Developmental competence for the primordial germ cell fate

Ufuk Günesdogan and M. Azim Surani

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

Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK.

Correspondence: u.gunesdogan@gurdon.cam.ac.uk and a.surani@gurdon.cam.ac.uk

Abstract

During mammalian embryonic development, the trophectoderm and primitive endoderm give rise to extraembryonic tissues, while the epiblast differentiates into all somatic lineages and the germline. Remarkably, only a few classes of signalling pathways induce the differentiation of these progenitor cells into diverse lineages. Accordingly, the functional outcome of a particular signal depends on the developmental competence of the target cells. Thus, developmental competence can be defined as the ability of a cell to integrate intrinsic and extrinsic cues to execute a specific developmental program towards a specific cell fate. Downstream of signalling, there is the combinatorial activity of transcription factors and their co-factors, which is modulated by the chromatin state of the target cells. Here we discuss the concept of developmental competence, and the factors that regulate this state with reference to the specification of mouse primordial germ cells.

1. Introduction

Although every cell in the organism harbours the same genetic information, each cell fate decision requires only a specific set of genes, which defines its form and function. Thus, a fundamental question in biology is how the genetic network in subsets of cells is differentially controlled to orchestrate the development of a whole organism. This involves the context-dependent role of key transcription factors, which bind to *cis*-regulatory DNA sequences to regulate cell fates.

Signalling pathways directly or indirectly control the activity and/or expression of transcription factors. Given the complexity of a developing embryo, it is intriguing that only a few classes of signalling pathways are required to drive the differentiation of all cell types of an organism. In the mammalian post-implantation epiblast, bone morphogenetic protein (BMP) and WNT signalling are not only required for the induction of mesoderm fate (Winnier et al., 1995), but also for the specification of primordial germ cells (PGCs) (Aramaki et al., 2013; Lawson et al., 1999; Ohinata et al., 2009), which are the precursors of the gametes. In prospective PGCs, the signals induce the expression of *Prdm1* (encoding BLIMP1), *Prdm14* and Tfap2c (encoding AP2ɣ). Each of these factors has distinct context-dependent roles in development, but in appropriately competent cells, the combination of them drives a PGC-specific transcriptional program.

The specification of PGCs is one example of the intricate interplay of intrinsic and extrinsic signals that results in the activation of signal-dependent transcription factors, which in turn can modulate the chromatin state of the target cells and activate the expression of lineage-specific transcription factors. Their combinatorial action is modified by co-factors and the chromatin state of their binding sites, suggesting that signalling pathways, the proteome and the chromatin state facilitate developmental competence.

Here we provide an overview of the factors that influence the acquisition of developmental competence during the specification of primordial germ cells (PGCs) from the posterior proximal epiblast in embryos. We also discuss *in vitro* model systems, which recapitulate these events starting with naïve pluripotent stem cells. We refer to mouse development unless stated otherwise. We first consider the acquisition of developmental competence of post-implantation epiblast cells towards both the mesoderm and PGC fate, and summarize in this context the complex

interplay of signalling pathways. We then discuss the combinatorial role of the key transcription factors of the PGC fate, BLIMP1, PRDM14, AP2ɣ, and their context-dependent roles in embryonic development. Finally, we discuss one target element of transcription factors, the transcriptional enhancer, and its role in refining the transcriptional output downstream of signalling.

2. Acquisition of developmental competence

2.1 Early embryonic development

Developmental competence follows after fertilisation and establishment of the totipotent zygote. After a few cell divisions, the zygote gives rise to the morula, which contains outer cells and smaller inner cells that will become trophectoderm (TE) and the inner cell mass (ICM) respectively, during the formation of the blastocyst (Arnold and Robertson, 2009). The ICM subsequently segregates into the primitive endoderm (PrE) and the epiblast. After blastocyst implantation, TE and PrE serve as the precursors of extraembryonic lineages, while the epiblast becomes the founder population of the embryo proper including the germline. Hence, there is a sequential commitment of developmental competence to spatially separated cell populations during early embryonic development. *In vivo* the competent state is transient, but can be captured in cells *in vitro* in embryonic stem cells (ESCs) from the pre-implantation epiblast (Evans and Kaufman, 1981; Martin, 1981), extraembryonic endoderm (XEN) cells from the PrE (Kunath, 2005) and trophoblast stem cells (TSCs) from the TE (Tanaka et al., 1998).

2.2 Primordial germ cells

Signalling from extraembryonic tissues induces the differentiation of mesoderm, ectoderm, definitive endoderm and PGCs from the post-implantation epiblast (Fig. 1A). The TE lineage forms the extraembryonic ectoderm (ExE), which is proximal to the epiblast. The PrE lineage gives rise to the visceral endoderm, which surrounds the epiblast and ExE. BMP4 signalling from the ExE induces PGCs in a few cells of the proximal posterior epiblast (Fig. 1A) (Lawson et al., 1999). Subsequently, PGCs migrate towards the prospective gonads, while they undergo extensive transcriptional and epigenetic changes before they enter meiosis to form the gametes, sperm and oocytes, respectively (Hackett et al., 2013; Hajkova et al., 2008; Kurimoto et al., 2015; Kurimoto et al., 2008; Magnúsdóttir et al., 2013; Seki et al., 2007). PGCs are unipotent as they differentiate either into eggs or sperm depending on the sex of the embryo. Specification of PGCs is also accompanied by re-expression of a number of

pluripotency genes (Kurimoto et al., 2008; Magnúsdóttir et al., 2013). Accordingly, PGCs from embryos at embryonic day (E) 7.5-E12.5 can de-differentiate to pluripotent embryonic germ cells (EGCs) in culture, and form testicular teratomas (Matsui et al., 1992; Resnick et al., 1992; Stevens, 1967). EGCs are very similar to ESCs (Leitch et al., 2010), except for their DNA methylation status at imprinted loci (Durcova-Hills et al., 2001; Shovlin et al., 2008; Tada et al., 1998). Nonetheless, PGCs are highly specialised cells, which do not contribute to chimeras after injection into embryos (Leitch et al., 2014), and from ~E12.5 onwards, they undergo differentiation only towards gametes. The precise role of pluripotency factors in early PGCs is not entirely clear except that they might help to promote epigenetic reprogramming (Papp and Plath, 2013).

The developmental competence for PGC specification is established in the post-implantation epiblast, but only in a subset of cells in the posterior of the embryo acquire PGC fate (Fig. 1A). However, distal epiblast cells transplanted to the posterior can also respond to BMP4 signalling and become PGCs (Tam and Zhou, 1996). Indeed, a large number of epiblast cells when separated from the extraembryonic tissues have the potential to become PGCs in response to high levels of BMP4 in *ex vivo* culture (Fig. 1B). The developmental competence for the PGC fate is restricted to a short developmental time window from ~E5.5-6.5 (Ohinata et al., 2009), prior to the formation of the primitive streak and the onset of gastrulation (Arnold and Robertson, 2009).

