recent advances in our understanding PGC specification mechanisms have laid the groundwork for the induction of PGCLCs from PSCs (Figures 1 and 2). *Blimp1* (also known as *Prdm1*) and *Prdm14* expression mark cells specified toward the germ cell lineage in the most proximal posterior epiblast cells (Ohinata et al., 2005; Vincent et al., 2005; Yamaji et al., 2008). Their specification into PGCs involves three key events: repression of the somatic mesodermal program, re-acquisition of pluripotent potential, and genome-wide epigenetic reprogramming (Saitou et al., 2002; Seki et al., 2005; Yabuta et al., 2006). *Blimp1* is required for all three events to occur, whereas *Prdm14* is essential for at least the latter two (Kurimoto et al., 2008; Yamaji et al., 2008).

Bone morphogenetic protein (BMP) 4, which is secreted from the extra-embryonic ectoderm (ExE), is essential for conferring germ cell fate to the most proximal posterior epiblast cells (Lawson et al., 1999). A key study subsequently revealed a signaling principle for the induction of mPGCs in the epiblast (Figure 2; Ohinata et al., 2009). In normal development, antagonists of BMP4, such as Cerberus 1 (CER1), which is secreted from the anterior visceral endoderm (AVE), prohibit mPGC specification



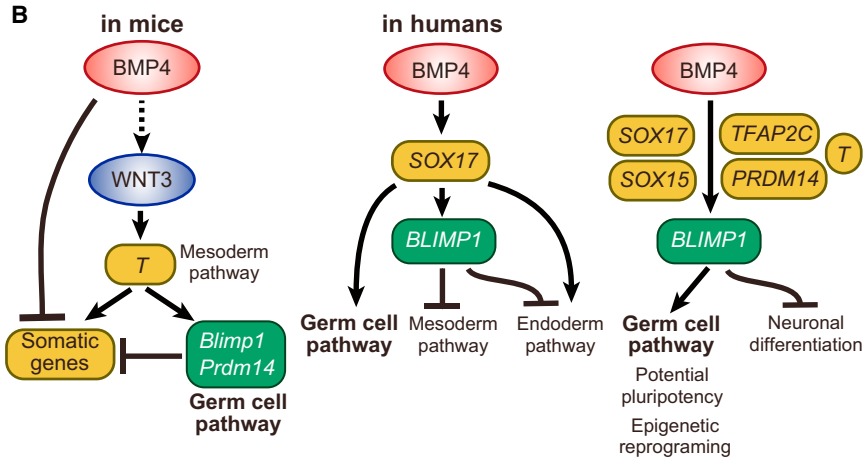


Figure 2. m/hPGC Specification in Early Post-implantation Embryos

(A) Schematics of mouse (left) and human (right) embryos at the stages for germ cell specification. Purple circles represent PGCs. Left: red and blue dotted circles show the BMP signal from ExE and the Wnt signal in the posterior VE and epiblast, respectively. AVE secretes inhibitory signals against PGC specification. Right: a hypothetical hPGC specification. A, anterior; P, posterior; EM, embryonic mesoderm.

(B) Proposed mechanisms for PGC(LC) specification in mice (Aramaki et al., 2013) (left) and humans (Irie et al., 2015; Sasaki et al., 2015) (center and right). In mice, BMP4 directly or indirectly activates WNT3, which activates a mesodermal TF, *T*. *T*, in turn, directly activates *Blimp1* and *Prdm14* (Aramaki et al., 2013) (left). BMP4 signaling appears to repress somatic programs activated by T. In humans, BMP4 initially activates *SOX17*, which is required for subsequent programs for hPGCLC specification, including *BLIMP1* expression. *BLIMP1* is required to repress meso/endoderm pathways (Irie et al., 2015) (center) or to activate the germline transcriptional circuit and repress neuronal differentiation (Sasaki et al., 2015) (right) in hPGCLCs.

of anterior epiblast cells. Consistently, in mutants that are defective in AVE formation, such as *Smad2* knockouts, *Blimp1* expression becomes widespread in epiblast cells. Accordingly, ex vivo culture experiments demonstrated that, when separated from the VE, essentially all epiblast cells from around E5.5–E6.0, but not those after E6.5, are competent for *Blimp1* expression and, subsequently, *Stella*, a marker for established mPGCs (Saitou et al., 2002; Sato et al., 2002), in response to BMP4. WNT3, which is secreted from the epiblast cells themselves partly in response to BMP4, is also essential for the induction of *Blimp1* and *Prdm14*. When cultured as floating aggregates in the presence of BMP4, stem cell factor (SCF), and other cytokines critical for the growth/survival of mPGCs, isolated E6.0 epiblast cells acquire properties of cells resembling mPGCs with regard to gene expression and epigenetic profiles (Ohinata et al., 2009). Remarkably, mPGC-like cells induced from the epiblast contribute to spermatogenesis when transplanted into a testis of a neonatal W/W^v mouse or when used for the generation of reconstituted testes followed by transplantation, and the resultant spermatozoa contribute to healthy offspring (Ohinata et al., 2009). By identifying a signaling principle in mPGC specification, this study established a robust strategy for reconstituting the mPGC specification pathway in vitro.

