How to make a primordial germ cell
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ABSTRACT
Primordial germ cells (PGCs) are the precursors of sperm and eggs, which generate a new organism that is capable of creating endless new generations through germ cells. PGCs are specified during early mammalian postimplantation development, and are uniquely programmed for transmission of genetic and epigenetic information to subsequent generations. In this Primer, we summarise the establishment of the fundamental principles of PGC specification during early development and discuss how it is now possible to make mouse PGCs from pluripotent embryonic stem cells, and indeed somatic cells if they are first rendered pluripotent in culture.

KEY WORDS: Epigenetic programming, Primordial germ cells, Specification, Transcription factors

Introduction
Primordial germ cells are highly specialised cells that are precursors of gametes, which, following meiosis, develop as haploid sperm and eggs that generate a new organism upon fertilisation. They transmit genetic and epigenetic information between generations and ensure the survival of a species. Although germ cells are set aside during early development in almost all animals, the mechanism of germ cell specification is not conserved among animals. Typically, specification of germ cells occurs either through the inheritance of preformed germ plasm (Weissmann, 1885), or is induced among equipotent cells by instructive signals. For example, germ cell specification in Xenopus and C. elegans occurs via the inheritance of germ plasm, whereas, in axolotls, germ cell specification occurs in animal cap cells in response to signals (Johnson et al., 2011; Niewkoop, 1979). In principle, both these mechanisms ensure suppression of somatic fate while promoting the onset of the germ cell programme. In mice, primordial germ cells (PGCs) originate from the early postimplantation epiblast cells, which also give rise to all somatic cells in response to signals from the extraembryonic tissues.

Mammalian sperm and eggs make an equal genetic contribution to a new organism, but their epigenetic contributions are unique and complementary. Both a male and a female genome are necessary for development to term because of the parent of origin-dependent ‘imprinting’ of the genome in the germ line (McGrath and Solter, 1984; Surani et al., 1984). Investigations of mammalian germ cells provide a unique insight into how epigenetic information with respect to ‘imprints’ is first erased and then re-initiated, and becomes heritable from the germ line into adulthood. Imprinting results in the parent of origin-dependent monoallelic expression of imprinted genes during development. Faulty imprints can lead to developmental, physiological and behavioural anomalies in mice, and result in diseases in humans (reviewed by Grossniklaus et al., 2013). The process of robust erasure and resetting of the epigenome in early PGCs also ensures that aberrant epigenetic information is not transmitted to the offspring. Nonetheless, there are recent reports suggesting that environmental factors can induce epigenetic changes that can be transmitted through the germline to subsequent generations with detrimental consequences (reviewed by Tomizawa and Sasaki, 2012). Studies on the germ cell lineage might test the validity of these claims or provide the mechanistic basis for them.

The resetting of the germine epigenome is also crucial for the establishment of the totipotent state following fertilisation. The underlying mechanisms involved may be applicable to the experimental reprogramming of the epigenome and to the manipulation of cell fates in vitro, and potentially to the reprogramming of the endogenous cells of diseased tissues. This has important implications for regenerative medicine and human diseases, including germ cell tumours. Germ cells also provide opportunities with which to uncover the mechanisms of chromatin modifications, as well as of DNA methylation/demethylation. How the factors involved induce significant epigenetic changes might be useful in other contexts during normal and aberrant development, and could lead to the development of novel therapeutic agents.

More directly, investigation of germ cells could lead to advances in reproductive medicine. For example, defective mitochondria transmitted by oocytes have major health implications; how they accumulate and are transmitted thereafter is of particular interest, as are the other causes of infertility. Advances in germ cell biology and genome editing, together with the ability to generate germ cells from pluripotent stem cells might lead to advances in assisted reproduction in some mammals.

Major advances over the past decade have been the identification of key regulators of PGC specification (reviewed by Leitch et al., 2013a), how the genetic network functions during PGC specification and during the onset of a unique epigenetic programme (Magnúsdóttir et al., 2013), and the establishment of an epigenetic ground state and the initiation of the imprinting cycle (Hackett et al., 2012; Hayashi et al., 2007). Here, we focus on the mechanism of PGC specification in mice, which is currently the best-characterised germ cell lineage among mammals. Its characterisation has led to the development of experimental systems that can be used to generate PGC-like cells from pluripotent stem cells, and potentially from any somatic cell via induced pluripotent stem cells (iPSCs), which in turn can give rise to viable gametes in vitro that are capable of generating a live organism (Hayashi and Surani, 2009; Hayashi et al., 2011; Hayashi et al., 2012). We are currently able to generate early germ cells and rudimentary oocyte-like structures in vitro (Hayashi and Surani, 2009), but further advances might lead to the ability to induce meiosis and advanced development of gametes in vitro.
The instructive nature of PGC specification in mice

