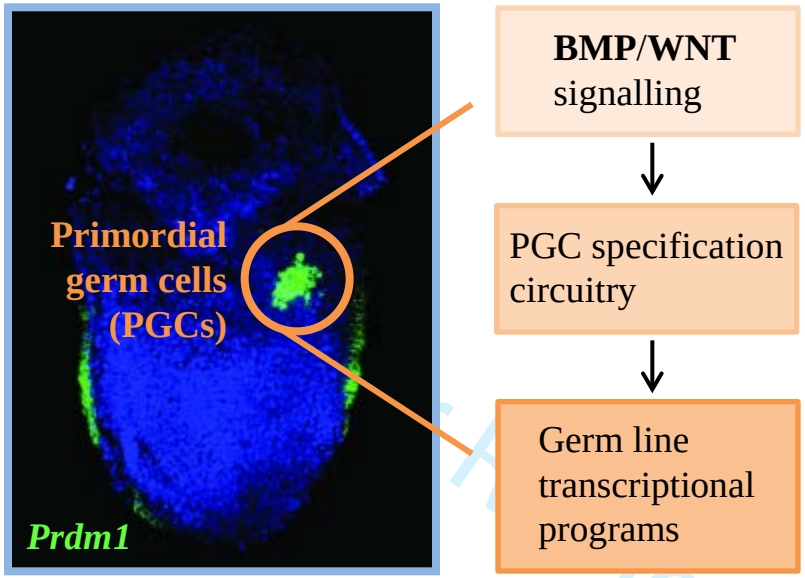
WIREs
SYSTEMS BIOLOGY AND MEDICINE

WILEY

Committing the primordial germ cell: an updated molecular perspective

Journal:	*WIREs Systems Biology and Medicine*
Manuscript ID	SYSBIO-385.R2
Wiley - Manuscript type:	Advanced Review
Date Submitted by the Author:	22-Jun-2018
Complete List of Authors:	Tan, Haihan; Institute of Molecular and Cell Biology Tee, Wee-Wei; Institute of Molecular and Cell Biology; National University Singapore Yong Loo Lin School of Medicine, Department of Physiology
Keywords:	germ cells, pluripotency
Choose 1-3 topics to categorize your article:	Stem Cell Biology and Regeneration (SAAB) < Developmental Biology (SAAA), Cell Fates (SCAB) < Biological Mechanisms (SCAA), Mammalian Physiology in Health and Disease (SBAB) < Physiology (SBAA)

Graphical Abstract



Mammalian primordial germ cells are induced by common signalling pathways. Downstream transcriptional events governing specification, however, diverge between species.

Committing the primordial germ cell: an updated molecular perspective

Authors

Haihan Tan

Institute of Molecular and Cell Biology, A*STAR, 61 Biopolis Drive, Proteos, Singapore 138673

hhtan@imcb.a-star.edu.sg

Wee-Wei Tee*

Institute of Molecular and Cell Biology, A*STAR, 61 Biopolis Drive, Proteos, Singapore 138673;

Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597

wwtee@imcb.a-star.edu.sg

Corresponding author: Wee-Wei Tee, wwtee@imcb.a-star.edu.sg

No conflicts of interest declared.

Abstract

The germ line is a crucial cell lineage that is distinct from somatic cells, and solely responsible for the trans-generational transmission of hereditary information in metazoan sexual reproduction. Primordial germ cells (PGCs) – the precursors to functional germ cells – are amongst the first cell types to be allocated in embryonic development, and this lineage commitment is a critical event in partitioning germ line and somatic tissues. Classically, mammalian PGC development has been largely informed by investigations on mouse embryos and embryonic stem cells. Recent findings from corresponding non-rodent systems, however, have indicated that murine PGC specification may not be fully archetypal. In this review, we outline the current understanding of molecular mechanisms in PGC specification, emphasising key transcriptional events, and focus on salient differences between early human and mouse PGC commitment. Beyond these latest findings, we also contemplate the future outlook of inquiries in this field, highlighting the importance of comprehensively understanding early fate decisions that underlie the segregation of this unique lineage.

Introduction

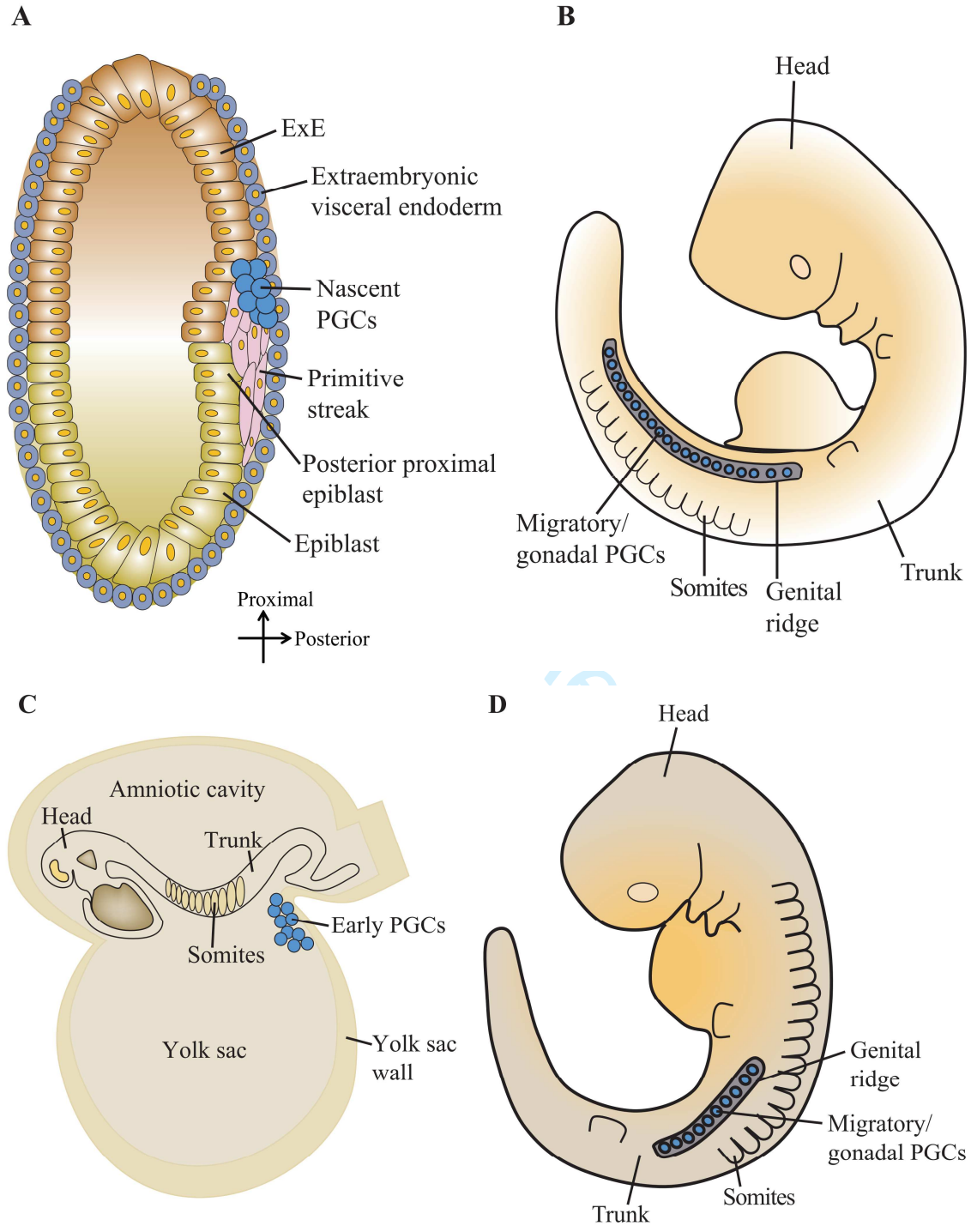
Reproduction is a defining feature of life, allowing for the survival and propagation of a species. Among metazoan systems, the germ line constitutes a critical cell type that permits reproduction and heredity. Germ cells – encompassing primordial germ cells (PGCs) and subsequently derived mature gametes – are set aside early in embryonic development, and represent the means through which genetic and epigenetic information are passed on to the next generation. The early segregation of the germ lineage portends a delineation between the germ line and the soma/somatic tissues, a concept anticipated by August Weissmann more than a century ago (Weismann *et al*., 1893), and indeed germ cells are the only known agents of hereditary transmission, to the exclusion of somatic cells.

The PGC state is the first overt commitment of the embryonic germ line, serving as the precursor cell type to functional gametes. In female embryos, PGCs mature into oogonia, which terminally differentiate into oocytes; in males, germ line counterparts are known as spermatogonia and spermatocytes respectively. Gametes in the terminally differentiated state can then fuse during fertilisation to reconstitute a totipotent zygote of the next generation. Over the course of germ cell specification, maturation, and differentiation, several processes must occur before functional gametes arise. First, an early PGC transcriptional network is established to specify PGC fate. Second, a naïve pluripotent state re-emerges and any somatic programmes are shut down. Third, global epigenetic changes occur to re-set the epigenome and re-activate quiescent sex chromosomes. Work on germ cell developmental dynamics and mechanisms has gathered at pace in the past decade, with a number of significant findings spotlighting the critical step of PGC commitment. Here we focus on current molecular knowledge of PGC specification, with emphasis on recent advances reported in the human PGC (hPGC) system.

An anatomical perspective of embryonic PGCs

The early identification and molecular investigation of PGCs in developing embryos centred around the mouse model, with murine embryos being a pre-eminently accessible and convenient model organism of choice. Mouse PGCs (mPGCs) can be first reliably detected by TNAP (tissue non-specific alkaline phosphatase) staining at around embryonic day 7.0 to 7.25 (E7.0-E7.25) in the extra-embryonic mesoderm just posterior to the primitive streak (Fig. 1A). mPGCs then appear as a cluster above the amnion by about E7.25 (Ginsburg *et al*., 1990; Lawson and Hage, 1994). The mPGCs then migrate through the hindgut into the genital ridge and gonadal anlage where they continue maturing (Fig. 1B) (Tam and Snow, 1981; Molyneaux *et al*., 2001). The PGC stage concludes when male cells mitotically arrest upon genital ridge colonisation and female cells enter meiosis from about E13.5 (Saitou and Yamaji, 2012).

Figure 1 | Anatomical observations of embryonic PGCs.



The embryonic study of hPGCs is unfortunately not as comprehensive as in the mouse embryo. Due to inaccessibility and unavailability of very early human embryonic material, there are no reports of human equivalents to the earliest TNAP-positive mPGCs in the epiblast (De Felici, 2013). It is, however, speculated that hPGCs are probably specified in the peri-gastrulation period at about week 2 of development (De Felici, 2013). The earliest hPGCs to be observed occurred in week 3 to week 4 embryos as morphologically distinct cells in the extra-embryonic yolk sac wall that were also positive for TNAP (Fig. 1C) (McKay *et al*., 1953; De Felici, 2013). Thereafter, hPGCs undergo processes similar to that in the mouse embryo, migrating through the nascent gut to the genital ridge by about week 6 (Fig. 1D), and marking the end of the PGC stage of development, when male and female cells begin mitotic arrest and meiotic entry respectively (Kurilo, 1981; Culty, 2009; De Felici, 2013).