Induction of mPGCLCs from mPSCs

Ying et al. (2008) have developed a method to culture mESCs under a serum-free, defined condition with inhibitors for the mitogen-activated protein kinase (MAPK) pathway and the glycogen synthase kinase 3 (GSK3) pathway, known as 2i. mESCs in 2i are highly uniform in gene expression for pluripotency and correspond to peri-implantation epiblast cells at E4.5 in gene expression and DNA methylation profiles (Boroviak et al., 2014; Marks et al., 2012), capturing the ground state of naive pluripotency. The elaboration of PSC culture conditions, development of functional assays for germ cells, and advances in understanding of the mechanism for germ cell specification have converged to permit the induction from mPSCs of mPGCLCs with the capacity to contribute to spermatogenesis and oogenesis and generate fertile offspring (Figure 1; Hayashi et al., 2011, 2012a).

The fact that the pre-gastrulating epiblast cells at E5.5–E6.0 specify in culture into PGC-like cells with the capacity for spermatogenesis and for contribution to offspring suggests that, when pre-gastrulating epiblast-like cells differentiate from mPSCs, such cells could serve as a precursor for the induction of mPGCLCs (Ohinata et al., 2009).

Initially, researchers expected epiblast stem cells (EpiSCs) to serve as such a precursor. EpiSCs are mPSCs derived from post-implantation epiblasts at around E5.5 in the presence of Activin A (ActA) and basic fibroblast growth factor (bFGF) (Brons

et al., 2007; Tesar et al., 2007). EpiSCs show distinct morphology, gene expression, and epigenetic properties, and although they form teratomas with cells of three germ layers, unlike mESCs, they are unable to contribute to chimeras. Thus, they have been proposed to represent a primed state of pluripotency (Hackett and Surani, 2014). However, EpiSCs turned out not to be an appropriate precursor for the induction of germ cell fate (Hayashi et al., 2011), most likely because, during their derivation, they acquire properties similar to those of epiblasts of relatively late developmental stages (Kojima et al., 2014), which have little, if any, competence for germ cell fate (Ohinata et al., 2009).

Accordingly, mESCs, when cultured in 2i and stimulated by ActA and bFGF over a short period, transiently resemble pre-gastrulating epiblast-like cells (EpiLCs) with a robust competence for germ cell fate (Hayashi et al., 2011). Reflecting the narrow time window over which epiblast cells can form PGC-like cells, EpiLCs induced for 2 days, but not 1 or 3 days, efficiently differentiate into mPGCLCs expressing *Blimp1*, *Prdm14*, and *Stella* with a procedure essentially identical to that used for the induction of PGC-like cells from the epiblast. The day 2 EpiLCs show a transcriptome very similar to that of the E5.75 epiblast. Moreover, the induction of mPGCLCs from day 2 EpiLCs involves the three key events associated with mPGC specification: reacquisition of pluripotency gene expression, such as *Sox2* and *Nanog*; transient upregulation of somatic mesodermal genes, such as *T* (*Brachyury*), *Hoxa1*, and *Hoxb1*; and downregulation of key epigenetic modifiers such as *Dnmt3a*, *Dnmt3b*, *Uhrf1*, and *Ehmt1*. Consequently, day 6 mPGCLCs exhibit a transcriptome and epigenetic properties highly similar to those of E9.5 mPGCs at a migrating stage. It is important to note that mPGCLCs do not upregulate genes such as *Dazl* (deleted in azoospermia-like) (Cooke et al., 1996) and *Ddx4* (Fujiwara et al., 1994), which are highly upregulated in mPGCs colonized in embryonic gonads (Hayashi et al., 2011; Kurimoto et al., 2015).

The function of mPGCLCs was first demonstrated in males. When transplanted into the neonatal testes of W/W^{v} mice, mPGCLCs induced from mESCs as well as miPSCs contributed to spermatogenesis, and the isolated spermatozoa from the testes, through intracytoplasmic sperm injection (ICSI) and transfer to foster mothers, contributed to fertile offspring (Figure 1C; Hayashi et al., 2011).

Hayashi et al. (2012a) subsequently validated the function of mPGCLCs as precursors for oocytes. They induced female (XX) mESCs into mPGCLCs, which were aggregated with somatic cells of embryonic ovaries to form reconstituted ovaries. Within the reconstituted ovary cultures, mPGCLCs upregulated genes such as *Ddx4* and, at around days 4–6 of aggregation culture, acquired gene expression similar to that of E12.5 female mPGCs, erased imprinting, and reactivated the inactive X chromosome. Upon further aggregation culture, mPGCLCs progressed into the prophase of the first meiotic division. Thus, mPGCLCs are fully competent to undergo oogenic development in vitro when cultured with embryonic ovarian somatic cells. When the reconstituted ovaries were transplanted under the ovarian bursa of nude mice for 4 weeks, mPGCLCs matured into fully grown oocytes that, through IVM and IVF, completed meiosis, formed zygotes, and contributed to fertile offspring.

Thus, the mPGCLC specification pathway reconstitutes a key developmental transition that originates from the peri-implantation epiblast at around E4.5 (mESCs/miPSCs), goes through the pre-gastrulating epiblast at around E5.5–E5.75 (EpiLCs), and reaches migrating mPGCs at E9.5 (day 6 PGCLCs). With the use of reconstituted ovaries, the pathway extends further, at least to the oogonia at the prophase of the first meiosis at around E15.5 (Figure 1C; Hayashi et al., 2011, 2012a).