The classical work on clonal analysis by Lawson and Hage showed that the progenitors of PGCs are located in the most proximal region of the postimplantation epiblast, close to the extraembryonic tissues (Fig. 1). These PGC precursors are detected here at embryonic day (E) 6.0-6.5, and eventually generate a founding population of ~40 PGCs at ~E7.25 (Chiquoine, 1954; Ginsburg et al., 1990; Lawson and Hage, 1994).

The key instructive signals for PGC specification are the bone morphogenetic proteins (BMP2, BMP4 and BMP8B), which originate from the extraembryonic tissues and act through SMAD1 and SMAD5 (Hayashi et al., 2002; Lawson et al., 1999; Tam and Snow, 1981; Ying and Zhao, 2001; Ying et al., 2000). The response of epiblast cells to PGC specification is BMP dose dependent in vitro (Lawson et al., 1999), as the number of PGCs decreases in proportion to the loss of BMP2 and BMP4 alleles. In vitro, BMP4 is sufficient for PGC induction, whereas WNT3 is required to induce competence for PGC fate in epiblast cells and enable their appropriate response to BMP signalling towards PGC specification (Ohinata et al., 2009). In principle, any postimplantation epiblast cell at this time of development can potentially acquire PGC fate under appropriate conditions, although this response is normally restricted in vivo by diverse signalling gradients, not only of the inductive BMP signals but also gradients of inhibitory signals from the distal and anterior visceral endoderm, such as cerberus 1 (CER1), dickkopf 1 (DKK1) and LEFTY1 (Ohinata et al., 2009; Tam and Zhou, 1996) (Fig. 1).

Intrinsic regulators of mouse PGC fate

The genetic basis for PGC specification has emerged only over the past decade following attempts to discover the key regulators of PGC fate by single cell analysis. Among the first genes to be identified was developmental pluripotency-associated 3 ([Dppa3 (previously Stella)] as the definitive marker of founder PGCs (Saitou et al., 2002; Sato et al., 2002), followed by Prdm1, which encodes BLIMP1, a PR domain zinc-finger protein that is a key regulator of PGC specification (Chang et al., 2002; Hayashi et al., 2007; Ohinata et al., 2005; Vincent et al., 2005). Expression of BLIMP1 in a few randomly distributed most proximal epiblast cells at ~E6.25 marks the onset of PGC specification, which fits with the origin of PGCs by clonal analysis (Lawson and Hage, 1994). BLIMP1 expression demarcates germ cells from the neighbouring somatic cells through the repression of the incipient mesodermal programme (Hayashi et al., 2007; Kurimoto et al., 2008; Ohinata et al., 2005). A mutation in BLIMP1 resulted in a small cluster of aberrant PGC-like cells at ~E8.5 that were more like the neighbouring somatic cells due to a failure to repress somatic gene expression and to induce PGC-specific genes (Ohinata et al., 2005).

A comparison between wild-type and mutant BLIMP1 PGC-like cells was subsequently pivotal for the identification of Prdm14 (a