2.3 Epiblast stem cells

The post-implantation epiblast can give rise to pluripotent epiblast stem cells (EpiSCs) *in vitro* in the presence of bFGF and Activin A (Brons et al., 2007; Tesar et al., 2007). EpiSCs can differentiate into derivates of the three germ layers, but the induction of PGCs is very inefficient (Hayashi and Surani, 2009). Also, they contribute very poorly, if at all, to chimeras after blastocyst injections (Brons et al., 2007; Han et al., 2010; Tesar et al., 2007). EpiSCs appear to be transcriptionally close to the ectoderm of the late gastrula (Kojima et al., 2014), suggesting that EpiSCs become heterogeneous in culture (Han et al., 2010; Tsakiridis et al., 2014), where the majority of cells progress to a later developmental stage and consequently loose the developmental competence to become PGCs. This is also reflected by their epigenetic state, since putative enhancer elements and promoters of genes important for somatic differentiation show enrichment for active histone marks (Factor et al., 2014; Kurimoto et al., 2015). The inhibition of WNT signalling results in

a more homogeneous population of EpiSC, which can contribute to the germline, when transplanted into the post-implantation embryo (Sugimoto et al., 2015; Sumi et al., 2013). Further studies are required to address the question whether EpiSCs maintained or derived under these culture conditions, can respond to BMP4 and give rise to PGCLCs efficiently as observed with epiblast-like cells (EpiLCs; see below).

2.4 Epiblast-like cells

ESCs cultured with a GSK3 inhibitor (CHIR99021), a MEK/ERK inhibitor (PD0325901) and leukemia inhibitory factor (LIF), represent a relative homogeneous population of naïve pluripotent cells (Ying et al., 2008), which are transcriptionally close to the pre-implantation epiblast (Boroviak et al., 2014). These ESCs give rise to EpiLCs following culture in bFGF and Activin A (Fig. 1C) (Hayashi et al., 2011). EpiLCs undergo extensive cell death after three days of culture and thus appear to represent a transient cell state. They are to some extent different from EpiSCs on transcriptional level, but similar to the pre-gastrulating epiblast (Hayashi et al., 2011). Accordingly, a large proportion of EpiLCs at day2 respond to BMP4 and become PGC-like cells (PGCLCs) very efficiently, which can give rise to sperm or oocytes after transplantation experiments (Hayashi et al., 2011; Hayashi et al., 2012). However, this response was not observed with EpiLCs at day1, suggesting that the transit to the competent state for the PGC fate is not completed at this stage. Future studies might reveal the key differences between day1 and day2 EpiLCs and what restricts or promotes the induction of the PGC fate as they progress towards the competence for germ cell and somatic fates.

2.5 Human PGCLCs

Recent studies have explored, whether human PGCLCs (hPGCLCs) can be derived from human ESCs (hESCs) *in vitro* (Irie et al., 2015; Sasaki et al., 2015; Sugawa et al., 2015). Irie et al. cultured human ESCs (hESCs) with four inhibitors (for GSK3, MEK, p38, JNK; '4i') together with LIF, bFGF and TGFβ (Gafni et al., 2013). hESCs in 4i can be directly induced into hPGCLCs upon addition of BMP2 or BMP4 (Fig. 1C), which are transcriptionally close to human PGCs from week 7 embryos four days after induction. Thus, hESCs in 4i gain and retain competence for germ cell fate while undergoing self-renewal. This is unlike mouse EpiLCs where the competence for PGCs occurs only transiently. Intriguingly, hESCs in 4i culture exhibit a gene expression profile that includes expression of mesoderm and primitive streak markers. Hence, the expression of lineage-specific markers such as T (also known as BRACHYURY) could render hESCs competent for the hPGCLC fate, since T

plays an important role in the specification of mice PGCLCs (see below). Consistent with this, hESCs cultured in conventional medium show a poor response to hPGCLC induction (Irie et al., 2015; Sugawa et al., 2015). Notably, the two hESC states, in 4i and conventional culture respectively, are reversible and depend on the appropriate environmental signal (Fig. 1C) (Irie et al., 2015).

3. Interplay of signalling pathways

3.1 BMP pathway

Signalling molecules pattern the embryo, and they act during specific time windows to induce developmental processes, which results in the differentiation of groups of cells. These pathways do not act individually but show a highly complex interplay with other signals. Thus, the balance of activating and inhibitory signals refines the temporal and spatial output during cell fate specification.

BMPs induce the receptor-mediated phosphorylation of the transcription factors SMAD1/5/8, which can form a complex with SMAD4 and subsequently translocate into the nucleus, and induces or represses transcription of target genes. The output of SMAD activation is modulated on different levels, since SMADs interact with many co-activators or -repressors. For example, the transcriptional co-activator p300/CBP interacts with SMAD1 to SMAD4 (Pouponnot et al., 1998). p300/CBP in turn not only interacts with transcription factors of all major families, but also has acetyltransferase activity (Holmqvist and Mannervik, 2014). It was shown to bind *cis*-regulatory sequences genome-wide, including putative enhancer elements (Visel et al., 2009), often resulting in the acetylation of histone H3 lysine 27 (H3K27ac), a hallmark of active enhancers (Creyghton et al., 2010). However, not all p300/CBP-bound enhancers are enriched for H3K27ac, suggesting that p300/CBP-bound enhancer elements become 'poised' in the first instance before activation. Therefore, the activity of the BMP pathway not only results in a direct activation or repression of target genes, but could also modulate the enhancer landscape and consequently the developmental competence of the target cells.

BMP4 is required for the induction of mesoderm as well as the PGC fate in the post-implantation epiblast (Lawson et al., 1999; Mishina et al., 1995; Winnier et al., 1995). Accordingly, a mutation in the *Bmp4* gene, results in embryonic lethality between E6.5-E9.5 due to early patterning defects (Mishina et al., 1995; Winnier et al., 1995). BMP4 has diverse roles during development and was recently also implicated in pre-

implantation embryos for the development of extra-embryonic lineages as well as for cell division (de Mochel et al., 2015; Graham et al., 2014).