The mouse paradigm for inducing embryonic PGCs

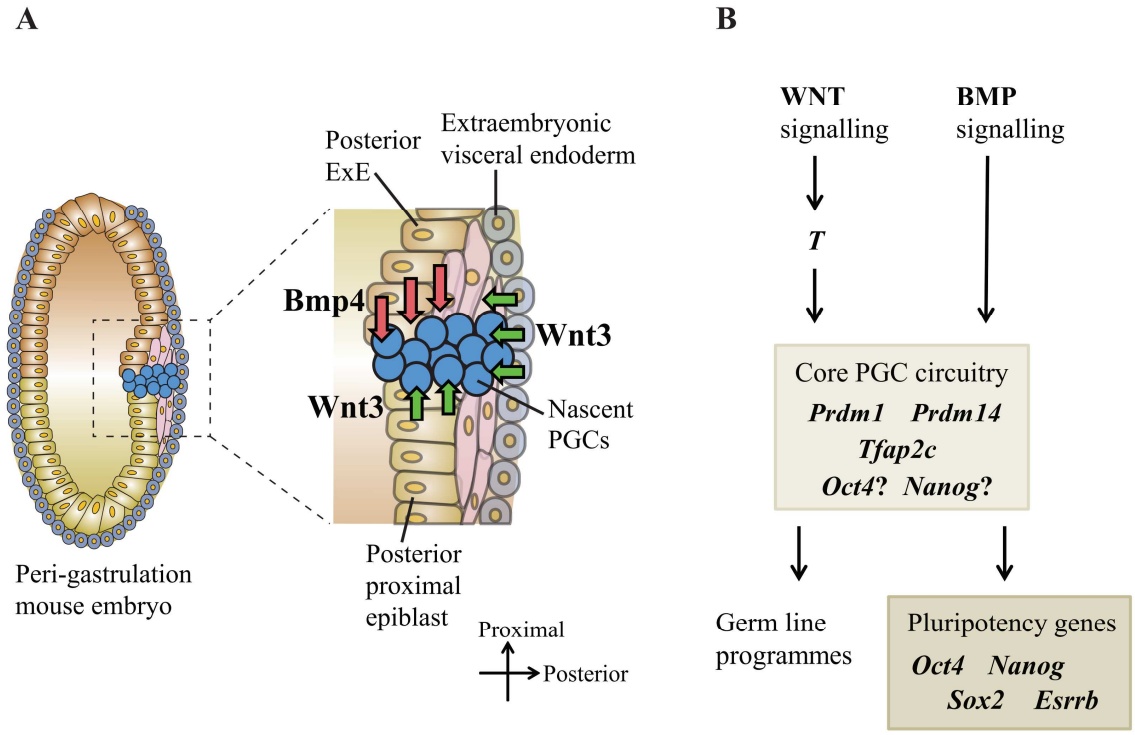
Metazoan germ lines are established through two major modes: preformation and induction. Preformation occurs in model organisms such as *C. elegans*, zebrafish, and *Xenopus*, among others, and involves the inheritance of maternally supplied germ lineage determinants by specifically located germ cell precursors. Induction occurs in species such as certain amphibians and insects, mice, and humans, and depends on extrinsic cell-to-cell signalling that induces the specification of PGCs from competent precursors (Extavour and Akam, 2003; Johnson *et al*., 2003; Johnson and Alberio, 2015; Whittle and Extavour, 2017). While induction is commonly viewed to be the more evolutionarily basal trait compared to preformation, all mammalian lineages studied so far utilise the inductive mode (Extavour and Akam, 2003; Johnson *et al*., 2003).

Given the relative ease of obtaining and analysing murine embryos from all stages of development, it is no surprise that the earliest investigations laying groundwork for future advances were performed in this tractable system. The initial specification of mPGCs may be abridged to three major processes occurring in and around the posterior proximal epiblast: (i) signalling pathways that impinge upon (ii) competent epiblast cells, which respond by (iii) upregulating key transcription factors that are the molecular drivers for germ line fate.

The posterior ExE and visceral endoderm of the peri-implantation mouse embryo express various Bone Morphogenetic Protein (BMP) isoforms that signal to the underlying proximal epiblast (Fig. 2A) (Beddington and Robertson, 1999; Ying and Zhao, 2001). A flurry of reports more than a decade ago indicated that genetic perturbations in the BMP pathway – be it through the loss of signalling

molecules such as *Bmp2* and *Bmp4*, or of downstream signal transducers like the *Alk2* receptor, and *Smad* transcriptional effectors of various isoforms – abrogated the mPGC lineage (Lawson *et al*., 1999; Ying *et al*., 2000; Chang and Matzuk, 2001; Tremblay *et al*., 2001; Ying and Zhao, 2001; Chu *et al*., 2004; de Sousa Lopes *et al*., 2004). This strongly hinted at the involvement of the BMP pathway in mPGC induction, which was confirmed by the robust induction of PGC fate from E6.0 epiblast cultured in the presence of Bmp ligands, and by the blockage of said induction through application of Cer1, a notable inhibitor of the BMP pathway (Ohinata *et al*., 2009).

Figure 2 | A molecular paradigm for mouse PGC specification.



Another major signalling pathway heavily involved in early mouse embryo patterning is the Wingless/INT-1 (WNT) pathway (Fig. 2A) (Liu *et al*., 1999). Having ascertained the role of BMP signalling in mPGC emergence, Ohinata *et al.* additionally revealed that *Wnt3* mutant embryos neither showed mPGC induction nor functional BMP signal transduction (Ohinata *et al*., 2009). Coupled with the observation that the Wnt3a cytokine was insufficient for PGC induction from *ex vivo* epiblasts (Ohinata *et al*., 2009), this suggested that WNT signalling in and around the posterior proximal epiblast conferred competence to respond to the BMP induction signal. A putative

molecular basis of this competence was thereafter uncovered as *T* (also known as *Brachyury*), one of the classical targets of canonical WNT signalling (Arnold *et al*., 2000). Genetic disruption of *T* indicated a failure in sustaining expression of the early mPGC marker *Prdm1* in mouse embryos, and also led to absence of expression of other mPGC markers *in vivo* (Aramaki *et al*., 2013). It is notable, however, that *T*-null embryos were shown to retain *Prdm1* expression at the earliest stages of mPGC emergence, and that *T*-null cells induced *in vitro* for PGC differentiation did not exhibit complete loss of PGC markers (Aramaki *et al*., 2013), indicating that while *T* contributes to PGC development, it may not be a requisite factor for specification.

The exact mechanisms of mPGC induction by the combination of BMP and WNT signalling remains to be elucidated. However, there is now extensive knowledge of the main molecular drivers of mPGC specification (Fig. 2B). Studies on knockout mice have identified the transcription factors *Prdm1* (also known as *Blimp1*), *Prdm14*, and *Tfap2c* (also known as *AP2γ*) as a potent triumvirate that are expressed in early mPGCs from E6.25 onwards, and whose loss results in embryos with impaired PGC specification and maintenance (Ohinata *et al*., 2005; Vincent *et al*., 2005; Yamaji *et al*., 2008; Weber *et al*., 2010). Further to this, these transcription factors also serve to repress somatic gene regulatory programmes, to facilitate re-expression of pluripotency genes, and to kickstart reprogramming of the epigenome – all fundamental processes of PGC commitment (Ohinata *et al*., 2005; Kurimoto *et al*., 2008; Yamaji *et al*., 2008; Weber *et al*., 2010; Grabole *et al*., 2013). Consequently, it is now a common consensus that *Prdm1*, *Prdm14*, and *Tfap2c* are the major players in molecularly specifying the early mPGC lineage (Fig. 2B).

Pluripotency-associated transcription factors, such as *Oct4* (also known as *Pou5f1*), *Nanog*, and *Sox2*, are upregulated in nascent mPGCs in an environment contemporaneous with the pro-PGC *Prdm1*, *Prdm14*, and *Tfap2c* axis (Fig. 2B). While *Oct4* embryonic expression persists in epiblast cells through their commitment to PGC fate, that of *Nanog* and *Sox2* first show downregulation in the epiblast by the peri-implantation stage, then are later re-expressed in the PGCs (Scholer *et al*., 1990; Avilion *et al*., 2003; Chambers *et al*., 2003). In agreement with this, single-cell analysis of inchoate mPGCs around the stages of their specification indicated that pluripotency genes like *Sox2* are first repressed in the developing epiblast, then upregulated in mPGCs (Yabuta *et al*., 2006). Besides their distinct role in ensuring mPGC reversion to a pluripotent/totipotent state, are these pluripotency factors also involved in PGC specification and/or development?

Earlier studies reported that conditional/constitutive disruption of each of the pluripotency factors *Nanog*, *Oct4*, and *Sox2* resulted in mPGC loss mainly through aberrant apoptosis or failure to proliferate (Kehler *et al*., 2004; Yamaguchi *et al*., 2009; Campolo *et al*., 2013), indicative of a role of these pluripotency factors in PGC development and survival. Additionally, whereas *Oct4* may play a role in PGC specification, *Nanog* appears to be dispensable (Chambers *et al*., 2007; Okamura *et al*., 2008). Indeed, *Nanog*-null mouse embryonic stem cells (mESCs) could still form PGCs at E11.5 in chimeric embryos, but exhibited reduced viability thereafter (Chambers *et al*., 2007). Notably, this compromised development of *Nanog*-null PGCs could be rescued by introducing exogenous *Esrrb* (Zhang *et al*., 2018), a key downstream target of Nanog (Festuccia *et al*., 2012), providing evidence for a *Nanog*-*Esrrb* hierarchy in mPGC development and highlighting functional conservation of the naïve pluripotency network in both ESCs and PGCs (Fig. 2B). Intriguingly, while *Nanog* is not strictly essential for germline development *in vivo*, its forced induction can enhance the formation of PGC-like cells (PGCLCs) *in vitro*, as detailed below (Murakami *et al*., 2016).

Extending the mouse paradigm to *in vitro* germ line specification

Detailed knowledge of the three principles of mPGC specification uncovered by studying the early embryo – extrinsic signalling, competence of receptive cells, and initiation of a specific germ line transcriptional regulatory programme – have recently been used to guide the establishment of protocols generating PGCLCs *in vitro*. In turn, these cell-based investigations have provided further corroboration of *in vivo* data to reinforce what we know of germ line specification. The earliest *in vitro* efforts endeavoured to differentiate murine germ cells from mESC cultures. These reported the formation of cells resembling PGCs, oocytes, and spermatids, which seemed to be capable of re-constituting blastocysts (Hubner *et al*., 2003; Toyooka *et al*., 2003; Geijsen *et al*., 2004). BMP signalling also seemed to enhance the derivation of these mouse PGCLCs (mPGCLCs) (Toyooka *et al*., 2003). However, the method of choice for generating these cells was random spontaneous differentiation, and the efficiency of differentiation proved to be very low.

The observation that the early peri-implantation embryonic epiblast (around E5.5 to E6.0) was competent for mPGC induction offered an initial clue to setting up a directed differentiation system for mPGCLCs (Ohinata *et al*., 2009). Pluripotent murine cells *in vitro* have been derived either as mESCs reflecting early embryonic cells of the inner cell mass with naïve pluripotency (E3.5 to E4.5), or as mouse epiblast stem cells (mEpiSCs) that mirror later post-implantation epiblast cells endowed with a primed pluripotent state (E5.75 to E6.5) (Brons *et al*., 2007; Nichols and Smith, 2009). Speculation that mEpiSCs, exhibiting a developmental state similar to germline-competent epiblast,

might provide a more permissive state for PGC induction, led to an attempt at mPGC generation from mEpiSCs; this however resulted in fairly low conversion efficiencies as well (Hayashi and Surani, 2009), perhaps attributable to the acquisition of later primitive streak characteristics at the expense of early epiblast traits (Kojima *et al*., 2014).