The mPGCLC Specification Pathway as an Experimental System

A number of key findings have been made using the mPGCLC induction system, which provides abundant experimental material that is unattainable from isolating mPGCs in vivo. Overexpressing key transcription factors (TFs) in EpiLCs, such as BLIMP1, PRDM14, and TFAP2C, is sufficient for inducing a PGC-like state. The so-called TF-PGCLCs display a robust capacity for spermatogenesis and contribute to offspring (Nakaki et al., 2013). Importantly, TFs induced a germ cell fate when overexpressed in EpiLCs but not in ESCs, indicating the importance of cellular context (e.g., epigenetic properties) for TF-PGCLC induction. Interestingly, PRDM14 alone was also sufficient, although less effectively than the three TFs, in inducing TF-PGCLCs, indicating that PRDM14 activates *Blimp1* and *Tfap2c* and that the three TFs function synergistically for TF-PGCLC induction (Nakaki et al., 2013). More recently, overexpression of NANOG alone in EpiLCs was shown to be sufficient for the induction of a PGC-like state (Murakami et al., 2016), indicating an unexpected transcriptional circuitry underlying a PGC phenotype. The consequences of overexpressing the three TFs have also been assessed in a teratocarcinoma model (Magnúsdóttir et al., 2013).

Notably, the TF-PGCLC induction bypasses transient activation of a somatic mesodermal program associated with mPGC specification, indicating the dispensability of such a program for the function of mPGC(LC)s (Nakaki et al., 2013). Interestingly, however, a study exploring the mechanism of action of BMP4 and WNT3 revealed that T (BRACHYURY), a mesodermal factor transiently upregulated upon mPGC specification, is essential for mPGC specification or mPGCLC induction by cytokines (Aramaki et al., 2013). Accordingly, T is a direct target of WNT3 signaling and, in turn, directly upregulates *Blimp1* and *Prdm14* as well as the mesodermal program by recruiting histone H3 lysine 27 acetylation (H3K27ac) for mPGC(LC) specification (Aramaki et al., 2013; Kurimoto et al., 2015). The involvement of mesodermal factor(s) for PGC specification appears to be a widely conserved mechanism among metazoans (Ewen-Campen et al., 2013; Extavour and Akam, 2003; Nieuwkoop, 1947).

The mPGCLC specification pathway was also exploited for the delineation of chromatin remodeling during mPGC specification (Kurimoto et al., 2015). The mPGCLC induction system will continue to serve as a platform by which to explore transcriptional, translational, and epigenetic regulation during mPGC development.

Induction from mPGCLCs to More Mature Germ Cells and Gametes

Induction to More Mature PGCs. The induction of mPGCLCs from mESCs is a transient process, and the resulting mPGCLCs can be maintained for no more than 10 days (Hayashi et al.,

2011). The long-term proliferation of mPGCLCs/mPGCs in culture is therefore a key unmet challenge and would open many possibilities in reproductive science. Mechanisms that support the proliferation of mPGCs should exist in vivo, especially between E9.5 and E12.5, because their numbers increase 100-fold during this period (Tam and Snow, 1981). A better understanding of this mechanism and the cytokines that stimulate mPGC survival/proliferation is needed to advance these efforts.

As discussed earlier, day 6 mPGCLCs show little, if any, expression of the so-called "germline genes" (Borgel et al., 2010; Weber et al., 2007), such as *Dazl*, *Ddx4*, *Mael*, and *Piwil2*, that are induced upon mPGC colonization of embryonic gonads and are critical for meiosis initiation in females and transposon repression in males (Figure 1). The germline genes are repressed in mPGCLCs by histone H3 lysine 27 tri-methylation (H3K27me3) (Kurimoto et al., 2015). Interestingly, in a C57BL/6 background, mPGCs require *Dazl* expression as a "license" to progress into meiosis or into pro-spermatogonia differentiation (Gill et al., 2011; Lin et al., 2008). As described earlier, aggregation with the somatic cells of embryonic gonads, especially with those of embryonic ovaries, promotes maturation of mPGCLCs into a state similar to E12.5 mPGCs with germline gene expression and then to meiotic oocytes (Hayashi et al., 2012a). Thus, the ability to maintain long-term proliferation of mPGCLCs/mPGCs in culture should provide a key opportunity to identify signals, based on the knowledge of the mechanism for sex determination of germ cells (Lin and Capel, 2015), that induce mPGCLCs into either spermatogenic or oogenic pathways without gonadal somatic cells. Key markers for spermatogenic pathways include *Nanos2*, a translational regulator essential for pro-spermatogonia differentiation (Suzuki and Saga, 2008; Tsuda et al., 2003), and *Plzf*, a TF critical for the maintenance of SSCs (Buaas et al., 2004; Costoya et al., 2004), whereas key markers for oogenic pathways include *Stra8*, a critical regulator for the initiation of meiosis (Baltus et al., 2006), and key genes for meiotic recombination, such as *Spo11* and *Sycp3* (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000; Yuan et al., 2000; Figure 1).