**Fig. 1. Specification of primordial germ cells.** Primordial germ cells (PGCs) originate from the postimplantation epiblast cells in vivo during embryonic days (E) ~6.5-7.5 (in vivo column) in response to BMP4 from the extra-embryonic ectoderm (ExE), with BMP2 from the visceral endoderm (VE) (left). They bind to receptors that phosphorylate SMAD1 and SMAD5, which dimerise with SMAD4, translocate to the nucleus and induce the key transcriptional regulators of PGCs. These events are recapitulated in vitro (in vitro column) with naïve pluripotent embryonic stem cells (ESCs), which acquire a primed competent state on day 2 when cultured in activin/FGF2. PGCs can be induced in these cells either by cytokines or directly by BLIMP1, PRDM14 and AP2γ, a helix-loop-helix transcription factor; BLIMP1 (PRDM1), a transcription factor with a PR and ZNF domain; BMP, bone morphogenetic protein; EGF, epidermal growth factor; FGF, fibroblast growth factor; ICM, inner cell mass; LIF, leukaemia inhibitory factor; PRDM14, transcription factor with a PR and ZNF domain; SCF, stem cell factor.
member of the Prdm family) (Grabole et al., 2013; Kurimoto et al., 2008; Yamaji et al., 2008). A mutation in Prdm14 led to the formation of aberrant PGCs that were lost by ~E11.5. The cells exhibited a defective epigenetic programme, as judged by the failure of the characteristic global erasure of H3K9me2 histone modification at ~E8.5, partly because the Ezh1-Ezh2-mediated H3K9 methylation activity was not repressed. The cells also failed to show genome-wide induction of the polycomb enzyme EZH2-mediated H3K27me3. This suggests that PRDM14 is important at least for the epigenetic programme in early germ cells (Hajkova et al., 2008; Hajkova et al., 2010; Yamaji et al., 2008; Seki et al., 2007). Furthermore, BLIMP1 expression is sustained by PRDM14, which might explain the induction of somatic genes in mutant cells (Grabole et al., 2013; Magnusdottir et al., 2012; Magnusdottir et al., 2013). PRDM14 also induces Dppa3 and Sox2 at ~E8.5. Thus, neither PGC-specific gene expression nor the re-initiation of pluripotency gene expression occurred in Prdm14-null cells (Grabole et al., 2013; Yamaji et al., 2008).

Finally, Tcfap2c, which encodes AP2γ (a direct target of BLIMP1), is also crucial for PGC specification (Kurimoto et al., 2008; Magnusdottir et al., 2013; Weber et al., 2010) because a mutation in this gene causes very early loss of PGCs. Although these cells remain to be fully characterised, it is possible that AP2γ might also be involved in the repression of somatic genes, including early mesodermal marker Hoxb1 (Weber et al., 2010). Thus, BLIMP1, PRDM14 and AP2γ together constitute a mutually interdependent transcriptional network for PGC specification (Magnusdottir et al., 2013; Nakaki et al., 2013).

PGCs versus soma: the underlying pluripotency of the unipotent germ cell lineage

Evidence suggests that at the onset of PGC specification, the postimplantation epiblast cells are destined for somatic fate, with an increase in DNA methylation and H3K9me2 histone modification, including the onset of X inactivation (Hackett et al., 2013); pluripotency genes, such as Nanog, Rex1 and Dppa3 are also repressed. BLIMP1 arrests this trend towards somatic fate at the onset of PGC specification, and initiates a reversion of some of these properties, including initiation of X-reactivation and re-expression of key pluripotency genes, such as Sox2 and Nanog.

Comprehensive re-expression of pluripotency genes is unique to cells that adopt PGC fate, which is evident to only a limited extent in somatic lineages as in neuronal progenitors where Sox2 promotes cell identity (Graham et al., 2003). The expression of pluripotency genes in PGCs is likely permissive for the initiation of an epigenetic programme, such as DNA demethylation (Hackett et al., 2013; Leitch et al., 2013b; Seisenberger et al., 2012). Despite the similarity to the inner cell mass (ICM) and embryonic stem cells (ESCs) with respect to the expression of pluripotency genes, PGCs are unique because they exhibit the imprinting cycle and have the potential for meiosis (reviewed by Surani et al., 2007). Genes such as Nanog are apparently necessary for the maintenance of early germ cells because mutant cells undergo apoptosis during migration (Chambers et al., 2007; Yamaguchi et al., 2009). OCT4 might have a role in establishing competence for PGC fate and thereafter, perhaps by priming of the appropriate enhancers (Kehler et al., 2004; Soufi et al., 2012). Expression of OCT4 in PGCs depends on the activation of its distal enhancer (Bao et al., 2009; Chen et al., 2008; Yeom et al., 1996), reflecting the key attributes of underlying pluripotency in PGCs, although they develop into only gametes, sperm and eggs.

Early PGCs can, however, undergo dedifferentiation into pluripotent embryonic germ cells (EGCs) in response to fibroblast growth factor (FGF)/leukaemia inhibitory factor (LIF) signalling in culture (Matsui et al., 1992). EGCs resemble ESCs, probably owing to the ‘erasure’ of the epigenetic memory of their origin from the postimplantation epiblast. Dedifferentiation of PGCs to EGCs entails rapid repression of BLIMP1 (Durcova-Hills et al., 2008; Leitch et al., 2010), suggesting that BLIMP1 might have a role in lineage restriction in PGCs; its repression is essential for the re-expression of Myc and Klf4, which are repressed in PGCs but are essential for the self-renewal of EGCs and ESCs (Durcova-Hills et al., 2008).