A breakthrough emerged with the finding that a short, controlled duration of pre-priming converted naïve mESCs into mouse epiblast-like cells (mEpiLCs), which proved to be a competent state allowing up to about 50% induction efficiency when exposed to BMP-containing inductive conditions (Fig. 2B) (Hayashi *et al*., 2011). Saliently, the mPGCLCs thus obtained could differentiate into functional gametes that gave rise to fertile offspring (Hayashi *et al*., 2011). The resulting stepwise differentiation procedure therefore had two main thrusts – (i) priming for an EpiLC state, followed by (ii) PGCLC induction with BMP signals. Where embryonic mPGC material was once a highly limiting resource, the burgeoning possibility of recapitulating the first stage of germ lineage formation *in vitro* now offered an abundance of biological material for investigations into the molecular mechanistics of germ cell specification,.

Notably, overexpressing the trio of pro-PGC transcription factors *Prdm1*, *Prdm14*, and *Tfap2c*, either individually or in various combinations in mEpiLCs, gave rise to similar mPGCLC differentiation efficiencies as BMP-based inductive conditions, confirming that these three factors are sufficient in committing the early mPGC (Fig. 2B) (Magnusdottir *et al*., 2013; Nakaki *et al*., 2013). The availability of mPGCLCs also permitted chromatin immunoprecipitation-sequencing (ChIP-seq) of these factors, revealing that they form an interdependent gene regulatory network that represses the somatic gene programme and activates germ line-associated genes (Magnusdottir *et al*., 2013). Curiously, the overexpression of *Nanog* in mEpiLCs also led to mPGCLC differentiation, and Nanog was found to bind and activate *Prdm1* and *Prdm14* expression (Murakami *et al*., 2016); conversely, differentiation of *Nanog*-null cells revealed a reduction in mPGCLC yield which could be rescued by ectopic *Nanog* or *Esrrb* expression (Murakami *et al*., 2016; Zhang *et al*., 2018). As the molecular details of BMP/WNT signalling crosstalk for competence and specification have yet to be clarified, it may be possible that *Nanog* acts in tandem with a defined signaling/transcriptional environment to promote germ lineage specification. Additional scrutiny of gene regulatory events downstream of BMP/WNT signalling will likely shed more light on auxiliary factors in mPGC specification pathways.

Mammalian PGC induction: beyond the murine narrative

While the accessibility of the mouse embryo has made it amenable to observational, genetic, and lineage tracing studies, leading to extensive knowledge of the molecular pathways responsible for mPGC commitment, the same cannot be said for human embryos. Due to ethical and moral considerations, coupled with a very limiting amount of embryonic material, it has been nigh on impossible to dissect mechanisms of early human PGC (hPGC) specification *in vivo* (De Felici, 2013). Critically, there is a paucity of information describing the earliest origins of hPGCs, with the earliest observations performed on week 3 to week 4 embryos, and the earliest molecular characterisations done on week 4 to week 7 embryos (De Felici, 2013; Gkountela *et al*., 2013; Gkountela *et al*., 2015; Guo *et al*., 2015; Tang *et al*., 2015).

Conventionally, elucidation of human embryonic development and differentiation processes has leaned heavily on extrapolation from the corresponding mouse pathways. While this has undoubtedly been a success in deciphering the early development of many a tissue, we note that mammalian germ lineage commitment initiates at the peri-gastrulation, peri-implantation stage, when murine and human embryonic morphology diverges greatly. For instance, mouse embryos develop as a cylindrical structure complete with a trophectoderm cap atop the epiblast, while human embryos form a planar, bilaminar disk structure whose epiblast lies adjacent to the abutting amnion epithelium (Behringer *et al*., 2000; Rossant, 2015). Given such major developmental differences at the timing of germ line allocation, it is perhaps unsurprising that there are variations in the mechanisms behind PGC emergence.

Owing to the lack of human embryo samples at the nascent stages of hPGC formation, inquiries into human germ cell induction and differentiation have mostly relied on *in vitro* modelling thus far. Similar to the murine system, early efforts randomly differentiated human ES cells (hESCs) in aggregated cells and isolated a low percentage of cells expressing germ cell markers. The supplementation of BMP ligands in culture conditions resulted in a small but consistent increase in differentiation efficiency (Clark *et al*., 2004; Kee *et al*., 2006). This intimated that the BMP signalling pathway was probably inductive as per the murine model (Fig. 3), and also that conventionally cultured hESCs, like mESCs, possessed low competence for germ line specification. Such non-permissiveness may likely be ascribed to the fact that hESCs are considered the equivalent of mEpiSCs (Nichols and Smith, 2009); in other words, hESCs are in a primed pluripotency state that may already have passed the window for germ line specification. To re-enable germ line competence, it hence appears that the canonical hESC pluripotent state must be somehow modified, just like in mouse pluripotent cells, and a series of recent publications has indeed shown this to be a

key step in inducing germ cells *in vitro* (Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015; von Meyenn *et al*., 2016 ; Irie and Surani, 2017).

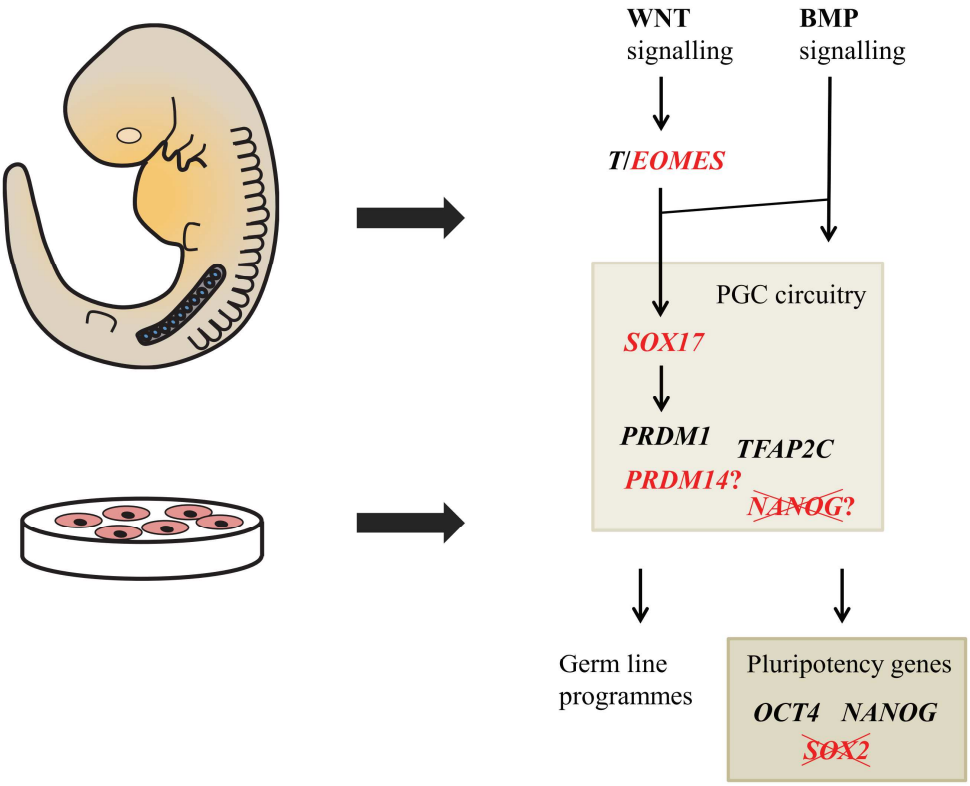
Using a specific cocktail of small molecules supplemented to human pluripotent stem cell (hPSC) culture, Irie and colleagues reported the generation of human PGCLCs (hPGCLCs) by directed differentiation, and also showed that hiPSCs resembled hESCs in their capacity for germ line differentiation (Gafni *et al*., 2013; Irie *et al*., 2015). Meanwhile, Sasaki and colleagues primed hiPSCs with a small molecule WNT agonist and Activin A, activating an incipient mesoderm-like cell state (iMeLC) that slightly upregulated early mesodermal genes and were capable of hPGCLC induction (Sasaki *et al*., 2015). The possibility that a very early primitive streak/mesoderm-like state could endow hPSCs with the ability to respond to pro-PGC signals was also explored by Sugawa *et al.*, who briefly stimulated hPSCs with Activin A to upregulate the primitive streak/early mesodermal gene *T* in hPSCs, then induced the cells in BMP^+ conditions to obtain specified hPGCLCs (Sugawa *et al*., 2015). Lessons from the murine *in vitro* model were also applied to hPSCs – hESCs were converted to human epiblast-like cells (hEpiLCs) with a procedure similar to that used for mouse counterparts, and hPGCLCs were generated upon subsequent addition of BMP^+ medium (Hayashi *et al*., 2011; von Meyenn *et al*., 2016). The latest accounts of *in vitro* differentiation have essentially replicated prior methodologies with slight modifications, if any, reinforcing the robustness of directed hPGCLC differentiation (Kobayashi *et al*., 2017; Mitsunaga *et al*., 2017; Yokobayashi *et al*., 2017).

All of the differentiation strategies discussed above may differ to various extents, yet they all share overarching principles that are also seen in the murine differentiation model. First, the only clear signalling modality for hPGCLC induction thus far is the BMP pathway, similar to the mouse system. The WNT pathway would be a logical alternative, but does not appear to be an inductive pathway as exogenous WNT3A fails to enhance hPGCLC specification efficiencies (Sugawa *et al*., 2015). Second, hPSCs must progress from a state of conventional pluripotency into a transitional state primed for PGC competence, with such competence arising concomitantly with primitive streak/nascent mesoderm traits that were explicitly reported in the intermediate cell types of Irie, Sasaki, and Sugawa *et al.* (Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015). Although von Meyenn and colleagues did not profile their intermediate cell type in detail, it would not be unexpected that these hEpiLCs exhibit expression of primitive streak/mesoderm markers (von Meyenn *et al*., 2016).

Mechanisms in PGC development: the human perspective

What then, are the molecular and transcriptional mechanisms underlying human germ line competence and subsequent commitment? A recent succession of analyses isolated migratory hPGCs dissected from ethically obtained human embryonic genital ridges and showed that the expression profile of week 4 to week 7 hPGCs were largely similar to their murine counterparts (Fig. 3). The upregulation of key genes, such as the pluripotency factors *OCT4* and *NANOG*, the early germ line markers *PRDM1*, *TFAP2C*, and *NANOS3*, the late germ line markers *DAZL* and *DDX4*/*VASA*, and the germ cell surface markers *cKIT* and *TNAP*, demonstrated that a core germ line transcriptional programme is shared by both human and mouse early PGCs (Gkountela *et al*., 2013; Gkountela *et al*., 2015; Guo *et al*., 2015; Tang *et al*., 2015). However, closer inspection of these data revealed noteworthy differences between species. For example, early hPGCs express the naïve pluripotency genes *KLF4* and *TFCP2L1*, the mesendodermal lineage factor *GATA4*, and the extra-embryonic trophectoderm regulator *TEAD4*, all of which are not seen, or expressed at relatively lower levels, in mPGCs or mPGCLCs (Tang *et al*., 2015; Canovas *et al*., 2017). These observations suggested that mechanistic findings from the murine model could not be imported wholesale into the human system, and this was borne out by *in vitro* hPGCLC investigations which we discuss below.

Figure 3 | Current outlook of human PGC specification.



The earliest week 4 to week 5 hPGCs analysed were already in the post-commitment stage of migration to the gonadal anlage. As experimental material from even earlier stages could not be obtained, the *in vitro* differentiation system for hPGCLCs proved a boon to studying the earliest germ lineage specification events. We have discussed above how the state of competence for successful pro-PGC induction correlates with hPSCs adopting early primitive streak/mesoderm-like attributes. Indeed, multiple studies have reported the emergence of primitive streak/mesoderm-associated genes such as *T* and *EOMES* prior to hPGCLC induction, and migratory hPGCs *in vivo* also express *T* (Gkountela *et al*., 2015; Guo *et al*., 2015; Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015). This raises the question of whether early mesoderm lineage factors are the molecular basis for hPGC competence, and recent reports provide some evidence in support of this postulation.