3.2 Nodal/BMP/WNT signalling network in the posterior epiblast

The initiation of gastrulation is marked by the appearance of the primitive streak in the post-implantation epiblast, the site where cells ingress to form the mesoderm. Not only BMP but also Nodal, FGF and WNT signalling are required to control this complex developmental process (Ciruna et al., 1997; Conlon et al., 1994; Dunn et al., 2004; Johansson and Wiles, 1995; Kelly et al., 2004; Liu et al., 1999; Mishina et al., 1995; Winnier et al., 1995; Yamaguchi et al., 1994). Together, these pathways regulate each other in feedback loops to mediate the activation of the mesoderm-specific transcriptional network (Fig. 1A). It should be noted that Nodal, like the BMPs, is a member of the TGFβ family and can act through SMAD2/3 (Arnold and Robertson, 2009). However, the SMAD2-dependent function controls the patterning of the anterior VE, which is a source of inhibitory signals such as *Cer1* against posteriorisation of the anterior epiblast (Brennan et al., 2001). The SMAD2-independent role of Nodal is based on the secretion of its precursor at high levels from the posterior epiblast to the ExE resulting in the expression of Nodal convertases and *Bmp4* (Ben-Haim et al., 2006). BMP4 is secreted from the ExE and activates the expression of *Wnt3* in the posterior epiblast, which in turn maintains *Nodal* expression. Accordingly, *Bmp4* and *Wnt3* expression is absent in *Nodal* mutant embryos (Brennan et al., 2001).

3.3 PGC induction

The BMP and WNT pathways also induce PGCs in the posterior epiblast proximal to the ExE (Fig. 1A) (Aramaki et al., 2013; Lawson et al., 1999; Ohinata et al., 2009). BMP4 and BMP2, which are structurally very similar, are sufficient to induce the PGC fate in *ex vivo* cultures of isolated whole epiblasts (Fig. 1B) (Ohinata et al., 2009). BMP8b, also secreted from the ExE, has an indirect role in PGC specification by restricting the development of the anterior VE. *Bmp8b* mutant embryos do not induce PGCs and exhibit an abnormal VE morphology with an expanded expression domain of inhibitory signals including *Cer1*, which are important for the anterior identity of the epiblast. Also, in *ex vivo* cultures of isolated epiblasts with the VE still attached, the addition of exogenous BMP4 without BMP8b does not result in the induction of PGCs (Fig. 1B), presumably due to the expansion of the inhibitory signals emanating from the VE. Thus, the primary signal for the induction of PGC fate is BMP4, which is supported by BMP2, while BMP8b indirectly affects this process.

WNT3 appears to be the mediator of BMP signalling to induce the PGC fate (Aramaki et al., 2013). *Wnt3a* alone does not induce PGCs in *ex vivo* epiblast culture. However, *Wnt3* mutant embryos fail to induce PGCs, although *Bmp4* expression in the ExE is not affected. A direct target of WNT3 is the transcription factor T, which is known to play an integral part in mesoderm development. In this developmental context, T binds to the enhancers of the genes encoding the key transcription factors of the germline *Prdm1* and *Prdm14* to activate their expression (Fig. 1D). It is important to note, however, that *T* mutant embryos show initial expression of BLIMP1 in the posterior epiblast, but not of PRDM14. This suggests that additional factors might be responsible for the initial upregulation of BLIMP1 in prospective PGCs, which then might be augmented by T. As suggested by Aramaki et al., one possible candidate could be the transcription factor EOMES, which shows moderate upregulation of BLIMP1 after PGCLC induction. T but not WNT3a is sufficient to induce PGCLCs. The pretreatment of EpiLCs with exogenous WNT3a results in a decrease of PGCLC induction efficiency via T, suggesting that WNT activates other targets, which could drive the EpiLCs towards a mesodermal fate rather than PGCLC fate providing a possible explanation for the distinct effects of T and WNT3a in the *in vitro* experiments.

The role of Nodal signalling in PGC specification has not been extensively explored. It is likely that Nodal has at least an indirect role in PGC specification, since it affects the expression of *Bmp4* and consequently *Wnt3* in the posterior epiblast (Brennan et al., 2001). However, after E6.0 the addition of exogenous Nodal or its inhibition in the epiblast did not affect PGC induction via BMP4 (Ohinata et al., 2009), suggesting that in this context Nodal does not play an important role just prior PGC specification.

Taken together, these findings demonstrate the interplay between different signalling pathways during post-implantation development inducing distinct cell lineages from the same population of epiblast cells. These cells do show at least similar developmental competence in the early post-implantation epiblast, but the signalling can potentially not only directly induce a particular cell fate, but also change the developmental competence of the cells. This is exemplified by BMP signalling, which is apparently required to create a permissive environment in epiblast cells, which can then respond to WNT signalling to induce PGCs (Aramaki et al., 2013). However, the exact mechanistic function of BMP-SMADs during PGC specification requires further investigation. It is important to note that subtle intrinsic, maybe stochastic,

differences in the post-implantation epiblast can prime subgroup of cells to respond differentially to the same signal.

4. Combinatorial action of transcription factors

4.1 Context-dependency

Transcription factors contain a DNA-binding domain and bind to short DNA sequences (8-21bp), referred to as transcription factor binding motif. However, the occurrence of binding motifs in the genome is not necessarily predictive for transcription factor occupancy, as usually the number of motifs vastly outnumbers actual binding sites of a transcription factor. Accordingly, many transcription factors show a highly cell type-specific binding pattern, which is due to the combinatorial action of transcription factors with co-factors, dose-dependent effects and chromatin state (including accessibility) of DNA binding sites (Spitz and Furlong, 2012). For example, TGFβ signalling is mediated via the SMAD2 and SMAD3 transcription factors and is required for diverse developmental processes. Accordingly, SMAD3 co-occupies different sets of regulatory sequences together with lineage-specific transcription factors; for example in ESCs with OCT4, in myotubes with MYOD1 and in pro-B cells with PU.1 (Mullen et al., 2011). In addition, the level of phosphorylated SMAD2 induced dictates its binding pattern to distinct sets of regulatory elements in ESCs, which is reflected in the differentiation into distinct lineages (Lee et al., 2011). Further, a comparison of multiple datasets for human transcription factors showed a correlation of transcription factor binding with nucleosome-depleted and DNase I-accessible regions (Wang et al., 2012). However, the accessibility of a DNA binding site can not only be the cause but also a direct consequence of transcription factor binding as it is the case upon binding of pioneer factors (Zaret and Carroll, 2011).

4.2 BLIMP1/PRDM14/AP2ɣ in PGCs

Upon specification of PGCs via the BMP and WNT pathway, the key transcription factors BLIMP1 and PRDM14 are upregulated (Fig. 1D). BLIMP1 mainly represses genes of the somatic lineages but can also act as an activator, as it induces the expression of *Tfap2c*. Similarly, BLIMP1 also induces the expression of AP2α during neural crest formation in zebrafish (Powell et al., 2013). The unique combinatorial action of BLIMP1/PRDM14/AP2ɣ in PGCs is critical in directing the early development of the germline. Accordingly, a mutation in any one of the three genes results in the loss of PGCs (Ohinata et al., 2005; Weber et al., 2010; Yamaji et al., 2008). Similar to the response of EpiLCs to BMP4, the induced expression of the

three factors specifies the PGC fate. In contrast, the expression of the three factors in ESCs does not result in PGCLC induction; instead these cells remain transcriptionally similar to ESCs (Nakaki et al., 2013). This clearly demonstrates the distinct developmental competence of ESCs and EpiLCs.