The Male Pathway. For reconstitution of the male pathway, a critical next step will be inducing a population of cells with SSC activity from mPGCLCs. Currently, SSC activity can only be defined by the transplantation assay (Table 1; Brinster, 2002), and there has been no single marker or combination of markers that prospectively define a "pure" SSC population, the identity of which has thus been a subject of intensive debate (Kanatsu-Shinohara and Shinohara, 2013; Kumar, 2014). Interestingly, recent live imaging analyses have shown that a population of spermatogonia positive for GFRα1, a receptor for glial cell-derived neurotrophic factor (GDNF), may collectively function as SSCs under physiological conditions (Hara et al., 2014). On the other hand, primary cell lines with robust SSC activity upon transplantation have been derived from neonatal or adult testes and designated as germline stem cells (GSCs) (Kanatsu-Shinohara et al., 2003; Kubota et al., 2004). GSCs exhibit exponential growth in culture in response to GDNF (Meng et al., 2000) and, remarkably, maintain their SSC activity over more than 2 years of culture (Kanatsu-Shinohara et al., 2005). Thus, the induction of a GSC-like state from mPGCLCs will be an important challenge.

Notably, spermatogenesis can be completed and maintained long-term (up to 6 months) in an ex vivo culture of neonatal mouse testes that initially only contain spermatogonia, and the resultant spermatozoa contribute to fertile offspring (Komeya et al., 2016; Sato et al., 2011a). Importantly, GSCs undergo spermatogenesis in the transplanted testes of neonatal W/W^v mice cultured under the same ex vivo conditions (Sato et al., 2011b). Thus, upon induction of a GSC-like state from mPGCLCs, such cells could be used for spermatogenesis ex vivo, opening a route to complete spermatogenesis from mPSCs under in vitro/ex vivo conditions. The possibility of direct in vitro induction of GSCs/a GSC-like state into meiosis by key factors for inducing meiosis, including retinoic acid (RA) (Anderson et al., 2008; Bowles et al., 2006; Koubova et al., 2006), should also be explored.

Recently, it was reported that, when co-cultured with dissociated cells from testes of neonatal W/W^v mice in the presence of RA, BMP2/4/7, and ActA, day 6 mPGCLCs enter into the first prophase of meiosis during the first 6 days, and when the co-culture medium was supplemented with follicle-stimulating hormone (FSH), bovine pituitary extract (BPE), and testosterone from day 6 of co-culture onward, the day 6 mPGCLC-derived cells completed meiosis over the subsequent 8 days, resulting in the generation of spermatid-like cells that, upon ICSI, contributed to fertile offspring (Zhou et al., 2016). This is a surprising result considering that the reported procedure entirely skips a period of more than 2 weeks of development—i.e., the period from mPGCs at around E9.5 ($\sim$day 6 mPGCLCs) to spermatogonia, initiating the first wave of spermatogenesis at around P10 (Figure 1; Hayashi et al., 2011; Bellvé et al., 1977). It is important to note that, during this period, male germ cells undergo epigenetic reprogramming, which would be critical for acquiring a proper androgenetic epigenome (Kato et al., 2007; Kobayashi et al., 2013; Kubo et al., 2015; Seisenberger et al., 2012). Hence, the validity of this study should be examined strictly by other laboratories.

The Female Pathway. As with the reconstitution of the female pathway, improvements of culture conditions for reconstituted ovaries (Hayashi et al., 2012a; Obata et al., 2002) and of IVG (Eppig and O'Brien, 1996; Hirao, 2011) may lead to the generation of oocytes that bear the capacity to complete meiosis and contribute to offspring. The optimization of these conditions could serve as a powerful basis for exploring key mechanisms underlying oogenesis. Considering crucial and complex roles of ovarian somatic cells (granulosa and theca cells) in the maturation of oocytes (Eppig and O'Brien, 1996; Hirao, 2011; Matzuk et al., 2002), the generation of fully functional oocytes from PSCs without the use of ovarian somatic cells would require further extensive investigation of the mechanism by which ovarian somatic cells support oogenesis.

Human Germ Cell Development

The investigation into the development of the human germ cell lineage dates back more than a century ago, when Fuss and Felix independently identified a group of cells with a unique morphology as hPGCs in week 4 embryos (Felix, 1911; Fuss, 1911, 1912; De Felici, 2013; Figure 3). Subsequently, by analyzing a substantial number of embryos, Politzer (1930, 1933) and Witshi (1948) defined a migratory pathway of hPGCs from the yolk sac endoderm through the hindgut endoderm