The usage of a number of different hiPSC clonal lines for iMeLC-based generation of hPGCLCs resulted in clear differences of conversion efficiencies. Remarkably, this variation was positively correlated with the expression levels of *T* and *EOMES*, with higher expression of said factors reflected in enhanced differentiation potential of the clone (Yokobayashi *et al*., 2017). This correlation was reproduced in hESCs induced with a 4i-primed intermediate stage, and additional

differentiations with *EOMES* mutant cells showed drastic reduction in hPGCLC specification, hinting that early mesoderm-associated transcription factors are necessary at least for the initial step of hPGCLC commitment (Fig. 3) (Chen *et al*., 2017). Exactly how the presence of factors such as T and EOMES engenders permissiveness of BMP-responding cells remains to be clarified. With a multitude of differentiation options available, investigations of *T* mutant hPSCs and of genomic binding events of relevant primitive streak/mesoderm factors are surely not far off, and it would be instructive to tease apart the confluence of BMP/WNT pathways with T and EOMES in the hPGC precursor.

While the basis of hPGC competence remains less understood, the molecular events of hPGC specification are more comprehensively documented. In a surprising landmark discovery, the transcription factor *SOX17* was implicated as a crucial upstream regulator of the early human germ lineage (Fig. 3) (Irie *et al*., 2015). The orthodox consensus view of *SOX17* is that it is a classical specifier of endodermal fate, and indeed it is extensively utilised as one of the earliest markers for endoderm lineage differentiation (Kanai-Azuma *et al*., 2002; D'Amour *et al*., 2005). This canonical role, coupled with the observation that murine *Sox17* is required not for mPGC specification but only for proper migration along gut endoderm, made its hPGC regulatory function unanticipated (Hara *et al*., 2009). Previous observations noted the presence of SOX17 protein and RNA in gonadal hPGCs between week 4 to week 8, but these samples were too advanced to represent the commitment phase of hPGC development (de Jong *et al*., 2008; Gkountela *et al*., 2015; Guo *et al*., 2015; Tang *et al*., 2015).

The pivotal finding that *SOX17* is one of the earliest genes to be specifically upregulated in nascent hPGCLCs *in vitro* was key to pinpointing its early role. *SOX17* is expressed in differentiating hPGCLCs after *T* and before *PRDM1* and *TFAP2C*, while *SOX17* knockout cells fail to induce hPGCLCs. Furthermore, this failure of induction can be rescued by conditional *SOX17* expression (Irie *et al*., 2015). Convincingly, overexpressing *SOX17* in wildtype hESCs could also activate germ cell programmes generating hPGCLCs at appreciable efficiencies even without BMP-based induction, reminiscent of analogous murine experiments overexpressing the *Prdm1*, *Prdm14*, and *Tfap2c* trio of pro-mPGC factors (Magnusdottir *et al*., 2013; Nakaki *et al*., 2013; Irie *et al*., 2015). These data robustly demonstrated that *SOX17* is necessary and sufficient for hPGC specification, placing it at the top of a genetic hierarchy driving hPGC fate in a competent milieu. Just as importantly, most other reports of *in vitro* hPGCLC differentiation thus far have independently verified early *SOX17* expression, which has now positioned *SOX17* as a major molecular footprint of

nascent hPGC identity (Sasaki *et al*., 2015; von Meyenn *et al*., 2016; Chen *et al*., 2017; Kobayashi *et al*., 2017; Mitsunaga *et al*., 2017).

The axis of *Prdm1*, *Prdm14*, and *Tfap2c* is recognised as the fundamental murine germ lineage specifier, as reviewed above. In humans, *PRDM1* is similarly a signature gene for hPGC commitment (Fig. 3). *PRDM1* is expressed *in vivo* in migratory and gonadal hPGCs (Gkountela *et al*., 2013; Gkountela *et al*., 2015; Guo *et al*., 2015; Tang *et al*., 2015), and is also among the earliest germ lineage factors upregulated upon *in vitro* hPGCLC induction, validating its common usage as a definitive hPGC fate marker (Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015; von Meyenn *et al*., 2016; Chen *et al*., 2017; Irie and Surani, 2017; Kobayashi *et al*., 2017; Mitsunaga *et al*., 2017). Attempts to differentiate *PRDM1* knockout/knockdown hESCs into hPGCLCs revealed their inability to adopt germ line fate, and in particular, other germ line genes like *NANOS3* were completely uninduced (Irie *et al*., 2015; Sugawa *et al*., 2015). While the *PRDM1*-deficient cells still produced a small number of $TNAP^{+}$ cells that could potentially indicate remnant hPGCLC commitment, analysis of this rare population showed low expression of the hPGC markers *TFAP2C*, *OCT4*, and *NANOG*, but significant activation of somatic genes like mesoderm and endoderm lineage factors (Irie *et al*., 2015; Tang *et al*., 2015). Could PRDM1, a known transcriptional repressor, thus act to suppress somatic activation in humans, equivalently to the murine system (Magnusdottir *et al*., 2013)?

The interpretation of this data in light of *SOX17* involvement is intriguing. *SOX17* is a classical endoderm specifier, yet is now also known to be a hPGC lineage driver – this dichotomy may be reconciled by latest reports showing that overexpression of *SOX17* in over-primed hESCs beyond a PGC-competent window indeed upregulated endoderm genes instead of hPGC genes (Kobayashi *et al*., 2017). In the absence of PRDM1, hPGC precursor factors such as T, EOMES, and SOX17 could possibly activate their respective canonical mesoderm and endoderm programmes without PRDM1-mediated suppression. Accordingly, high dosage of ectopic *SOX17* in germ line-differentiating hPSCs leads to aberrant expression of endoderm markers, which is corrected by simultaneously applying a comparable dose of *PRDM1* (Kobayashi *et al*., 2017). Taken together, *PRDM1* can be considered as an early pro-hPGC factor, but unlike its counterpart in the mouse system, it is likely not a specifying factor for hPGC fate, as *PRDM1* overexpression in primed hPSCs failed to efficiently generate hPGCLCs (Kobayashi *et al*., 2017). That early specification role has probably been taken over by *SOX17* in a re-wiring of regulatory networks for human germ line specification.

Prdm14 is critical for mPGC differentiation, but its human orthologue appears to have a diminished role in hPGC commitment. *In vivo* expression data from migratory hPGCs showed that *PRDM14* may be expressed at lower levels than the pluripotent epiblast or hPSCs, whereas extrapolation from the murine model would predict equivalent expression between PSC and PGC states (Gkountela *et al*., 2015; Guo *et al*., 2015; Sugawa *et al*., 2015; Tang *et al*., 2015). This was confirmed by hPGCLC differentiations uncovering low expression levels of *PRDM14* during and after lineage specification (Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015; von Meyenn *et al*., 2016; Mitsunaga *et al*., 2017). *PRDM14* is a known, potent pluripotency-promoting factor in both mouse and human cells (Tsuneyoshi *et al*., 2008; Gillich *et al*., 2012), and its downregulation at the cusp of hPGCLC induction has been suggested as a means to facilitate germ line fate acquisition (Irie *et al*., 2015). Subsequent maintenance of a low-*PRDM14* state could suggest that this transcription factor has been made redundant for hPGC fate as opposed to the murine model. In support, knockdown of *PRDM14* in hPSCs induced for germ cell differentiation showed no effect on hPGCLC induction (Sugawa *et al*., 2015), although we note that this knockdown was incomplete, implying that residual *PRDM14* might still retain biological functionality. Therefore, without investigation on cells bearing complete loss of *PRDM14*, its role in hPGC specification remains uncertain (Fig. 3).

The human orthologue of the third factor in the murine pro-PGC axis, *TFAP2C*, is possibly the least characterised among the trio. Like its murine counterpart, it is upregulated in migratory and gonadal hPGCs *in vivo* (Gkountela *et al*., 2015; Guo *et al*., 2015; Tang *et al*., 2015), and is also regularly used as a marker of hPGCLC commitment *in vitro* (Irie *et al*., 2015; Sasaki *et al*., 2015; Kobayashi *et al*., 2017; Mitsunaga *et al*., 2017). Tracking the dynamics of *TFAP2C* expression over time in hPGCLC specification revealed that it is activated slightly after *PRDM1*, placing it downstream of *PRDM1* in the hPGC genetic programme (Fig. 3) (Irie *et al*., 2015; Kobayashi *et al*., 2017). Recent preliminary work also showed that forced expression of *TFAP2C* in competent hPSCs did not lead to hPGCLC generation at appreciable efficiencies, even in conjunction with *PRDM1* overexpression (Kobayashi *et al*., 2017). This seems to suggest that while *TFAP2C* is retained in the hPGC regulatory network, it is insufficient on its own for germ line fate acquisition, warranting more in-depth study of this factor.

Downstream of the core PGC specifying factors, the re-expression of pluripotency genes in the human germ line is somewhat similar to that in mouse, albeit with one conspicuous difference. The likes of *OCT4* and *NANOG* – classical pluripotency genes – are well-known to be expressed in migrating hPGCs and hPGCLCs undergoing germ line commitment, mirroring their behaviour in

mPGCs and mPGCLCs (Fig. 3). Additionally, their upregulation is observed after the induction of early hPGC factors like *SOX17* and *PRDM1*, denoting their deployment downstream of the *SOX17*/*PRDM1*/*TFAP2C* hPGC specification axis. However, another quintessential pluripotency factor, *SOX2*, is virtually absent in hPGCs and hPGCLCs, contrary to its presence and necessity in the murine system (Fig. 3) (de Jong *et al*., 2008; Gkountela *et al*., 2013; Gkountela *et al*., 2015; Guo *et al*., 2015; Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015; Tang *et al*., 2015; von Meyenn *et al*., 2016; Chen *et al*., 2017; Kobayashi *et al*., 2017; Mitsunaga *et al*., 2017).

Most reports so far have only addressed the lack of *SOX2* in a descriptive manner, and to our knowledge, *SOX2* has not been subjected to experimental manipulation in the hPGCLC setting. Besides its role in maintaining the pluripotent state, *SOX2* is also known for its role in neurectoderm differentiation, postulated to be a default differentiation pathway of hPSCs (Vallier *et al*., 2004; Sarkar and Hochedlinger, 2013). Speculation that *SOX2* and *PRDM14* may be genetic interactors has suggested that their low expression levels may be linked to a repressive programme for neural fate in hPGCLC precursors (Sugawa *et al*., 2015), but this is far from certain, necessitating at least some preliminary inquiries into the biology of *SOX2* during germ cell specification.

Besides *SOX2*, attempts have recently been made to recapitulate the murine finding that overexpression of *Nanog* alone can induce mPGCLCs without the introduction of core germ lineage specifiers (Murakami *et al*., 2016), but the human system has proven refractory, with ectopic *NANOG* expression unable to generate hPGCLCs (Kobayashi *et al*., 2017). Additionally, the very recent finding of *Esrrb* acting downstream of *Nanog* in mPGCs/mPGCLCs seems to be irrelevant to the human system, as *ESRRB* has been shown to be absent from hPGCs/hPGCLCs (Tang *et al*., 2015; Zhang *et al*., 2018). Although there is substantially less investigation into the roles of pluripotency factors in hPGC fate allocation compared to mPGCs, it is clear that the pluripotency network in hPGCs and hPGCLCs may deviate from the murine version. How extensive and significant this re-configuration is remains to be investigated.