4.3 BLIMP1

BLIMP1, PRDM14 and AP2ɣ are not only expressed in the germline, but have different roles during development. BLIMP1 was initially discovered as a transcriptional repressor of the human IFNβ promoter (Keller and Maniatis, 1991). It has a critical role in B and T lymphocytes as well as in multiple other lineages during development (Bikoff et al., 2009). For example, it was recently shown that BLIMP1 is involved in the differentiation of trophoblast subtypes (Mould et al., 2012). Accordingly, *Prdm1* mutant embryos arrest at E10.5 of embryonic development due to placental defects (Vincent et al., 2005), which demonstrates that the same transcription factor is not only critical for the development of embryonic but also extraembryonic lineages. BLIMP1 has a zinc finger DNA binding domain as well as a PR domain, which has similarity to the SET domains of histone methyltransferases, but appears to be catalytically inactive (Ancelin et al., 2006). Rather, BLIMP1 associates with distinct chromatin modifiers depending on the developmental context including histone deacetylases (Yu et al., 2000), G9a H3 methyl transferase (Gyory et al., 2004), a lysine-specific demethylase (LSD1) (Su et al., 2009) and the arginine methyltransferase PRMT5 (Ancelin et al., 2006). In PGCLCs as well as in P19 embryonal carcinoma (P19EC) cells, which were used as a model for PGCs, BLIMP1 binds mainly promoters of cell cycle and somatic genes resulting in their repression, which implies that BLIMP1 supresses somatic differentiation, paving the way for epigenetic programming and expression of pluripotency genes (Kurimoto et al., 2015; Magnúsdóttir et al., 2013). Interestingly, BLIMP1-bound regions at day2 of PGCLC differentiation, which are still present in day6 PGCLCs, accumulate the repressive polycomb repressive complex (PRC2)-catalysed modification H3K27me3, suggesting that BLIMP1 might directly or indirectly recruit PRC2 (Kurimoto et al., 2015). This is consistent with the data on P19EC cells, which shows that BLIMP1 has a high overlap with PRC binding regions in ESCs (Magnúsdóttir et al., 2013). There might also be a link to the regulation of *Mad2l2*, since in *Mad2l2* mutants, the process of global enrichment of H3K27me3 in PGCs is abrogated and PGCs are lost by E9.5 (Pirouz et al., 2013).

4.4 PRDM14

PRDM14 also belongs to the family of PR-domain containing proteins with zinc-finger domains. But as for BLIMP1, any histone methyltransferase activity for PRDM14 has not so far been demonstrated. PRDM14 interacts with TETs (Okashita et al., 2013), which mediate the conversion of 5-methylcytosine to 5-hydroxymethylcyotsine to promote DNA demethylation (Hackett and Surani, 2013). PRDM14 also activates the expression of pluripotency factors, represses FGF signalling and components of the DNA methylation machinery to maintain naïve pluripotency (Grabole et al., 2013; Ma et al., 2011; Nakaki and Saitou, 2014; Yamaji et al., 2013). PRDM14 exerts its repressive function at least in part by interacting with components of the PRC2 complex, which is also required for X chromosome reactivation in female embryos (Chan et al., 2013; Payer et al., 2013; Yamaji et al., 2013). Concomitant with the role of PRDM14 in pluripotency, it is expressed in pre-implantation embryos including the ICM at the blastocyst stage, before its expression ceases upon implantation. It then becomes specifically upregulated in PGCs (Burton et al., 2013; Kurimoto et al., 2008; Yamaji et al., 2008). Nevertheless, the role of PRDM14 during early embryonic development requires further investigation, since a null mutation in the *Prdm14* gene results in viable, but sterile mice, which however depends on the genetic background (Payer et al., 2013). In PGCs, it appears that while BLIMP1 represses somatic differentiation, PRDM14 mainly binds putative enhancer elements to induce re-expression of pluripotency factors as well as to promote epigenetic programming including global DNA demethylation (Grabole et al., 2013; Magnúsdóttir et al., 2013). However, to characterise the function of PRDM14 in detail, it will be interesting to map the binding sites of PRDM14 directly in PGCs or PGCLCs. It is important to note that while *Prdm14* alone is sufficient to induce mouse PGCLC fate *in vitro* (Nakaki et al., 2013), it appears that its function is not conserved. At the onset of human PGCLC induction, the expression of *Prdm14* is initially repressed, suggesting that it might not play a prominent role at early stages of human PGC development (Irie et al., 2015; Sasaki et al., 2015; Sugawa et al., 2015). Interestingly, the transcription factor SOX17, which is required for endoderm development, is involved in hPGCLC induction, since loss of *SOX17* abrogates hPGCLC specification and its induced expression is sufficient to upregulate germ cell markers (Irie et al., 2015). This suggests that in humans a different combination of transcription factors including SOX17 and BLIMP1 drive the PGC fate.

4.5 AP2ɣ

AP2ɣ, a member of the activator protein-2 (AP-2) family, with a DNA-binding, dimerization and a transactivation domain, has like BLIMP1 and PRDM14 multiple

context-dependent roles during embryonic development. A mutation in *Tfap2c* results in embryonic lethality at E7.0-9.0 due to placental defects (Auman et al., 2002; Werling and Schorle, 2002), since AP2ɣ is involved in the maintenance of the TE lineage (Kuckenberg et al., 2012). Interestingly, in the developmental context of this extraembryonic lineage AP2ɣ interacts with SOX2; together they mediate self-renewal and the control of the transcriptional network of TSCs *in vitro* (Adachi et al., 2013), which are derived from TE cells (Tanaka et al., 1998). This also provides an example for the context-dependent role of the pluripotency factor SOX2, which integrates LIF-STAT signalling in ESCs and FGF-ERK signalling in TSCs (Adachi et al., 2013). In PGCs, the expression of *Tfap2c* is induced by BLIMP1 shortly after specification, where AP2ɣ binds promoters and enhancers, acting cooperatively with BLIMP1 and PRDM14 to induce genes essential for the PGC lineage (Magnúsdóttir et al., 2013; Schemmer et al., 2013). Of note, SOX2 is also induced in PGCs and the conditional loss of *Sox2* results in the loss of PGCs (Campolo et al., 2013; Kurimoto et al., 2008; Magnúsdóttir et al., 2013). Thus, it could be that AP2ɣ and SOX2 also act together in PGCs as in TSCs.