The tripartite transcriptional network for PGC specification

The tripartite network of BLIMP1, AP2γ and PRDM14 is important not only for the initiation of PGC specification, but also for the unfolding epigenetic programme that ensues. To identify direct targets of BLIMP1 and AP2γ, the embryonal carcinoma cell line P19 (P19EC) was used as a surrogate system because PGCs are limited in number and difficult to culture and manipulate. P19EC cells are appropriate because they originate from postimplantation epiblast cells, the precursors of PGCs (McBurney, 1993; Spivakov and Fisher, 2007). Notably, expression of BLIMP1, PRDM14 and AP2γ in P19EC cells, both individually and in different combinations, elicits expression of several PGC genes and induces repression of somatic genes (Magnusdottir et al., 2013).

Bioinformatics analysis has provided a wealth of information on the potential targets of the tripartite transcriptional network. Single cell transcriptome data of normal and mutant PGCs, combined with the information on the targets of BLIMP1 and AP2γ in P19EC, as well as of PRDM14 in ESCs (Ma et al., 2011), has revealed that the germ cell programme is executed by a high degree of cooperative activity between the three factors. This enables initiation of several programmes in early PGCs, including the repression of somatic genes. For example, the migratory programme, as well as expression of PGC-specific genes, is executed mainly by PRDM14 and AP2γ, whereas PRDM14 and BLIMP1 collaborate in both the induction and repression of epigenetic modifiers.

The majority of the AP2γ-bound genes were also bound by BLIMP1 and PRDM14 (Fig. 2), to facilitate changes in gene expression (Fig. 3). AP2γ is enriched both on distal elements as well as on promoters, unlike BLIMP1, which is predominantly bound to promoters, whereas PRDM14 binds mainly to distal regulatory regions (Ma et al., 2011). It is possible that PRDM14 and BLIMP1 make the initial engagement with genomic binding sites to initiate transcriptional changes, which are then ‘locked in’ by AP2γ (Fig. 2C). BLIMP1 also binds to cell cycle and multiple transcriptional regulators, including all four Hox gene loci (HoxA, HoxB, HoxC and HoxD), possibly to ‘protect’ PGCs with their underlying pluripotency from responding to various extrinsic signals as they migrate to the gonads.

Activation of the PGC programme

A pivotal role of BLIMP1 is to induce Tcfap2c expression directly (Kurimoto et al., 2008; Magnusdottir et al., 2013; Weber et al., 2010). How BLIMP1 can both repress and induce different targets is unknown, but it might recruit transcriptional co-repressors and co-activators under some specific conditions (Ancelin et al., 2006; Ren et al., 1999; Su et al., 2009; Yu et al., 2000). Notably, in zebrafish, BLIMP1 directly activates Tfap2a (encoding AP2a), which is crucial for the specification of neural crest cells (Powell et al., 2013). The induction of AP2γ by BLIMP1 is crucial for the combinatorial role of PRDM14 and AP2γ in the induction of PGC-specific genes such as Dnd1, as well as Nanos3 (Fig. 3B). They also bind to the
distal enhancer of *Pou5f1* (previously *Oct4*), to maintain its expression in PGCs. Overall, PRDM14 probably contributes to epigenetic reprogramming in PGCs, as shown by its potential for reprogramming of epiblast stem cells (EpiSCs) to naive ESCs (Gillich et al., 2012). PRDM14 alone and in collaboration with AP2γ regulates a multitude of genes involved in cell-cell adhesion and migration (Magnúsdóttir et al., 2013; Ma et al., 2011).

**The genetic network for PGC specification initiates the epigenetic programme**

The epigenetic programme induced primarily by BLIMP1 and PRDM14 leads to global DNA demethylation towards an epigenetic ground state in early PGCs (Hackett et al., 2013; Hajkova et al., 2008; Hajkova et al., 2010; Ohno et al., 2013; Seisenberger et al., 2012) (Fig. 4). For example, the repression of *Uhrf1* (Bostick et al., 2007) and *Dmmt3b* by BLIMP1 and PRDM14 promotes DNA replication-coupled DNA demethylation in PGCs. Additionally, TET1 and TET2, which are bound by PRDM14, catalyse hydroxylation of 5-methyl-cytosine, which provides an additional parallel redundant mechanism for DNA demethylation in PGCs (Hackett et al., 2013; Ma et al., 2011; Ohno et al., 2013; Seisenberger et al., 2012). There is potentially a role for a base excision repair mechanism (Hajkova et al., 2008) that could contribute to the erasure of imprints. Although PRDM14 contributes to DNA hypomethylation in naïve pluripotent ESCs, imprints are retained in these cells (Leitch et al., 2013b).