The molecular basis of PGC commitment in an evolutionary perspective

Extensive recent utilisation of *in vitro* hPGCLC differentiation procedures has revealed that while many pathways and factors critical to PGC/PGCLC development have been conserved, some notable dissimilarities in deployment of PGC-associated factors have repeatedly been observed. Perhaps the most significant finding is that a *SOX17*/*PRDM1* cascade has replaced the murine *Prdm1*/*Prdm14*/*Tfap2c* trio as the major axis for hPGC induction (compare Fig. 2B to Fig. 3). Could

this phenomenon be a reflection of morphogenetic divergence in the peri-gastrulating embryo? The mouse peri-gastrulation embryo presents as an egg cylinder, while the human equivalent manifests as a bilaminar disc. Importantly, the cylindrical morphology of rodent embryos appears to be quite unique to that clade, as the embryos of many other mammalian lineages are disc-like (Irie *et al*., 2014).

Very recent work has looked at PGC origination around the timing of gastrulation in pig and non-human primate embryos, whose embryonic morphologies are bilaminar and similar to human embryos. Notably, early pre-migratory porcine PGCs (pPGCs) and cynomolgus monkey PGCs (cyPGCs) bore a transcription factor signature essentially equivalent to that in hPGCs/hPGCLCs, such as *SOX17*, *PRDM1*, *TFAP2C*, *OCT4*, *NANOG*, and lack of *SOX2* (Sasaki *et al*., 2016; Kobayashi *et al*., 2017). Additionally, initial reports of stepwise *in vitro* generation of porcine and cynomolgus monkey PGCLCs from their respective pluripotent stem cells also demonstrated that these factors are similarly involved in germ lineage induction in cells of these species (Wang *et al*., 2016; Kobayashi *et al*., 2017). It is therefore probable that cylindrical and bilaminar embryos possess dissimilarly wired transcriptional circuitry for promoting PGC fate.

From an evolutionary developmental perspective, what could be a likely reason behind alteration of the early pro-PGC gene regulatory network? A school of thought in PGC development holds that PGCs segregate very early from nascent mesoderm, and hence may be mesodermal derivatives (Extavour and Akam, 2003; Johnson *et al*., 2003; Pilato *et al*., 2013). Molecularly, this may be supported by the observation of *T* and *EOMES* expression preceding canonical pro-PGC factors, where *T* and *EOMES* are traditionally considered to be mesodermal genes and reported as such (Guo *et al*., 2015; Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015; Yokobayashi *et al*., 2017).

Another possible interpretation of *T*/*EOMES* expression is that they delineate an early mesendoderm precursor population, given that *T* and *EOMES* are widely reported as mesendoderm factors (Kubo *et al*., 2004; Tada *et al*., 2005; Costello *et al*., 2015; Song *et al*., 2016; Wang *et al*., 2017). In this scenario, it may be that *T*/*EOMES* activity in nascent mesendoderm could have activated the endoderm lineage factor *SOX17* at some point in the evolutionary past, which was in turn co-opted into a *PRDM1*-based regulatory network for PGC fate. We note, on the other hand, that there remains a lack of consensus on the nature of mesendoderm in mice (Tzouanacou *et al*., 2009), and perhaps *T*/*EOMES* may merely demarcate a more general pre-streak epiblast cell population. It is nonetheless interesting that the loss of *PRDM1* function in differentiating PGCs/PGCLCs increased

expression of mesoderm and endoderm factors, hinting that PRDM1 may suppress early somatic programmes to switch cells into PGC fate, and that a co-opted *SOX17* somewhat retains its original endodermal function (Irie *et al*., 2015). Considering a recent account of *SOX17* expression in PGCs of the basal rodent *Lagostomus maximus*, it may even be plausible that the mouse specification mechanism lacking *SOX17* could be a derived trait, as the *Muridae* clade to which mice belong is evolutionarily young among rodents (Fabre *et al*., 2012; Leopardo and Vitullo, 2017).

Besides the puzzling nature of *SOX17* involvement, there remain plenty of mysteries surrounding hPGC fate allocation. For instance, there are no ready explanations regarding the lack of necessity for *SOX2*, and the curtailed expression of *PRDM14*. Additionally, the observation of *SOX17*-related *SOX15* expression in early-stage hPGCs/hPGCLCs (de Jong *et al*., 2008; Guo *et al*., 2015; Sasaki *et al*., 2015; Mitsunaga *et al*., 2017) raises further possibilities – while it may be possible that *SOX15* may be acting redundantly with *SOX17* to ensure a robust fate transition, it may also be plausible that *SOX15* has uniquely important roles in hPGC commitment that have not been uncovered. Over and above the still-uncertain individual roles of these transcription factors discussed above, there is an acute lack of understanding of the ways in which these factors physically bind and regulate expression of one another, and of downstream target genes, warranting a range of further investigations.

Peculiarly, a recent finding that non-human primate PGCs seem to have origins in the amnion instead of the epiblast introduces yet another enigma. While the earliest indications of non-primate mammalian PGCs so far place their emergence at the posterior epiblast where the incipient primitive streak forms, cynomolgus monkey PGCs clearly arise from the dorsal amnion that is physically separate from the posterior epiblast. Even more curiously, this region of the dorsal amnion expresses *T* prior to gastrulating epiblast (Sasaki *et al*., 2016). Murine *T* is known to be expressed early in extra-embryonic tissue abutting the epiblast before the primitive streak forms, and is also implicated in gastrulation-associated cell movement (Rivera-Perez and Magnuson, 2005; Turner *et al*., 2014). Further to this, posterior epiblast ingressing through the primitive streak does contribute to the amnion in the mouse (Tam and Beddington, 1987; Kinder *et al*., 1999). In light of the association between the amnion, epiblast, and *T*, a possible role of T could be to engage cell motility programmes pertinent to the critical migratory behaviour of PGCs (Molyneaux and Wylie, 2004; Turner *et al*., 2014).

The prospective outlook in PGC research

To address knowledge gaps in early human/primate PGC commitment, we propose that a judicious strategy would be to take extensive advantage of *in vitro* PGC directed differentiation techniques, established for human/primate/porcine/murine systems, to generate abundant material at each stage in pre-PGC and PGC development. Up until now, hPGCLCs appear to represent a transient cell state that is incapable of *in vitro* maintenance, as these cells extinguish PGC features beyond about a week in culture (Irie *et al*., 2015; Sasaki *et al*., 2015; Sugawa *et al*., 2015). Whether culture conditions can be modified to indefinitely maintain the hPGCLC state is an open question.

Additional potential improvements that may be implemented include differentiation in monolayers instead of aggregates, and transdifferentiation. All current differentiation approaches utilise embryoid bodies as the starting point for induction; while this may have proven fairly successful, the aggregate nature of the starting material may bring about issues such as diffusional non-uniformity of inductive compounds and inconvenient manipulation steps (Bratt-Leal *et al*., 2009; Mummery *et al*., 2012). Development of monolayer differentiation methods would likely enhance yields of hPGCLCs while improving final purity, and increase the accessibility of such directed differentiations to more investigators. In parallel, a second methodological improvement would be to enable transdifferentiation of non-stem cells to hPGCLCs. Latest efforts have reported limited success (Canovas *et al*., 2017), but these are still early days, and we expect that future breakthroughs may allow convenient use of somatic cells for hPGCLC generation. For now, aggregate-based differentiations remain as the prevailing protocol, and the rapid induction of hPGCLCs (within two to four days) in such procedures still enables speedy procurement of material for experimentation.

Without constrictive limitation of sample material, genetic manipulation of signalling pathways and transcription factors can be more easily achievable, and critically, ChIP-seq of key transcription factors can be robustly performed to flesh out genetic linkages within the pro-PGC network, much like comparable work in the murine system (Magnusdottir *et al*., 2013). The sum result of this would be to unequivocally clarify if and how human germ lineage segregation differs from other mammals. Such clarity, combined with advances in *ex vivo* pre-gastrulation embryonic culture (Deglincerti *et al*., 2016; Shahbazi *et al*., 2016), opens up the real prospect that stepwise hPGC commitment may be systematically confirmed in the embryo, setting forth a gold standard for hPGC differentiation.

Looking further ahead, these advances pave the way for modelling and understanding germ line-associated diseases and syndromes, such as cancers arising from dysregulated germ cells and infertility due to loss of germ cells. We anticipate that gene regulatory networks underpinning post-

hPGC maturation into oogonia/spermatogonia, and thereafter functional gametes, will be elucidated in the near future. While the clinical usage of laboratory-generated gametes will likely be ethically and scientifically fraught (Ishii and Saitou, 2017), basic biological knowledge gleaned from gamete differentiation could be used for purposes such as diagnostic screening, reproductive toxicology, and even endangered species preservation through gamete banking.

Conclusion

August Weissmann's hypothesised germ line/soma barrier was first proposed more than a century ago (Weismann *et al*., 1893). In the modern era, with the aid of contemporary methodologies, the germ cell field has begun to tease apart the molecular players that segregate the germ lineage and lay the foundation of Weissmann's barrier. Although the barrier has been conceptually challenged to a certain extent by the usage of reprogrammed somatic cells (iPSCs) to recapitulate the germ lineage, this constitutes a laboratory phenomenon with no correlate in the natural world. Future work into germ line sequestration will undoubtedly provide more clues uncovering the basis of the germ line/soma barrier, and of the development and evolutionary history of this fascinating lineage that is so vital for reproductive propagation.

Acknowledgments

The W.-W.T. Lab is supported by the Singapore National Research Foundation Fellowship [grant number NRF-NRFF2016-06], and the Biomedical Research Council, Agency for Science, Technology and Research [grant number 1531C00144]. We thank Dennis Tan for excellent help with figure preparations.

Conflict of Interest

The authors have declared no conflicts of interest for this article.

Figure Legends

Figure 1 | Anatomical observations of embryonic PGCs.

Fig. 1. (A) Schematic diagram of the peri-gastrulation mouse embryo, indicating nascent mPGCs observed in the posterior embryo. **(B)** Schematic diagram of the mouse embryo at about E9.5, showing migratory, committed mPGCs colonising the genital ridge. **(C)** Schematic diagram of the

week 3 human embryo, with the earliest observed hPGCs in the yolk sac wall. **(D)** Schematic diagram of the week 5 to 6 human embryo, indicating migratory hPGCs populating the genital ridge. Diagrams are not to scale. ExE; extraembryonic ectoderm. PGC; primordial germ cell.

Figure 2 | A molecular paradigm for mouse PGC specification.

Fig. 2. (A) In the peri-gastrulation/implantation mouse embryo, BMP and WNT signals emanate from the posterior ExE, visceral endoderm, and the proximal epiblast itself, impinging and acting on the posterior proximal epiblast. These signals represent competence and inductive modalities to drive receptive epiblast cells towards germ lineage fate. **(B)** A wealth of *in vivo* and *in vitro* data has unveiled the molecular pathways and players through which the mPGC lineage is specified. This flowchart demonstrates the major factors involved. ExE; extraembryonic ectoderm. PGC; primordial germ cell.

Figure 3 | Current outlook of human PGC specification.

Fig. 3. Combined *in vitro* and *in vivo* findings have uncovered a hPGC specification mechanism distinct from that of the mouse. This flowchart includes the main, best-characterised factors in pro-hPGC pathways; factors in red are the key elements that differ between mPGC and hPGC development. Of note, *SOX17* lies upstream of *PRDM1* in the PGC specification circuitry, and the roles of *PRDM14* and *NANOG* in specifying hPGCs are unclear. Additionally, *EOMES* has yet to be characterised as a molecular player in mPGC commitment, while *SOX2* is not expressed in committed hPGCs. PGC; primordial germ cell.