In summary, the function of many transcription factors is not restricted to one particular lineage, but there is a lineage-specific combination of proteins that cooperatively define a unique functional outcome. Thus, the developmental competence of a cell is also defined by its proteome, because the presence or absence of co-factors, e.g. chromatin modifiers, defines the functional output of transcription factors that were initially induced by signalling. To gain insight into the context-dependent roles of transcription factors in future studies, it is key to perform biochemical studies to identify co-factors of critical transcription factors.

5. Transcriptional enhancer elements

5.1 Enhancer setup

Transcription factors bind *cis*-regulatory DNA sequences including enhancer elements to control transcription. It appears that enhancers show a highly cell type specific epigenetic setup, which could facilitate the binding of transcription factors and thus potentially contribute to the developmental competence of a cell. It is important to note that the presence of histone modifications at enhancers could also be a consequence of transcription factor binding (Calo and Wysocka, 2013). The generation of specific histone mutants would be the most stringent way to test for the functional role of histone modifications. Since canonical histone genes are present in

many copies in the genome of higher eukaryotes, the generation of defined histone mutants is technically challenging, and was achieved for example in *Drosophila* but not in mammals so far (Günesdogan et al., 2010). However, recent advances in genome editing tools including the CRISPR-Cas9 technology have enabled the locus-specific editing of histone modifications in mammalian cells. For example, the guide RNA-mediated recruitment of a fusion protein, which consists of the catalytically inactive Cas9 (dCas9) protein and the catalytic domain of the acetyltransferase p300, results in enrichment of H3K27ac at selected enhancers and transcriptional activation of target genes (Hilton et al., 2015). Further, the fusion of dCas9 to the lysine-specific demethylase LSD1 results in a decrease of H3K4me2 concomitantly with a decrease in H3K27ac at target enhancers as well as reduced target gene expression (Kearns et al., 2015). The apparent interdependence of H3K4me2 and H3K27ac suggests recruitment of histone deacetylases via LSD1 (Mendenhall et al., 2013). Taken together, these and other studies demonstrate elegantly that the combinatorial presence of histone modifications is required for the activity of enhancer elements and might thus be involved in the acquirement of developmental competence. Nevertheless, there are some limitations of such experiments that need to be considered, since histone modifiers usually have many non-histone targets. Also, the binding of dCas9 to an enhancer could spatially interfere with the binding of transcription factors, which could also effect target gene expression.

5.2 Enhancer setup and developmental competence

Recent studies characterised the dynamic histone modification enrichment at enhancers during the differentiation of multi-potent progenitor cells, e.g. during haematopoiesis (Lara-Astiaso et al., 2014) or hESC differentiation (Wang et al., 2015). Wang et al. profiled the enrichment of H3K4me1 and H3K27ac at enhancers during the sequential differentiation of hESCs towards pancreatic endoderm cells (Fig. 2). Enhancers enriched only for H3K4me1 were categorised as ‘poised’, while enhancers enriched for both H3K4me1 and H3K27ac were categorised as ‘active’ (Creyghton et al., 2010; Heintzman et al., 2007). A subset of enhancers became poised at the gut tube (GT) stage, which upon differentiation into different lineages became activated. This suggests that the poised state of enhancers can contribute or define the developmental competence of GT cells. In addition, poised enhancers are bound by the pioneer transcription factor FOXA1, which can modulate local chromatin structure to mediate enhancer function (Zaret and Carroll, 2011). While knockdown of *FOXA1* did not affect H3K4me1 enrichment at tested enhancers,

further studies are required to determine the precise role of FOXA1 in setting up the poised state, considering reported redundancy between FOXA proteins (Gao et al., 2008; Li et al., 2009; Wan et al., 2005).

5.3 Enhancer-promoter contacts

Enhancers do not always target the nearest promoter, but also act in a long-range fashion. Thereby, the intervening sequence between enhancer and promoter loops out, bringing these elements in close spatial proximity. This 'interaction' could allow the delivery of enhancer-bound transcription factors and RNA polymerase II to the promoter to activate or enhance transcription. The regulation of promoter activity appears to be very complex, since promoters often interact with multiple enhancers, and promoters and enhancers also interact with each other (Ghavi-Helm et al., 2014; Schoenfelder et al., 2015a).

The PRC1 component RING1B binds promoters in ESCs, which are predominantly in contact with poised enhancers that are enriched for H3K4me1, H3K27me3 and p300 (Fig. 3A) (Schoenfelder et al., 2015b). In *Ring1A/B* knockout cells, these enhancers loose H3K27me3, but gain the active H3K27ac mark concomitantly with the transcriptional induction of associated genes (Fig. 3B). Similarly, inactive promoters also interact with poised enhancers, which then become selectively activated upon differentiation in *Drosophila* (Ghavi-Helm et al., 2014). Thus, a feature of poised enhancers could be the 'preformation' of enhancer-promoter contacts, which could facilitate the quick response to signal-dependent and/or lineage-specific transcription factors.

5.4 Enhancer setup for the PGC fate

In mice, the gain of developmental competence for the PGC fate is established during the differentiation of ESCs to EpiLCs (Hayashi et al., 2011). This transition is accompanied by the dynamic reorganisation of the enhancer landscape displaying distinct sets of histone modifications accompanied by differential binding of transcription factors (Buecker et al., 2014). Notably, OCT4 exhibits a marked cell type-specific genomic occupancy, with enrichment peaks close to genes associated with naïve pluripotency in ESCs, close to genes associated with the post-implantation epiblast in EpiLCs, and shared between both cell types. This redistribution of OCT4 correlates with H3K27ac and p300 enrichment. ESC-specific OCT4 binding sites are also enriched for H3K27ac in PGCLCs, suggesting that OCT4 controls similar subsets of genes in both cell types (Kurimoto et al., 2015). The

dynamic behaviour of OCT4 can be explained by a recurring concept for gene expression control. While OCT4 is expressed at similar levels in ESCs and EpiLCs, its binding is modulated by other cell type-specific transcription factors such as OTX2 in EpiLCs (Buecker et al., 2014; Yang et al., 2014). Interestingly, the comparison of BLIMP1 binding targets to the transcriptional consequences of loss of *Blimp1* during the reversion of PGCs to EGCs revealed that BLIMP1 binds to and restricts the expression of a subset of OCT4 and other pluripotency transcription factor targets. This suggests that BLIMP1 limits the pluripotency network and maintains the lineage restriction of PGCs (Nagamatsu et al., 2015). Accordingly, BLIMP1 is repressed early during EGC derivation, prior to the acquisition of functional pluripotency (Durcova-Hills et al., 2008; Leitch et al., 2010).

In sum, histone modifications at enhancer elements can predict their activity and in some examples also the developmental competence of a cell. However, the functional implications of these marks are less clear; e.g. how does the presence of H3K4me1 results in the poised state of an enhancer? The underlying mechanism could be that the presence of H3K4me1 results in an open chromatin structure at putative enhancer elements, prevents the binding of transcriptional repressor, or inhibits DNA methylation indirectly (Fig. 2) (Calo and Wysocka, 2013), enabling the binding of activating transcription factors upon signalling. In agreement with this, recent studies show that the induced loss or gain of these modifications can influence enhancer activity.