The transcriptional network for PGC specification also regulates histone modifications, as PRDM14 induces H3K9 histone demethylases Kdm3a and Kdm4b. This induction, together with the repression of *Ehmt1*, ensures rapid and global loss of H3K9me2, and could account for the re-expression of pluripotency genes and promote DNA demethylation (Liu et al., 2013; Rothbart et al., 2012). The repression of *Kdm6b* demethylase by PRDM14 and BLIMP1 contributes to the global increase in H3K27me, which is also seen in the inner cell mass of blastocysts. Furthermore, PRDM14 induces *Hdac6*, which, unlike its orthologues, does not seem to participate in transcriptional regulation, but promotes cell mobility, an important aspect of PGC biology (Li et al., 2013). The re-activation of the inactive X-chromosome in PGCs might be facilitated by PRDM14 binding to intron 1 of *Xist* and to *Rnf12* (*Rlim* – Mouse Genome Informatics), an activator of *Xist* (Barakat et al., 2011; Ma et al., 2011; Shin et al., 2010).

**Generating PGCs in vitro: recapitulating development**

The increasing knowledge of PGC specification in *vivo* has led to attempts to mimic the process *in vitro* (Hayashi and Surani, 2009; Hayashi et al., 2011; Magnúsdóttir et al., 2013; Matsui et al., 1992; Nakaki et al., 2013; Ohinata et al., 2009; Ohinata et al., 2009; Tam and Zhou, 1996). Initial attempts were made with the whole postimplantation epiblast, with or without the visceral endoderm (VE) still attached, as well as with or without the extraembryonic ectoderm (ExE), which was cultured in the presence of cytokines (Lawson et al., 1999). These studies were then comprehensively validated using *Prdm1*, *Prdm14* and *Dppa3* promoters as reporters (Ohinata et al., 2009), which established that most, if not all, epiblast cells are competent for PGC fate in the presence of BMP4. The efficiency increased with stem
cell factor (SCF), epidermal growth factor (EGF), LIF and BMP8B, partly due to increased cell viability in vitro because some factors, such as LIF, are not implicated in PGC specification in vivo. The efficiency increased when the VE and ExE were removed. This response to cytokines for PGC specification was predictably abrogated in the absence of SMAD1 and SMAD5, eliminating the possibility of indirect effects of BMP signalling molecules in PGC induction. The in vitro generated PGCs could develop into viable gametes in vivo that were able to give rise to viable offspring (Ohinata et al., 2009).

Importantly PGC-like cells could also be generated starting with naïve pluripotent ESCs that were first cultured for 2 days in basic FGF (bFGF) and activin A, followed by other cytokines, including BMP4, BMP8B, SCF, EGF and LIF, to obtain PGC-like cells (Hayashi et al., 2011). BMP4 is probably the most important cytokine for the induction of PGCs, whereas other factors, such as LIF and SCF, aid the survival of PGCs, which currently can be maintained for only 5-6 days in vitro. The initial culture of ESCs in bFGF and activin A signalling molecules drives them towards a postimplantation epiblast character (Guo et al., 2009). These epiblast-like cells (EpiLCs) form embryoid bodies when cultured as cell aggregates on non-adherent surfaces. The embryoid bodies respond to BMP4 and other cytokines and start to develop as PGC-like cells from day 2 onwards, resulting in 40-60% of cells...
becoming PGCs after about 6 days (Fig. 1). These in vitro-generated PGCs can develop into viable sperm and oocytes (Hayashi et al., 2011; Hayashi et al., 2012). Although this in vitro method is appropriate for studying PGC specification, these cells do not exhibit imprint erasure or enter meiosis in vitro, whereas they can undergo gametogenesis in vivo when introduced back into the gonads of fully grown animals (Hayashi et al., 2011; Hayashi et al., 2012).