References

Aramaki, S., Hayashi, K., Kurimoto, K., Ohta, H., Yabuta, Y., Iwanari, H., Mochizuki, Y., Hamakubo, T., Kato, Y., Shirahige, K. *et al*. (2013) 'A mesodermal factor, T, specifies mouse germ cell fate by directly activating germline determinants', *Dev Cell* 27(5): 516-29.

Arnold, S. J., Stappert, J., Bauer, A., Kispert, A., Herrmann, B. G. and Kemler, R. (2000) 'Brachyury is a target gene of the Wnt/beta-catenin signaling pathway', *Mech Dev* 91(1-2): 249-58.

Avilion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N. and Lovell-Badge, R. (2003) 'Multipotent cell lineages in early mouse development depend on SOX2 function', *Genes Dev* 17(1): 126-40.

Beddington, R. S. and Robertson, E. J. (1999) 'Axis development and early asymmetry in mammals', *Cell* 96(2): 195-209.

Behringer, R. R., Wakamiya, M., Tsang, T. E. and Tam, P. P. (2000) 'A flattened mouse embryo: leveling the playing field', *Genesis* 28(1): 23-30.

Bratt-Leal, A. M., Carpenedo, R. L. and McDevitt, T. C. (2009) 'Engineering the embryoid body microenvironment to direct embryonic stem cell differentiation', *Biotechnol Prog* 25(1): 43-51.
Brons, I. G., Smithers, L. E., Trotter, M. W., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S. M., Howlett, S. K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R. A. *et al*. (2007) 'Derivation of pluripotent epiblast stem cells from mammalian embryos', *Nature* 448(7150): 191-5.
Campolo, F., Gori, M., Favaro, R., Nicolis, S., Pellegrini, M., Botti, F., Rossi, P., Jannini, E. A. and Dolci, S. (2013) 'Essential role of Sox2 for the establishment and maintenance of the germ cell line', *Stem Cells* 31(7): 1408-21.
Canovas, S., Campos, R., Aguilar, E. and Cibelli, J. B. (2017) 'Progress towards human primordial germ cell specification in vitro', *Mol Hum Reprod* 23(1): 4-15.
Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S. and Smith, A. (2003) 'Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells', *Cell* 113(5): 643-55.
Chambers, I., Silva, J., Colby, D., Nichols, J., Nijmeijer, B., Robertson, M., Vrana, J., Jones, K., Grotewold, L. and Smith, A. (2007) 'Nanog safeguards pluripotency and mediates germline development', *Nature* 450(7173): 1230-4.
Chang, H. and Matzuk, M. M. (2001) 'Smad5 is required for mouse primordial germ cell development', *Mech Dev* 104(1-2): 61-7.
Chen, D., Liu, W., Lukianchikov, A., Hancock, G., Zimmerman, J., Lowe, M., Kim, R., Galic, Z., Irie, N., Surani, M. A. *et al*. (2017) 'Germline competency of human embryonic stem cells depends on EOMESODERMIN', *Biol Reprod*.
Chu, G. C., Dunn, N. R., Anderson, D. C., Oxburgh, L. and Robertson, E. J. (2004) 'Differential requirements for Smad4 in TGFbeta-dependent patterning of the early mouse embryo', *Development* 131(15): 3501-12.
Clark, A. T., Bodnar, M. S., Fox, M., Rodriquez, R. T., Abeyta, M. J., Firpo, M. T. and Pera, R. A. (2004) 'Spontaneous differentiation of germ cells from human embryonic stem cells in vitro', *Hum Mol Genet* 13(7): 727-39.
Costello, I., Nowotschin, S., Sun, X., Mould, A. W., Hadjantonakis, A. K., Bikoff, E. K. and Robertson, E. J. (2015) 'Lhx1 functions together with Otx2, Foxa2, and Ldb1 to govern anterior mesendoderm, node, and midline development', *Genes Dev* 29(20): 2108-22.
Culty, M. (2009) 'Gonocytes, the forgotten cells of the germ cell lineage', *Birth Defects Res C Embryo Today* 87(1): 1-26.
D'Amour, K. A., Agulnick, A. D., Eliazer, S., Kelly, O. G., Kroon, E. and Baetge, E. E. (2005) 'Efficient differentiation of human embryonic stem cells to definitive endoderm', *Nat Biotechnol* 23(12): 1534-41.
De Felici, M. (2013) *Origin, Migration, and Proliferation of Human Primordial Germ Cells*.
de Jong, J., Stoop, H., Gillis, A. J., van Gurp, R. J., van de Geijn, G. J., Boer, M., Hersmus, R., Saunders, P. T., Anderson, R. A., Oosterhuis, J. W. *et al*. (2008) 'Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications', *J Pathol* 215(1): 21-30.
de Sousa Lopes, S. M., Roelen, B. A., Monteiro, R. M., Emmens, R., Lin, H. Y., Li, E., Lawson, K. A. and Mummery, C. L. (2004) 'BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo', *Genes Dev* 18(15): 1838-49.
Deglincerti, A., Croft, G. F., Pietila, L. N., Zernicka-Goetz, M., Siggia, E. D. and Brivanlou, A. H. (2016) 'Self-organization of the in vitro attached human embryo', *Nature* 533(7602): 251-4.
Extavour, C. G. and Akam, M. (2003) 'Mechanisms of germ cell specification across the metazoans: epigenesis and preformation', *Development* 130(24): 5869-84.
Fabre, P. H., Hautier, L., Dimitrov, D. and Douzery, E. J. (2012) 'A glimpse on the pattern of rodent diversification: a phylogenetic approach', *BMC Evol Biol* 12: 88.

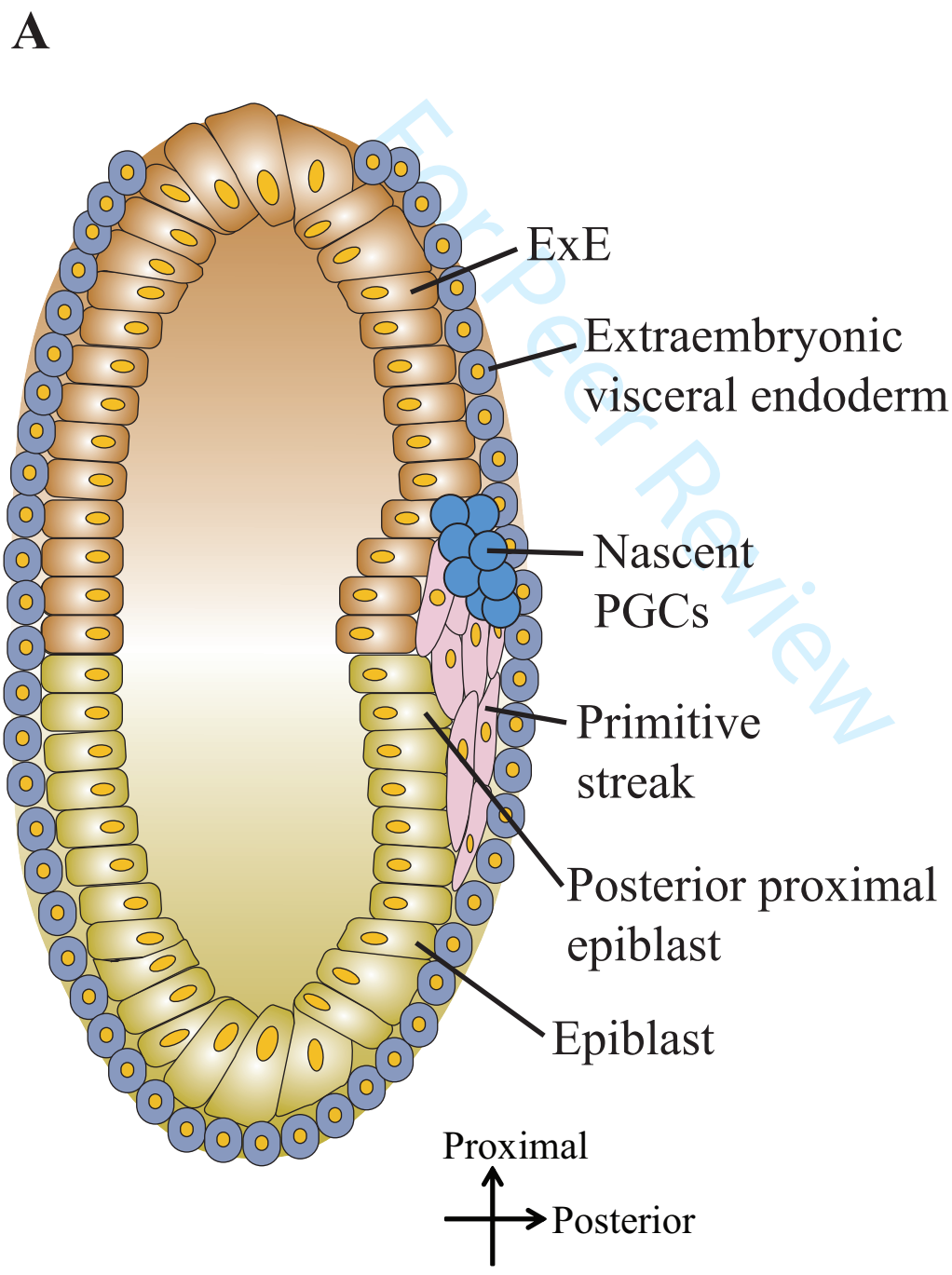
Festuccia, N., Osorno, R., Halbritter, F., Karwacki-Neisius, V., Navarro, P., Colby, D., Wong, F., Yates, A., Tomlinson, S. R. and Chambers, I. (2012) 'Esrrb is a direct Nanog target gene that can substitute for Nanog function in pluripotent cells', *Cell Stem Cell* 11(4): 477-90.
Gafni, O., Weinberger, L., Mansour, A. A., Manor, Y. S., Chomsky, E., Ben-Yosef, D., Kalma, Y., Viukov, S., Maza, I., Zviran, A. *et al*. (2013) 'Derivation of novel human ground state naive pluripotent stem cells', *Nature* 504(7479): 282-6.
Geijsen, N., Horoschak, M., Kim, K., Gribnau, J., Eggan, K. and Daley, G. Q. (2004) 'Derivation of embryonic germ cells and male gametes from embryonic stem cells', *Nature* 427(6970): 148-54.
Gillich, A., Bao, S., Grabole, N., Hayashi, K., Trotter, M. W., Pasque, V., Magnusdottir, E. and Surani, M. A. (2012) 'Epiblast stem cell-based system reveals reprogramming synergy of germline factors', *Cell Stem Cell* 10(4): 425-39.
Ginsburg, M., Snow, M. H. and McLaren, A. (1990) 'Primordial germ cells in the mouse embryo during gastrulation', *Development* 110(2): 521-8.
Gkountela, S., Li, Z., Vincent, J. J., Zhang, K. X., Chen, A., Pellegrini, M. and Clark, A. T. (2013) 'The ontogeny of cKIT+ human primordial germ cells proves to be a resource for human germ line reprogramming, imprint erasure and in vitro differentiation', *Nat Cell Biol* 15(1): 113-22.
Gkountela, S., Zhang, K. X., Shafiq, T. A., Liao, W. W., Hargan-Calvopina, J., Chen, P. Y. and Clark, A. T. (2015) 'DNA Demethylation Dynamics in the Human Prenatal Germline', *Cell* 161(6): 1425-36.
Grabole, N., Tischler, J., Hackett, J. A., Kim, S., Tang, F., Leitch, H. G., Magnusdottir, E. and Surani, M. A. (2013) 'Prdm14 promotes germline fate and naive pluripotency by repressing FGF signalling and DNA methylation', *EMBO Rep* 14(7): 629-37.
Guo, F., Yan, L., Guo, H., Li, L., Hu, B., Zhao, Y., Yong, J., Hu, Y., Wang, X., Wei, Y. *et al*. (2015) 'The Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells', *Cell* 161(6): 1437-52.
Hara, K., Kanai-Azuma, M., Uemura, M., Shitara, H., Taya, C., Yonekawa, H., Kawakami, H., Tsunekawa, N., Kurohmaru, M. and Kanai, Y. (2009) 'Evidence for crucial role of hindgut expansion in directing proper migration of primordial germ cells in mouse early embryogenesis', *Dev Biol* 330(2): 427-39.
Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S. and Saitou, M. (2011) 'Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells', *Cell* 146(4): 519-32.
Hayashi, K. and Surani, M. A. (2009) 'Self-renewing epiblast stem cells exhibit continual delineation of germ cells with epigenetic reprogramming in vitro', *Development* 136(21): 3549-56.
Hubner, K., Fuhrmann, G., Christenson, L. K., Kehler, J., Reinbold, R., De La Fuente, R., Wood, J., Strauss, J. F., 3rd, Boiani, M. and Scholer, H. R. (2003) 'Derivation of oocytes from mouse embryonic stem cells', *Science* 300(5623): 1251-6.
Irie, N. and Surani, M. A. (2017) 'Efficient Induction and Isolation of Human Primordial Germ Cell-Like Cells from Competent Human Pluripotent Stem Cells', *Methods Mol Biol* 1463: 217-226.
Irie, N., Tang, W. W. and Azim Surani, M. (2014) 'Germ cell specification and pluripotency in mammals: a perspective from early embryogenesis', *Reprod Med Biol* 13(4): 203-215.
Irie, N., Weinberger, L., Tang, W. W., Kobayashi, T., Viukov, S., Manor, Y. S., Dietmann, S., Hanna, J. H. and Surani, M. A. (2015) 'SOX17 is a critical specifier of human primordial germ cell fate', *Cell* 160(1-2): 253-68.
Ishii, T. and Saitou, M. (2017) 'Promoting In Vitro Gametogenesis Research with a Social Understanding', *Trends Mol Med* 23(11): 985-988.
Johnson, A. D. and Alberio, R. (2015) 'Primordial germ cells: the first cell lineage or the last cells standing?', *Development* 142(16): 2730-9.
Johnson, A. D., Drum, M., Bachvarova, R. F., Masi, T., White, M. E. and Crother, B. I. (2003) 'Evolution of predetermined germ cells in vertebrate embryos: implications for macroevolution', *Evol Dev* 5(4): 414-31.