6. Concluding remarks

Developmental competence for cell fate decisions involves multiple interconnected aspects including intrinsic and extrinsic signalling, transcription factors, chromatin modifiers, enhancer landscape amongst others. The latter might include DNA methylation, cell cycle progression and cell-cell contacts. In PGCs, the downstream effectors SMADs and T of the BMP and WNT pathway, respectively, activate the expression of BLIMP1/PRDM14, which drive the transcriptional program of the PGC fate. There are many more binding sites for these factors, which could activate the expression of essential co-factors and/or change the epigenetic state of enhancer elements in prospective PGCs. Future studies will benefit from the recent development of new techniques based on CRISPR/Cas9 for genome and epigenome editing, which might shed light on the underlying control mechanisms for a robust and refined transcriptional output to determine cell fates.

References

Adachi, K., Nikaido, I., Ohta, H., Ohtsuka, S., Ura, H., Kadota, M., Wakayama, T., Ueda, H. R. and Niwa, H. (2013). Context-dependent wiring of Sox2 regulatory networks for self-renewal of embryonic and trophoblast stem cells. *Mol. Cell* 52, 380–392.

Ancelin, K., Lange, U. C., Hajkova, P., Schneider, R., Bannister, A. J., Kouzarides, T. and Surani, M. A. (2006). Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nat. Cell Biol.* 8, 623–630.

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, 516–529.

Arnold, S. J. and Robertson, E. J. (2009). Making a commitment: cell lineage allocation and axis patterning in the early mouse embryo. *Nature Reviews Molecular Cell Biology* 10, 91–103.

Auman, H. J., Nottoli, T., Lakiza, O., Winger, Q., Donaldson, S. and Williams, T. (2002). Transcription factor AP-2gamma is essential in the extra-embryonic lineages for early postimplantation development. *Development* 129, 2733–2747.

Ben-Haim, N., Lu, C., Guzman-Ayala, M., Pescatore, L., Mesnard, D., Bischofberger, M., Naef, F., Robertson, E. J. and Constam, D. B. (2006). The nodal precursor acting via activin receptors induces mesoderm by maintaining a source of its convertases and BMP4. *Dev. Cell* 11, 313–323.

Bikoff, E. K., Morgan, M. A. and Robertson, E. J. (2009). An expanding job description for Blimp-1/PRDM1. *Curr. Opin. Genet. Dev.* 19, 379–385.

Boroviak, T., Loos, R., Bertone, P., Smith, A. and Nichols, J. (2014). The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification. *Nat. Cell Biol.* 16, 516–528.

Brennan, J., Lu, C. C., Norris, D. P., Rodriguez, T. A., Beddington, R. S. and Robertson, E. J. (2001). Nodal signalling in the epiblast patterns the early mouse embryo. *Nature* 411, 965–969.

Brons, I. G. M., Smithers, L. E., Trotter, M. W. B., 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, 191–195.

Buecker, C., Srinivasan, R., Wu, Z., Calo, E., Acampora, D., Faial, T., Simeone, A., Tan, M., Swigut, T. and Wysocka, J. (2014). Reorganization of enhancer patterns in transition from naive to primed pluripotency. *Cell Stem Cell* 14, 838–853.

Burton, A., Muller, J., Tu, S., Padilla-Longoria, P., Guccione, E. and Torres-Padilla, M.-E. (2013). Single-cell profiling of epigenetic modifiers identifies PRDM14 as an inducer of cell fate in the mammalian embryo. *Cell Rep* 5, 687–701.

Calo, E. and Wysocka, J. (2013). Modification of enhancer chromatin: what, how, and why? *Mol. Cell* 49, 825–837.

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, 1408–1421.

Chan, Y.-S., Göke, J., Lu, X., Venkatesan, N., Feng, B., Su, I.-H. and Ng, H.-H. (2013). A PRC2-Dependent Repressive Role of PRDM14 in Human Embryonic Stem Cells and Induced Pluripotent Stem Cell Reprogramming. *Stem Cells* 31, 682–692.

Ciruna, B. G., Schwartz, L., Harpal, K., Yamaguchi, T. P. and Rossant, J. (1997). Chimeric analysis of fibroblast growth factor receptor-1 (Fgfr1) function: a role for FGFR1 in morphogenetic movement through the primitive streak. *Development* 124, 2829–2841.

Conlon, F. L., Lyons, K. M., Takaesu, N., Barth, K. S., Kispert, A., Herrmann, B. and Robertson, E. J. (1994). A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. *Development* 120, 1919–1928.

Creyghton, M. P., Cheng, A. W., Welstead, G. G., Kooistra, T., Carey, B. W., Steine, E. J., Hanna, J., Lodato, M. A., Frampton, G. M., Sharp, P. A., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21931–21936.

de Mochel, N. S. R., Luong, M., Chiang, M., Javier, A. L., Luu, E., ToshihikoFujimori, MacGregor, G. R., Cinquin, O. and Cho, K. W. Y. (2015). Developmental Biology. *Dev. Biol.* 397, 45–55.

Dunn, N. R., Vincent, S. D., Oxburgh, L., Robertson, E. J. and Bikoff, E. K. (2004). Combinatorial activities of Smad2 and Smad3 regulate mesoderm formation and patterning in the mouse embryo. *Development* 131, 1717–1728.

Durcova-Hills, G., Ainscough, J. and McLaren, A. (2001). Pluripotential stem cells derived from migrating primordial germ cells. *Differentiation* 68, 220–226.

Durcova-Hills, G., Tang, F., Doody, G., Tooze, R. and Surani, M. A. (2008). Reprogramming primordial germ cells into pluripotent stem cells. *PLoS ONE* 3, e3531.

Evans, M. J. and Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292, 154–156.

Factor, D. C., Corradin, O., Zentner, G. E., Saiakhova, A., Song, L., Chenoweth, J. G., McKay, R. D., Crawford, G. E., Scacheri, P. C. and Tesar, P. J. (2014). Short Article. *Stem Cell* 14, 854–863.

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, 282–286.

Gao, N., LeLay, J., Vatamaniuk, M. Z., Rieck, S., Friedman, J. R. and Kaestner, K. H. (2008). Dynamic regulation of Pdx1 enhancers by Foxa1 and Foxa2 is essential for pancreas development. *Genes Dev.* 22, 3435–3448.

Ghavi-Helm, Y., Klein, F. A., Pakozdi, T., Ciglar, L., Noordermeer, D., Huber, W. and Furlong, E. E. M. (2014). Enhancer loops appear stable during development and are associated with paused polymerase. *Nature* 1–22.