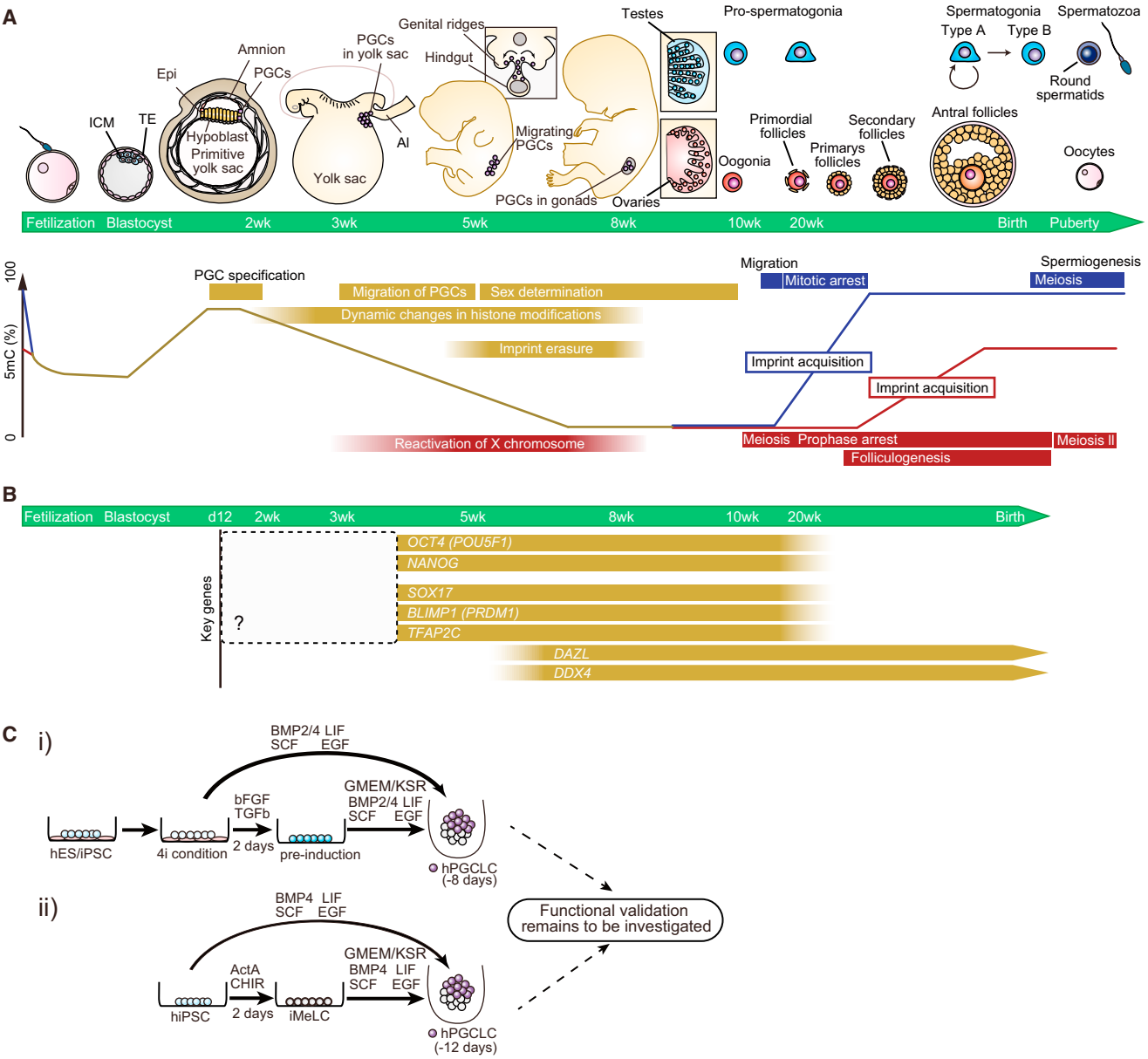


Figure 3. Human Germ Cell Development and Its Reconstitution from hPSCs

(A) Top: schematic of the development of the mouse germ cell lineage. Bottom: key developmental events associated with human germ cell development with the dynamics of the 5mC levels.

(B) Expression of key genes. Expression during the period in a dotted square remains to be determined. A key difference from mice is the repression of *SOX2* and the expression of *SOX17*.

(C) Schematic of the induction of hPGCLCs from hPSCs as demonstrated by Irie et al. (2015) (i) and Sasaki et al. (2015) (ii). (i) hESCs/iPSCs in 4i (inhibitors for MAPK, GSK3, p38, and JNK) on feeders are pre-induced by tumor necrosis factor β (TGF-β) and bFGF for 2 days, and the pre-induced cells are then induced into hPGCLCs by the same method as for mPGCLC induction. hESCs/iPSCs in 4i are also directly induced into hPGCLCs. (ii) Feeder-free hiPSCs are induced into iMeLCs by ActA and CHIR99021 (CHIR) for 2 days. iMeLCs are then induced into hPGCLCs by the same method as for mPGCLC induction.

and dorsal mesentery to the genital ridges (Figure 3). The migratory pathway of hPGCs is thus analogous to that of mPGCs.

In embryonic testes, which begin to differentiate from around weeks 5–6 with the expression of *SRY* and *SOX9* (Hanley et al., 2000), hPGCs continue to express key pluripotency markers such as POU5F1, NANOG, and SSEA1 and to proliferate, at least during the first trimester (weeks 10–12) (Culty, 2009). Thereafter, during the second trimester, most, but not all, hPGCs appear to enter progressively into mitotic arrest and initiate differentiation into pro-spermatogonia (Culty, 2009). Some of the pro-spermatogonia translocate to the basal compartment of seminiferous tubules and differentiate into spermatogonia during the embryonic period (Culty, 2009). In embryonic ovaries, which begin to differentiate from around weeks 6–8 with the expression of *FOXL2* and *RSPO1* (Duffin et al., 2009; Tomaselli et al., 2011), hPGCs continue to proliferate at least until