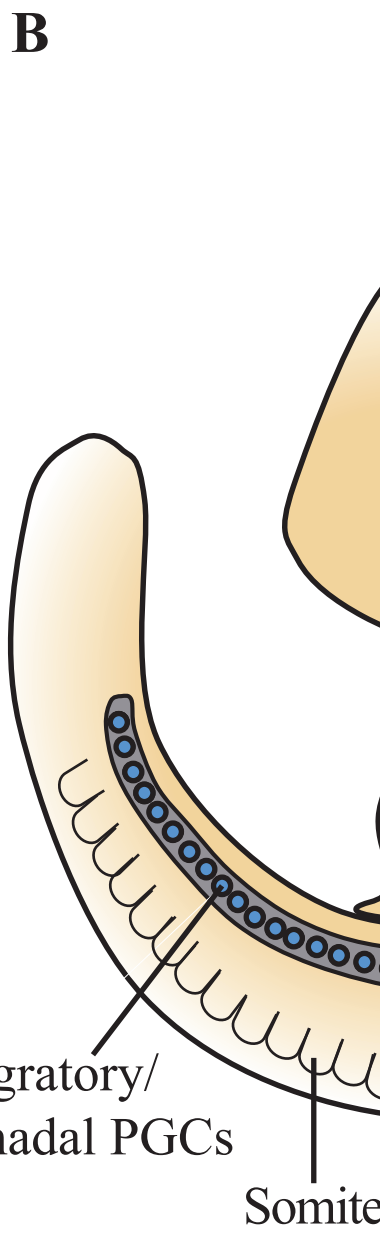
Kanai-Azuma, M., Kanai, Y., Gad, J. M., Tajima, Y., Taya, C., Kurohmaru, M., Sanai, Y., Yonekawa, H., Yazaki, K., Tam, P. P. *et al*. (2002) 'Depletion of definitive gut endoderm in Sox17-null mutant mice', *Development* 129(10): 2367-79.
Kee, K., Gonsalves, J. M., Clark, A. T. and Pera, R. A. (2006) 'Bone morphogenetic proteins induce germ cell differentiation from human embryonic stem cells', *Stem Cells Dev* 15(6): 831-7.
Kehler, J., Tolkunova, E., Koschorz, B., Pesce, M., Gentile, L., Boiani, M., Lomeli, H., Nagy, A., McLaughlin, K. J., Scholer, H. R. *et al*. (2004) 'Oct4 is required for primordial germ cell survival', *EMBO Rep* 5(11): 1078-83.
Kinder, S. J., Tsang, T. E., Quinlan, G. A., Hadjantonakis, A. K., Nagy, A. and Tam, P. P. (1999) 'The orderly allocation of mesodermal cells to the extraembryonic structures and the anteroposterior axis during gastrulation of the mouse embryo', *Development* 126(21): 4691-701.
Kobayashi, T., Zhang, H., Tang, W. W. C., Irie, N., Withey, S., Klisch, D., Sybirna, A., Dietmann, S., Contreras, D. A., Webb, R. *et al*. (2017) 'Principles of early human development and germ cell program from conserved model systems', *Nature* 546(7658): 416-420.
Kojima, Y., Kaufman-Francis, K., Studdert, J. B., Steiner, K. A., Power, M. D., Loebel, D. A., Jones, V., Hor, A., de Alencastro, G., Logan, G. J. *et al*. (2014) 'The transcriptional and functional properties of mouse epiblast stem cells resemble the anterior primitive streak', *Cell Stem Cell* 14(1): 107-20.
Kubo, A., Shinozaki, K., Shannon, J. M., Kouskoff, V., Kennedy, M., Woo, S., Fehling, H. J. and Keller, G. (2004) 'Development of definitive endoderm from embryonic stem cells in culture', *Development* 131(7): 1651-62.
Kurilo, L. F. (1981) 'Oogenesis in antenatal development in man', *Hum Genet* 57(1): 86-92.
Kurimoto, K., Yabuta, Y., Ohinata, Y., Shigeta, M., Yamanaka, K. and Saitou, M. (2008) 'Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice', *Genes Dev* 22(12): 1617-35.
Lawson, K. A., Dunn, N. R., Roelen, B. A., Zeinstra, L. M., Davis, A. M., Wright, C. V., Korving, J. P. and Hogan, B. L. (1999) 'Bmp4 is required for the generation of primordial germ cells in the mouse embryo', *Genes Dev* 13(4): 424-36.
Lawson, K. A. and Hage, W. J. (1994) 'Clonal analysis of the origin of primordial germ cells in the mouse', *Ciba Found Symp* 182: 68-84; discussion 84-91.
Leopardo, N. P. and Vitullo, A. D. (2017) 'Early embryonic development and spatiotemporal localization of mammalian primordial germ cell-associated proteins in the basal rodent Lagostomus maximus', *Sci Rep* 7(1): 594.
Liu, P., Wakamiya, M., Shea, M. J., Albrecht, U., Behringer, R. R. and Bradley, A. (1999) 'Requirement for Wnt3 in vertebrate axis formation', *Nat Genet* 22(4): 361-5.
Magnusdottir, E., Dietmann, S., Murakami, K., Gunesdogan, U., Tang, F., Bao, S., Diamanti, E., Lao, K., Gottgens, B. and Azim Surani, M. (2013) 'A tripartite transcription factor network regulates primordial germ cell specification in mice', *Nat Cell Biol* 15(8): 905-15.
McKay, D. G., Hertig, A. T., Adams, E. C. and Danziger, S. (1953) 'Histochemical observations on the germ cells of human embryos', *Anat Rec* 117(2): 201-19.
Mitsunaga, S., Odajima, J., Yawata, S., Shioda, K., Owa, C., Isselbacher, K. J., Hanna, J. H. and Shioda, T. (2017) 'Relevance of iPSC-derived human PGC-like cells at the surface of embryoid bodies to prechemotaxis migrating PGCs', *Proc Natl Acad Sci U S A*.
Molyneaux, K. and Wylie, C. (2004) 'Primordial germ cell migration', *Int J Dev Biol* 48(5-6): 537-44.
Molyneaux, K. A., Stallock, J., Schaible, K. and Wylie, C. (2001) 'Time-lapse analysis of living mouse germ cell migration', *Dev Biol* 240(2): 488-98.
Mummery, C. L., Zhang, J., Ng, E. S., Elliott, D. A., Elefanty, A. G. and Kamp, T. J. (2012) 'Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview', *Circ Res* 111(3): 344-58.