Grabole, N., Tischler, J., Hackett, J. A., Kim, S., Tang, F., Leitch, H. G., Magnúsdóttir, E. and Surani, M. A. (2013). Prdm14 promotes germline fate and naive pluripotency by repressing FGF signalling and DNA methylation. *EMBO Rep.* 14, 629–637.

Graham, S. J. L., Wicher, K. B., Jedrusik, A., Guo, G., Herath, W., Robson, P. and Zernicka-Goetz, M. (2014). BMP signalling regulates the pre-implantation development of extra-embryonic cell lineages in the mouse embryo. *Nature Communications* 5, 5667.

Günesdogan, U., Jäckle, H. and Herzig, A. (2010). A genetic system to assess in vivo the functions of histones and histone modifications in higher eukaryotes. *EMBO Rep.* 11, 772–776.

Gyory, I., Wu, J., Fejér, G., Seto, E. and Wright, K. L. (2004). PRDI-BF1 recruits the histone H3 methyltransferase G9a in transcriptional silencing. *Nat. Immunol.* 5, 299–308.

Hackett, J. A. and Surani, M. A. (2013). DNA methylation dynamics during the mammalian life cycle. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 368, 20110328.

Hackett, J. A., Sengupta, R., Zylicz, J. J., Murakami, K., Lee, C., Down, T. A. and Surani, M. A. (2013). Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* 339, 448–452.

Hajkova, P., Ancelin, K., Waldmann, T., Lacoste, N., Lange, U. C., Cesari, F., Lee, C., Almouzni, G., Schneider, R. and Surani, M. A. (2008). Chromatin dynamics during epigenetic reprogramming in the mouse germ line. *Nature* 452, 877–881.

Han, D. W., Tapia, N., Joo, J. Y., Greber, B., Araúzo-Bravo, M. J., Bernemann, C., Ko, K., Wu, G., Stehling, M., Do, J. T., et al. (2010). Epiblast Stem Cell Subpopulations Represent Mouse Embryosof Distinct Pregastrulation Stages. *Cell* 143, 617–627.

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, 3549–3556.

Hayashi, K., Ogushi, S., Kurimoto, K., Shimamoto, S., Ohta, H. and Saitou, M. (2012). Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. *Science* 338, 971–975.

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, 519–532.

Heintzman, N. D., Stuart, R. K., Hon, G., Fu, Y., Ching, C. W., Hawkins, R. D., Barrera, L. O., Van Calcar, S., Qu, C., Ching, K. A., et al. (2007). Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* 39, 311–318.

Hilton, I. B., D'Ippolito, A. M., Vockley, C. M., Thakore, P. I., Crawford, G. E., Reddy, T. E. and Gersbach, C. A. (2015). Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat Biotechnol* 33, 510–517.

Holmqvist, P.-H. and Mannervik, M. (2014). Genomic occupancy of the transcriptional co-activators p300 and CBP. *Transcription* 4, 18–23.

Irie, N., Weinberger, L., Tang, W. W. C., 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, 253–268.

Johansson, B. M. and Wiles, M. V. (1995). Evidence for involvement of activin A and bone morphogenetic protein 4 in mammalian mesoderm and hematopoietic development. *Mol. Cell. Biol.* 15, 141–151.

Kearns, N. A., Pham, H., Tabak, B., Genga, R. M., Silverstein, N. J., Garber, M. and Maehr, R. (2015). Functional annotation of native enhancers with a Cas9-histone demethylase fusion. *Nat. Methods* 12, 401–403.

Keller, A. D. and Maniatis, T. (1991). Identification and characterization of a novel repressor of beta-interferon gene expression. *Genes Dev.* 5, 868–879.

Kelly, O. G., Pinson, K. I. and Skarnes, W. C. (2004). The Wnt co-receptors Lrp5 and Lrp6 are essential for gastrulation in mice. *Development* 131, 2803–2815.

Kojima, Y., Kaufman-Francis, K., Studdert, J. B., Steiner, K. A., Power, M. D., Loebel, D. A. F., 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, 107–120.

Kuckenberg, P., Kubaczka, C. and Schorle, H. (2012). The role of transcription factor Tcfap2c/TFAP2C in trophectoderm development. *Reproductive BioMedicine Online* 25, 12–20.

Kunath, T. (2005). Imprinted X-inactivation in extra-embryonic endoderm cell lines from mouse blastocysts. *Development* 132, 1649–1661.

Kurimoto, K., Yabuta, Y., Hayashi, K., Ohta, H., Kiyonari, H., Mitani, T., Moritoki, Y., Kohri, K., Kimura, H., Yamamoto, T., et al. (2015). Quantitative Dynamics of Chromatin Remodeling during Germ Cell Specification from Mouse Embryonic Stem Cells. *Cell Stem Cell* 16, 517–532.

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, 1617–1635.

Lara-Astiaso, D., Weiner, A., Lorenzo-Vivas, E., Zaretsky, I., Jaitin, D. A., David, E., Keren-Shaul, H., Mildner, A., Winter, D., Jung, S., et al. (2014). Chromatin state dynamics during blood formation. *Science* 345, 943–949.

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, 424–436.

Lee, K. L., Lim, S. K., Orlov, Y. L., Yit, L. Y., Yang, H., Ang, L. T., Poellinger, L. and Lim, B. (2011). Graded Nodal/Activin Signaling Titrates Conversion of Quantitative Phospho-Smad2 Levels into Qualitative Embryonic Stem Cell Fate Decisions. *PLoS Genet* 7, e1002130.

Leitch, H. G., Blair, K., Mansfield, W., Ayetey, H., Humphreys, P., Nichols, J., Surani, M. A. and Smith, A. (2010). Embryonic germ cells from mice and rats exhibit properties consistent with a generic pluripotent ground state. *Development* 137, 2279–2287.

Leitch, H. G., Okamura, D., Durcova-Hills, G., Stewart, C. L., Gardner, R. L., Matsui, Y. and Papaioannou, V. E. (2014). On the fate of primordial germ cells injected into early mouse embryos. *Dev. Biol.* 385, 155–159.

Li, Z., White, P., Tuteja, G., Rubins, N., Sackett, S. and Kaestner, K. H. (2009). Foxa1 and Foxa2 regulate bile duct development in mice. *J. Clin. Invest.* 119, 1537–1545.

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, 361–365.

Ma, Z., Swigut, T., Valouev, A., Rada-Iglesias, A. and Wysocka, J. (2011). Sequence-specific regulator Prdm14 safeguards mouse ESCs from entering extraembryonic endoderm fates. *Nature Publishing Group* 18, 120–127.

Magnúsdóttir, E., Dietmann, S., Murakami, K., Günesdogan, 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, 905–915.

Martin, G. R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 78, 7634–7638.

Matsui, Y., Zsebo, K. and Hogan, B. L. (1992). Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell* 70, 841–847.