weeks 10–11, when they begin to enter into meiosis to differentiate into oocytes (Kurilo, 1981). The proliferation of hPGCs appears to continue at least until week 20 (Kurilo, 1981), and, therefore, mitotically active hPGCs and oocytes in the first prophase of meiosis co-exist for a relatively long period of time in embryonic ovaries. Notably, folliculogenesis progresses during the embryonic period, and mature follicles are occasionally formed before birth (Kurilo, 1981). Thus, the overall developmental dynamics of human germ cells in the embryonic period are similar to those of mouse germ cells, but human germ cells appear to show greater heterogeneity/asynchronicity in their developmental timing and require a much longer time for their developmental transitions. Moreover, during their relatively long gestational period, human germ cells undergo some of the events that occur only after birth in mice. The number of germ cells is around ~100 at week 3, which increases to around ~1,000 at week 4 and ~150,000 in males and ~450,000 in females at week 9 (De Felici, 2013; Mamsen et al., 2011; Politzer, 1930, 1933; Witschi, 1948). Clearly, further studies are warranted regarding the development of human/non-human primate embryonic germ cells.

Remarkably, recent studies have investigated epigenetic reprogramming, particularly genome-wide DNA demethylation dynamics, in human germ cells (Gkountela et al., 2013, 2015; Guo et al., 2015; Tang et al., 2015). Although the extent and the timing of DNA demethylation reported by these studies are somewhat different, the general consensus is that hPGCs undergo epigenetic reprogramming that is similar overall to that seen in mPGCs (Figure 3). Accordingly, hPGCs exhibit substantial demethylation as early as week 5 around their colonization of embryonic gonads (genome-wide 5-methylcytosine [5mC] level, ~20%) and undergo further demethylation thereafter (genome-wide 5mC level, ~5% at week 9), and, as a consequence, hPGCs exhibit much lower genome-wide 5mC levels than inner cell mass cells of the blastocysts (Smith et al., 2012). In parallel, the erasure of parental imprints and X reactivation in females occurs with some human-specific dynamics. There are also some regions that evade genome-wide DNA demethylation, and these DNA demethylation "escapees" may contribute to the transgenerational epigenetic inheritance. Consistent with the findings in mice (Kagiwada et al., 2013; Seisenberger et al., 2012; Seki et al., 2005), hPGCs/gonadal germ cells display low levels of histone H3 lysine 9 di-methylation (H3K9me2) and histone H3 lysine 9 tri-methylation (H3K9me3) as well as of DNMT3A/3B and UHRF1, suggesting that the mechanism for epigenetic reprogramming in human and mouse germ cells is conserved.

On the other hand, to date, the origin of hPGCs and the mechanism for hPGC specification has been unknown because of difficulties in analyzing earlier embryos (week 2). Human early post-implantation embryos bear a layer of disc-shaped epiblasts that, on the dorsal side, are continuous with the amnion and, on the ventral side, are underlined by a layer of hypoblasts that contribute to the secondary yolk sac (Figure 2). Additionally, they bear an extra-embryonic mesenchyme that is presumably a hypoblast derivative (Enders and King, 1988; Luckett, 1978) and lies between the trophectoderm (cytotrophoblasts and syncytiotrophoblasts) and the amnion (Figure 2). Thus, the epiblasts are physically separated by the amnion and the extra-embryonic mesenchyme from the trophectoderm, whose counterpart in mice acts as a critical signaling center for mPGC specification (Figure 2). hPGC specification should therefore involve a mechanism distinct from mPGC specification. Studies using appropriate primate models will provide critical insights into the mechanism for hPGC specification, which in turn will serve as a basis for establishing/validating the strategy for induction of the human germ cell lineage from hPSCs. It is also important to point out that, in rabbit early post-implantation embryos with disc-shaped epiblasts, rPGCs marked by *Blimp1* have been identified in the posterior epiblasts, BMP2 is expressed in hypoblasts, and BMP4 is expressed within posterior epiblasts (Hopf et al., 2011).

Gametogenesis from PSCs in Humans

A number of studies have reported the induction of human germ cells from hESCs/hiPSCs (Chen et al., 2007; Clark et al., 2004; Park et al., 2009; Tilgner et al., 2008, 2010; West et al., 2010). The strategies include random differentiation in embryoid body or two-dimensional culture, differentiation in the presence of BMPs, and differentiation on feeder cells prepared from embryonic testes, all followed by identification of cells marked by *DDX4*, a gene expressed in hPGCs colonized in embryonic gonads. However, these studies bore several weaknesses. The induction was often inefficient, the induced cells were poorly characterized (validation of the *DDX4* reporter was insufficient), and it was difficult or impossible to monitor the process of hPGC specification.

More recent work has shown that overexpression of *DAZ* family genes or *DAZL* and *DDX4*, all of which are translational regulators, in hESCs/hiPSCs promotes entry into meiosis and, with an efficiency of ~2%, generates haploid cells within 2 weeks in differentiation culture (Kee et al., 2009; Medrano et al., 2012; Panula et al., 2011). Other studies have also reported induction of meiosis and haploid cells by spontaneous differentiation of hiPSCs for 3 weeks, followed by further differentiation with RA for another 3 weeks (Eguizabal et al., 2011), or by culturing hESCs under GSC culture conditions (Easley et al., 2012). The induction of meiosis and haploid cells from hPSCs is a remarkable achievement. However, these studies also suffer from an inefficiency of induction and insufficient characterization of the induced cells. Overall, more precise characterizations, including careful comparison with the processes in vivo, of each induction step would be necessary for these studies.