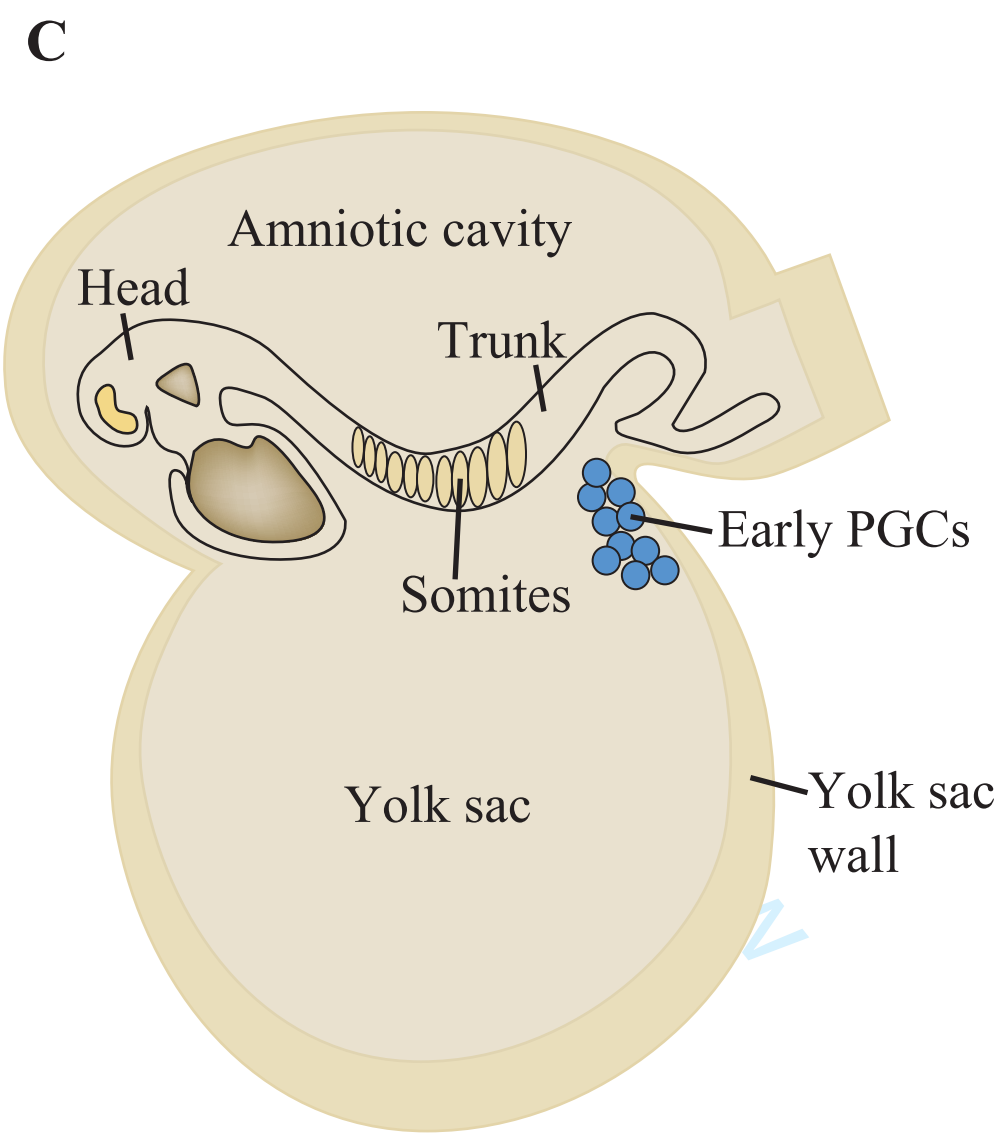
Murakami, K., Gunesdogan, U., Zylicz, J. J., Tang, W. W. C., Sengupta, R., Kobayashi, T., Kim, S., Butler, R., Dietmann, S. and Surani, M. A. (2016) 'NANOG alone induces germ cells in primed epiblast in vitro by activation of enhancers', *Nature* 529(7586): 403-407.
Nakaki, F., Hayashi, K., Ohta, H., Kurimoto, K., Yabuta, Y. and Saitou, M. (2013) 'Induction of mouse germ-cell fate by transcription factors in vitro', *Nature* 501(7466): 222-6.
Nichols, J. and Smith, A. (2009) 'Naive and primed pluripotent states', *Cell Stem Cell* 4(6): 487-92.
Ohinata, Y., Ohta, H., Shigeta, M., Yamanaka, K., Wakayama, T. and Saitou, M. (2009) 'A signaling principle for the specification of the germ cell lineage in mice', *Cell* 137(3): 571-84.
Ohinata, Y., Payer, B., O'Carroll, D., Ancelin, K., Ono, Y., Sano, M., Barton, S. C., Obukhanych, T., Nussenzweig, M., Tarakhovsky, A. *et al*. (2005) 'Blimp1 is a critical determinant of the germ cell lineage in mice', *Nature* 436(7048): 207-13.
Okamura, D., Tokitake, Y., Niwa, H. and Matsui, Y. (2008) 'Requirement of Oct3/4 function for germ cell specification', *Dev Biol* 317(2): 576-84.
Pilato, G., D'Urso, V., Viglianisi, F., Sammartano, F., Sabella, G. and Lisi, O. (2013) 'The problem of the origin of primordial germ cells (PGCs) in vertebrates: historical review and a possible solution', *Int J Dev Biol* 57(11-12): 809-19.
Rivera-Perez, J. A. and Magnuson, T. (2005) 'Primitive streak formation in mice is preceded by localized activation of Brachyury and Wnt3', *Dev Biol* 288(2): 363-71.
Rossant, J. (2015) 'Mouse and human blastocyst-derived stem cells: vive les differences', *Development* 142(1): 9-12.
Saitou, M. and Yamaji, M. (2012) 'Primordial germ cells in mice', *Cold Spring Harb Perspect Biol* 4(11).
Sarkar, A. and Hochedlinger, K. (2013) 'The sox family of transcription factors: versatile regulators of stem and progenitor cell fate', *Cell Stem Cell* 12(1): 15-30.
Sasaki, K., Nakamura, T., Okamoto, I., Yabuta, Y., Iwatani, C., Tsuchiya, H., Seita, Y., Nakamura, S., Shiraki, N., Takakuwa, T. *et al*. (2016) 'The Germ Cell Fate of Cynomolgus Monkeys Is Specified in the Nascent Amnion', *Dev Cell* 39(2): 169-185.
Sasaki, K., Yokobayashi, S., Nakamura, T., Okamoto, I., Yabuta, Y., Kurimoto, K., Ohta, H., Moritoki, Y., Iwatani, C., Tsuchiya, H. *et al*. (2015) 'Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells', *Cell Stem Cell* 17(2): 178-94.
Scholer, H. R., Dressler, G. R., Balling, R., Rohdewohld, H. and Gruss, P. (1990) 'Oct-4: a germline-specific transcription factor mapping to the mouse t-complex', *EMBO J* 9(7): 2185-95.
Shahbazi, M. N., Jedrusik, A., Vuoristo, S., Recher, G., Hupalowska, A., Bolton, V., Fogarty, N. N. M., Campbell, A., Devito, L., Ilic, D. *et al*. (2016) 'Self-organization of the human embryo in the absence of maternal tissues', *Nat Cell Biol* 18(6): 700-708.
Song, L., Chen, J., Peng, G., Tang, K. and Jing, N. (2016) 'Dynamic Heterogeneity of Brachyury in Mouse Epiblast Stem Cells Mediates Distinct Response to Extrinsic Bone Morphogenetic Protein (BMP) Signaling', *J Biol Chem* 291(29): 15212-25.
Sugawa, F., Arauzo-Bravo, M. J., Yoon, J., Kim, K. P., Aramaki, S., Wu, G., Stehling, M., Psathaki, O. E., Hubner, K. and Scholer, H. R. (2015) 'Human primordial germ cell commitment in vitro associates with a unique PRDM14 expression profile', *EMBO J* 34(8): 1009-24.
Tada, S., Era, T., Furusawa, C., Sakurai, H., Nishikawa, S., Kinoshita, M., Nakao, K. and Chiba, T. (2005) 'Characterization of mesendoderm: a diverging point of the definitive endoderm and mesoderm in embryonic stem cell differentiation culture', *Development* 132(19): 4363-74.
Tam, P. P. and Beddington, R. S. (1987) 'The formation of mesodermal tissues in the mouse embryo during gastrulation and early organogenesis', *Development* 99(1): 109-26.
Tam, P. P. and Snow, M. H. (1981) 'Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos', *J Embryol Exp Morphol* 64: 133-47.

Tang, W. W., Dietmann, S., Irie, N., Leitch, H. G., Floros, V. I., Bradshaw, C. R., Hackett, J. A., Chinnery, P. F. and Surani, M. A. (2015) 'A Unique Gene Regulatory Network Resets the Human Germline Epigenome for Development', *Cell* 161(6): 1453-67.
Toyooka, Y., Tsunekawa, N., Akasu, R. and Noce, T. (2003) 'Embryonic stem cells can form germ cells in vitro', *Proc Natl Acad Sci U S A* 100(20): 11457-62.
Tremblay, K. D., Dunn, N. R. and Robertson, E. J. (2001) 'Mouse embryos lacking Smad1 signals display defects in extra-embryonic tissues and germ cell formation', *Development* 128(18): 3609-21.
Tsuneyoshi, N., Sumi, T., Onda, H., Nojima, H., Nakatsuji, N. and Suemori, H. (2008) 'PRDM14 suppresses expression of differentiation marker genes in human embryonic stem cells', *Biochem Biophys Res Commun* 367(4): 899-905.
Turner, D. A., Rue, P., Mackenzie, J. P., Davies, E. and Martinez Arias, A. (2014) 'Brachyury cooperates with Wnt/beta-catenin signalling to elicit primitive-streak-like behaviour in differentiating mouse embryonic stem cells', *BMC Biol* 12: 63.
Tzouanacou, E., Wegener, A., Wymeersch, F. J., Wilson, V. and Nicolas, J. F. (2009) 'Redefining the progression of lineage segregations during mammalian embryogenesis by clonal analysis', *Dev Cell* 17(3): 365-76.
Vallier, L., Reynolds, D. and Pedersen, R. A. (2004) 'Nodal inhibits differentiation of human embryonic stem cells along the neuroectodermal default pathway', *Dev Biol* 275(2): 403-21.
Vincent, S. D., Dunn, N. R., Sciammas, R., Shapiro-Shalef, M., Davis, M. M., Calame, K., Bikoff, E. K. and Robertson, E. J. (2005) 'The zinc finger transcriptional repressor Blimp1/Prdm1 is dispensable for early axis formation but is required for specification of primordial germ cells in the mouse', *Development* 132(6): 1315-25.
von Meyenn, F., Berrens, R. V., Andrews, S., Santos, F., Collier, A. J., Krueger, F., Osorno, R., Dean, W., Rugg-Gunn, P. J. and Reik, W. (2016) 'Comparative Principles of DNA Methylation Reprogramming during Human and Mouse In Vitro Primordial Germ Cell Specification', *Dev Cell* 39(1): 104-115.
Wang, H., Xiang, J., Zhang, W., Li, J., Wei, Q., Zhong, L., Ouyang, H. and Han, J. (2016) 'Induction of Germ Cell-like Cells from Porcine Induced Pluripotent Stem Cells', *Sci Rep* 6: 27256.
Wang, Q., Zou, Y., Nowotschin, S., Kim, S. Y., Li, Q. V., Soh, C. L., Su, J., Zhang, C., Shu, W., Xi, Q. *et al*. (2017) 'The p53 Family Coordinates Wnt and Nodal Inputs in Mesendodermal Differentiation of Embryonic Stem Cells', *Cell Stem Cell* 20(1): 70-86.
Weber, S., Eckert, D., Nettersheim, D., Gillis, A. J., Schafer, S., Kuckenberg, P., Ehlermann, J., Werling, U., Biermann, K., Looijenga, L. H. *et al*. (2010) 'Critical function of AP-2 gamma/TCFAP2C in mouse embryonic germ cell maintenance', *Biol Reprod* 82(1): 214-23.
Weismann, A., Parker, W. N. and Rōnníeldt, H. (1893) *The germ-plasm: a theory of heredity*, New York,: C. Scribner's sons.
Whittle, C. A. and Extavour, C. G. (2017) 'Causes and evolutionary consequences of primordial germ-cell specification mode in metazoans', *Proc Natl Acad Sci U S A* 114(23): 5784-5791.
Yabuta, Y., Kurimoto, K., Ohinata, Y., Seki, Y. and Saitou, M. (2006) 'Gene expression dynamics during germline specification in mice identified by quantitative single-cell gene expression profiling', *Biol Reprod* 75(5): 705-16.
Yamaguchi, S., Kurimoto, K., Yabuta, Y., Sasaki, H., Nakatsuji, N., Saitou, M. and Tada, T. (2009) 'Conditional knockdown of Nanog induces apoptotic cell death in mouse migrating primordial germ cells', *Development* 136(23): 4011-20.
Yamaji, M., Seki, Y., Kurimoto, K., Yabuta, Y., Yuasa, M., Shigeta, M., Yamanaka, K., Ohinata, Y. and Saitou, M. (2008) 'Critical function of Prdm14 for the establishment of the germ cell lineage in mice', *Nat Genet* 40(8): 1016-22.
Ying, Y., Liu, X. M., Marble, A., Lawson, K. A. and Zhao, G. Q. (2000) 'Requirement of Bmp8b for the generation of primordial germ cells in the mouse', *Mol Endocrinol* 14(7): 1053-63.

Ying, Y. and Zhao, G. Q. (2001) 'Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse', *Dev Biol* 232(2): 484-92.
Yokobayashi, S., Okita, K., Nakagawa, M., Nakamura, T., Yabuta, Y., Yamamoto, T. and Saitou, M. (2017) 'Clonal variation of human induced pluripotent stem cells for induction into the germ cell fate', *Biol Reprod* 96(6): 1154-1166.
Zhang, M., Leitch, H. G., Tang, W. W. C., Festuccia, N., Hall-Ponsele, E., Nichols, J., Surani, M. A., Smith, A. and Chambers, I. (2018) 'Esrrb Complementation Rescues Development of Nanog-Null Germ Cells', *Cell Rep* 22(2): 332-339.






C
Amniotic cavity
Head
Trunk
Somites
Early PGCs
Yolk sac
Yolk sac wall
D
Tru