The PGC in vitro culture system has allowed the interrogation of several key regulators of PGC specification. In recent studies, two independent groups forced the expression of BLIMP1, AP2γ and PRDM14, and found that these proteins could directly induce PGC-like fate in vitro in the absence of cytokines (Magnúsdóttir et al., 2013; Nakaki et al., 2013). This is consistent with the role of these proteins in vivo (summarised in Fig. 3). Importantly, the somatic programme was not upregulated in the absence of cytokines during the direct induction of the PGC-like cells (Magnúsdóttir et al., 2013; Nakaki et al., 2013). Although PRDM14 alone was also able to induce PGC-like cells at a low frequency in vitro (Nakaki et al., 2013), this apparently occurred concomitant with the induction of endogenous Prdm1 and Tfat2c (the gene encoding AP2γ). This does not rule out the possibility that all three factors are necessary for PGC fate. The absence of somatic gene upregulation during the direct induction of PGC-like fate does not obviate the need for BLIMP1 in PGC specification in vitro, in part because it directly induces AP2γ, as well as inducing the epigenetic programme together with PRDM14. Thus, the role of BLIMP1 extends beyond the repression somatic genes per se. For example, BLIMP1 and PRDM14 together are essential for the repression of Uhrf1, which is crucial for DNA demethylation in PGCs and for the re-expression of pluripotency genes (Magnúsdóttir et al., 2013) (Fig. 4B). Furthermore, BLIMP1 represses Myc, which permits exit from pluripotency and development of the restricted but dynamic nature of the early germ cell lineage (Lin et al., 2012).

Conclusions

Investigations from over a decade of research have led to major advances in mouse germ cell biology, which will provide the basis for studies on germ cells of mammalian species, including humans. The identification of the key regulators of PGC specification, BLIMP1, PRDM14 and AP2γ, which together constitute the tripartite genetic network for specification PGCs, represents an important advance. There is also a greater and precise understanding of their combinatorial roles in the repression of genes of the somatic programme, induction of the germ cell genes and in the initiation of epigenetic modifications in early germ cells. BLIMP1 is expressed first and it is the key regulator of PGC specification, not least because it induces AP2γ, and together with PRDM14, they control all the major aspects of PGC specification and the key attributes of early germ cells, such as the epigenetic programme (Magnúsdóttir et al., 2013; Nakaki et al., 2013; Ohinata et al., 2005; Weber et al., 2010; Yamaji et al., 2008). The germine also represents an elegant system combining an underlying pluripotency and lineage restriction that is critically balanced by the co-expression of the appropriate transcriptional regulators.

The major advances in mammalian germ cell biology have led to the exploration of PGC specification from pluripotent stem cells. These PGC-like cells can progress towards the formation of rudimentary oocyte-like structures (Hayashi and Surani, 2009). Starting with naïve ESCs, such PGC-like cells are formed, although they do not exhibit some key properties such as the erasure of imprints or meiosis, but can undergo gametogenesis under appropriate conditions in vivo to form viable gametes that are capable of generating a live organism (Hayashi et al., 2011; Hayashi et al., 2012). Notably, the three key regulators of PGC specification can directly induce PGC-like fate in vivo in the absence of cytokines (Magnúsdóttir et al., 2013; Nakaki et al., 2013), although such direct induction of PGC-like cells occurs only from the appropriately primed epiblast cells but not from the naïve ESCs. This suggests the importance of appropriate priming of cells for PGC fate. Indeed, loss of competence also occurs in the pluripotent EpiSC, which is due to the increase in DNA methylation and includes the promoters of PGC genes (Bao et al., 2009; Gillich et al., 2012). These in vivo models might be useful for investigating the molecular prerequisites of competence for PGC-like fate and its counterpart, the somatic fate. It is also possible to induce PGC-like fate in iPSCs derived from somatic cells. This might make it possible to generate viable gametes from somatic cells, perhaps by direct transdifferentiation of somatic cells into germ cells with the three key regulators of PGCs.

Further studies will show how some mutations, both naturally occurring and introduced, influence the development and properties of the germ cell lineage. This may allow investigation into the long-term consequences of transgenerational inheritance of genetic and epigenetic information. The causes of some forms of infertility might also be uncovered, as well as the factors implicated in germ
cell tumours such that amelioration of these diseases may one day be possible.

Finally, the comprehensive epigenetic programming, including global DNA demethylation, observed in early germ cells provides a unique opportunity with which to gain knowledge of key enzymes and epigenetic modifiers that promote the epigenetic ground state (Hackett et al., 2013; Hajkova et al., 2008; Hajkova et al., 2010; Ng et al., 2013). These mechanisms might be applicable more widely for the experimental manipulation of the epigenome and cell fates of normal and diseased tissues, through the erasure and re-initiation of novel epigenetic information.

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Competing interests

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