Induction from hPSCs of hPGCLCs and Beyond

hPGCLCs. The pathway for the induction of mPGCLCs and more mature germ cells from mPSCs serves as a paradigm for inducing a human germ cell fate from hPSCs. However, there are some properties of hPSCs that differ from mPSCs that must be considered to successfully apply the principles of mPGCLC induction to a human system. hPSCs cultured under conventional conditions bear characteristics similar to EpiSCs, but not to mESCs, with regard to gene expression, epigenetic properties, and cytokine dependence, representing a primed pluripotency of post-gastrulating epiblasts with biased differentiation potential with little, if any, capacity to contribute to chimeras (Rossant, 2015). Considering that EpiSCs essentially lack competence for induction into a germ cell fate (Hayashi et al., 2011), hPSCs may also be devoid of this capacity. A number

of attempts have been made to identify the conditions for converting a primed state of human or primate PSCs into an mPSC-like naive state (Chan et al., 2013; Chen et al., 2015; Fang et al., 2014; Gafni et al., 2013; Takashima et al., 2014; Theunissen et al., 2014; Ware et al., 2014), but, so far, the results have been somewhat controversial and rather have suggested inherent species differences between humans and mice (Rossant, 2015). However, hPSCs do show differences from EpiSCs, and the early studies described above point to a potential competence of hPSCs to acquire the germ cell fate. A precise comparison of the properties of human or primate PSCs with those of epiblast cells in an appropriate primate model will provide critical insights into the competence of hPSCs.

Recently, Irie et al. (2015) showed that hESCs/hiPSCs cultured with inhibitors for four kinases (MAPK, GSK3, p38, and c-Jun N-terminal kinase [JNK]), which have been used to culture cells in a naive state of human pluripotency (Gafni et al., 2013), are efficiently (up to ~50%) induced into hPGCLCs using a procedure identical to that for mPGCLC induction, whereas hPSCs cultured under conventional conditions are unable to form hPGCLCs (Figure 2). The hPGCLCs induced by Irie et al. (2015) exhibit gene expression similar to week 7 hPGCs but, as with the case for mPGCLCs, are negative for genes such as *DDX4* and *DAZL*, suggesting that the hPGCLCs correspond to hPGCs at an early stage. Remarkably, these authors found that SOX17 acts upstream of BLIMP1 and is essential for hPGCLC specification. According to their model, SOX17 promotes the expression of genes for hPGC and endoderm development, whereas BLIMP1 promotes the expression of genes for hPGC development and represses genes for mesoderm and endoderm development.

On the other hand, Sasaki et al. (2015) demonstrated that hiPSCs cultured under a feeder-free, defined condition that are in a primed state of pluripotency first differentiate into incipient mesoderm-like cells (iMeLCs) and then into hPGCLCs (up to ~60%) using the identical procedure as that used for mPGCLC induction (Figure 2). The authors showed evidence that hiPSCs bear intermediate properties between EpiLCs and EpiSCs. The hPGCLCs induced by Sasaki et al. (2015) exhibit gene expression similar to gonadal PGCs of cynomolgus monkeys as well as to week 7 hPGCs and to the hPGCLCs induced by Irie et al. (2015) and are negative for *DDX4* and *DAZL*. The authors found that BLIMP1, which represses somatic programs in mice, activates and stabilizes a germline transcriptional circuit and represses a ''neuronal differentiation'' program.

The demonstration that hPGCLCs truly represent an in vitro counterpart of hPGCs will require further experiments, including a demonstration of the competence of hPGCLCs for epigenetic reprogramming, meiosis, and maturation into spermatogonia or oocytes. Nonetheless, these two studies serve as a foundation for the step-by-step reconstitution of human germ cell development in vitro and for exploring the key mechanisms in hPGC specification. In contrast, the cells induced by Sugawa et al. (2015) showed substantial differences in gene expression from the hPGCLCs induced by Irie et al. (2015) and Sasaki et al. (2015) and thus would likely not be hPGCLCs. Upon further investigation, Irie et al. (2015) noted that the 4i hPSC expression profile shares many genes associated with mesoderm development and is enriched for ''pattern specification process,'' ''muscle organ development,'' and ''regulation of cell migration.'' Notably, genes upregulated in iMeLCs relative to their levels in the hiPSCs induced by Sasaki et al. (2015) are also enriched with those for cell migration and pattern specification process. We speculate that a peri-gastrulating, mesoderm-like state might serve as an efficient precursor for the human germ cell lineage.

SOX17 has previously been considered a key factor in endoderm development both in mice and humans (Kanai-Azuma et al., 2002; Séguin et al., 2008; Yasunaga et al., 2005), and it is therefore surprising that SOX17 was identified as one of the most upstream TFs for hPGCLC specification. *Sox17* exhibits transient upregulation upon mPGC specification but wanes as early as E7.5 and would not have a role in mPGCs (Kurimoto et al., 2008). In this regard, it is important to note that SOX2 is downregulated, most likely by BMP4 signaling but not by BLIMP1 (Sasaki et al., 2015; Lin et al., 2014), and appears to be dispensable in hPGCs (de Jong et al., 2008; Perrett et al., 2008) and upon hPGCLC specification (Irie et al., 2015; Sasaki et al., 2015). SOX2 is a core TF for pluripotency in mice and humans (Avilion et al., 2003; Boyer et al., 2005); its expression is regained during mPGC specification (Kurimoto et al., 2008; Yamaji et al., 2008). SOX2 is also essential for mouse germ cell development (Campolo et al., 2013). Thus, redundancy of SOX2 in human germ cells and recruitment of SOX17 as a key factor for hPGC specification is an eminent example of the mechanistic divergence between mice and humans.

Compared with mPGC(LC) specification (Hayashi et al., 2011; Kurimoto et al., 2008), hPGCLC (and, presumably, hPGC) specification is accompanied by a much milder upregulation of the somatic mesodermal program and instead involves downregulation of a program for ''neuron development,'' which may be an intrinsic property associated with hPSCs (Sasaki et al., 2015). Accordingly, although BLIMP1 plays an essential role in repressing the somatic mesodermal program in mPGCs (Kurimoto et al., 2008), BLIMP1 is also critical for repressing neuron development in hPGCLCs (Sasaki et al., 2015). Such divergence between mice and humans would perhaps reflect a divergence of the developmental program of post-implantation epiblast cells. It would therefore be an important challenge to study such a process in non-human primate models.

Beyond hPGCLCs. The induction of more mature germ cells from hPGCLCs is an open field and poses a key challenge. hPGCLCs are negative for genes such as *DDX4*, *DAZL*, and *PIWIL2* and are in a state prior to extensive epigenetic reprogramming, corresponding to early hPGCs just after specification (Irie et al., 2015; Sasaki et al., 2015). As has been demonstrated for mPGCLCs, aggregation culture with somatic cells of embryonic gonads (reconstituted testis or ovary) would be an obvious possibility, although aggregation with mouse cells may not work because of species differences, and access to embryonic gonads of humans or non-human primates is limited. It would therefore be important to identify key cytokines that promote the maturation of hPGCLCs. It would also be an important option to explore the induction of gonadal somatic cells (i.e., Sertoli cells or granulosa cells) from PSCs or by TF-mediated trans-differentiation (Buganim et al., 2012). Considering the paucity of knowledge regarding the origin of and mechanism for the development of gonadal somatic cells (Lin and Capel, 2015), such

studies will require careful investigation using the mouse and appropriate primates as model organisms. The establishment of a method for long-term culture of hSSCs (Valli et al., 2014) and for IVG of human immature follicles should also be key priorities.

Potential Applications and Ethical Considerations

Successful in vitro gametogenesis from hPSCs should provide highly valuable information on all aspects of human germ cell development that are difficult to analyze, including the mechanisms for transcriptional control, epigenetic reprogramming, meiosis, and genome stability, which have already been partially clarified using the mouse system and hPGCLC induction. Such knowledge, in turn, should be instructive in discerning the causes and consequences of diseases arising from anomalies in germ cell development, including infertility, impaired development/physiology, and a diverse array of genetic/epigenetic disorders within offspring. A system for in vitro gametogenesis should also serve as a platform to identify potential drugs for such diseases as well as to screen chemicals for reproductive toxicity (Hayashi et al., 2012b).

In addition to such applications, one might consider using the gametes generated from hPSCs to treat infertility. In this regard, a critical technological concern would be the genetic mutations that would be expected to accumulate in the somatic cells from which hiPSCs are derived. It has been shown that the germ cell lineage exhibits a lower mutation rate than somatic lineages (Murphey et al., 2013). SSCs appear to bear a robust system for minimizing genetic mutations, whereas female germ cells cease mitotic divisions that accumulate such mutations early in their development (Rahbari et al., 2016), and this might contribute to a reduction in the potential transmission of deleterious mutations. In considering the use of such cells, therefore, it will be essential to carefully scrutinize the genome sequences of the hiPSCs that could be used as starting material as well as of the resultant gametes. The epigenetic profiles of the resultant gametes or embryos should also be carefully monitored to prevent the transmission of any deleterious epimutations. These two technological requirements should at least be strictly met when considering the use of artificial gametes. Moreover, even when all the technological concerns are resolved, the use of artificial gametes presents huge ethical and sociological concerns that have been discussed in detail elsewhere (Ishii et al., 2013) and should also be widely discussed at the level of the individual and society (Pearson, 2008). The rapidly evolving genome-editing technologies may also be applied to hiPSCs and gametes derived from them, but the use of such cells should also require careful discussion (Callaway, 2016).

Conclusions

Recent years have seen rapid progress in gametogenesis from PSCs in vitro, particularly with the mouse system. This progress is largely attributable to the significant accumulation of knowledge on the mechanisms of germ cell development, the development of many lines of reproductive technologies, and the elaboration of the culture protocols for PSCs in this model organism. The translation of this technology to humans has been underway and requires a better understanding of the mechanisms for human germ cell development, which would be complemented through analysis of germ cell development in non-human primate models. In turn, the successful in vitro reconstitution of human germ cell development would provide salient information regarding the mechanism of human germ cell development and the disease states arising from its anomalies. Moreover, should PSCs be successfully derived and information on germ cell development collected, the technology might be extended to many other mammalian species, which could have a major effect on the preservation of species diversity.

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