Mendenhall, E. M., Williamson, K. E., Reyon, D., Zou, J. Y., Ram, O., Joung, J. K. and Bernstein, B. E. (2013). Locus-specific editing of histone modifications at endogenous enhancers. *Nat Biotechnol* 31, 1133–1136.

Mishina, Y., Suzuki, A., Ueno, N. and Behringer, R. R. (1995). Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev.* 9, 3027–3037.

Mould, A., Morgan, M. A. J., Li, L., Bikoff, E. K. and Robertson, E. J. (2012). Blimp1/Prdm1 governs terminal differentiation of endovascular trophoblast giant cells and defines multipotent progenitors in the developing placenta. *Genes Dev.* 26, 2063–2074.

Mullen, A. C., Orlando, D. A., Newman, J. J., Lovén, J., Kumar, R. M., Bilodeau, S., Reddy, J., Guenther, M. G., DeKoter, R. P. and Young, R. A. (2011). Master Transcription Factors Determine Cell-Type-SpecificResponses to TGF-b Signaling. *Cell* 147, 565–576.

Nagamatsu, G., Saito, S., Takubo, K. and Suda, T. (2015). Stem Cell Reports. *Stem Cell Reports* 5, 111–124.

Nakaki, F. and Saitou, M. (2014). PRDM14: a unique regulator for pluripotency and epigenetic reprogramming. *Trends in Biochemical Sciences* 39, 289–298.

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, 222–226.

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, 571–584.

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, 207–213.

Okashita, N., Kumaki, Y., Ebi, K., Nishi, M., Okamoto, Y., Nakayama, M., Hashimoto, S., Nakamura, T., Sugasawa, K., Kojima, N., et al. (2013). PRDM14 promotes active DNA demethylation through the Ten-eleven translocation (TET)-mediated base excision repair pathway in embryonic stem cells. *Development* 141, 269–280.

Papp, B. and Plath, K. (2013). Epigenetics of reprogramming to induced pluripotency. *Cell* 152, 1324–1343.

Payer, B., Rosenberg, M., Yamaji, M., Yabuta, Y., Koyanagi-Aoi, M., Hayashi, K., Yamanaka, S., Saitou, M. and Lee, J. T. (2013). Tsix RNA and the Germline Factor, PRDM14,Link X Reactivation and Stem Cell Reprogramming. *Mol. Cell* 52, 805–818.

Pirouz, M., Pilarski, S. and Kessel, M. (2013). A critical function of Mad2l2 in primordial germ cell development of mice. *PLoS Genet* 9, e1003712.

Pouponnot, C., Jayaraman, L. and Massagué, J. (1998). Physical and functional interaction of SMADs and p300/CBP. *J. Biol. Chem.* 273, 22865–22868.

Powell, D. R., Hernandez-Lagunas, L., LaMonica, K. and Artinger, K. B. (2013). Prdm1a directly activates foxd3 and tfap2a during zebrafish neural crest specification. *Development* 140, 3445–3455.

Resnick, J. L., Bixler, L. S., Cheng, L. and Donovan, P. J. (1992). Long-term proliferation of mouse primordial germ cells in culture. *Nature* 359, 550–551.

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, 178–194.

Schemmer, J., Araúzo-Bravo, M. J., Haas, N., Schäfer, S., Weber, S. N., Becker, A., Eckert, D., Zimmer, A., Nettersheim, D. and Schorle, H. (2013). Transcription Factor TFAP2C Regulates Major Programs Required for Murine Fetal Germ Cell Maintenance and Haploinsufficiency Predisposes to Teratomas in Male Mice. *PLoS ONE* 8, e71113.

Schoenfelder, S., Furlan-Magaril, M., Mifsud, B., Tavares-Cadete, F., Sugar, R., Javierre, B.-M., Nagano, T., Katsman, Y., Sakthidevi, M., Wingett, S. W., et al. (2015a). The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. *Genome Res.* 25, 582–597.

Schoenfelder, S., Sugar, R., Dimond, A., Javierre, B.-M., Armstrong, H., Mifsud, B., Dimitrova, E., Matheson, L., Tavares-Cadete, F., Furlan-Magaril, M., et al. (2015b). Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. *Nat. Genet.* 1–11.

Seki, Y., Yamaji, M., Yabuta, Y., Sano, M., Shigeta, M., Matsui, Y., Saga, Y., Tachibana, M., Shinkai, Y. and Saitou, M. (2007). Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. *Development* 134, 2627–2638.

Shovlin, T. C., Durcova-Hills, G., Surani, A. and McLaren, A. (2008). Heterogeneity in imprinted methylation patterns of pluripotent embryonic germ cells derived from pre-migratory mouse germ cells. *Dev. Biol.* 313, 674–681.

Spitz, F. and Furlong, E. E. M. (2012). Transcription factors: from enhancer binding to developmental control. *Nat. Rev. Genet.* 13, 613–626.

Stevens, L. C. (1967). Origin of testicular teratomas from primordial germ cells in mice. *J. Natl. Cancer Inst.* 38, 549–552.

Su, S.-T., Ying, H.-Y., Chiu, Y.-K., Lin, F.-R., Chen, M.-Y. and Lin, K.-I. (2009). Involvement of histone demethylase LSD1 in Blimp-1-mediated gene repression during plasma cell differentiation. *Mol. Cell. Biol.* 29, 1421–1431.

Sugawa, F., Araúzo-Bravo, M. J., Yoon, J., Kim, K.-P., Aramaki, S., Wu, G., Stehling, M., Psathaki, O. E., Hübner, K. and SchOler, H. R. (2015). Human primordial germ cell commitment in vitro associates with a unique PRDM14 expression profile. *EMBO J.* 34, 1009–1024.

Sugimoto, M., Kondo, M., Koga, Y., Shiura, H., Ikeda, R., Hirose, M., Ogura, A., Murakami, A., Yoshiki, A., Chuva de Sousa Lopes, S. M., et al. (2015). A simple and robust method for establishing homogeneous mouse epiblast stem cell lines by wnt inhibition. *Stem Cell Reports* 4, 744–757.

Sumi, T., Oki, S., Kitajima, K. and Meno, C. (2013). Epiblast ground state is controlled by canonical Wnt/β-catenin signaling in the postimplantation mouse embryo and epiblast stem cells. *PLoS ONE* 8, e63378.

Tada, T., Tada, M., Hilton, K., Barton, S. C., Sado, T., Takagi, N. and Surani, M. A. (1998). Epigenotype switching of imprintable loci in embryonic germ cells. *Dev. Genes Evol.* 207, 551–561.

Tam, P. P. and Zhou, S. X. (1996). The allocation of epiblast cells to ectodermal and germ-line lineages is influenced by the position of the cells in the gastrulating mouse embryo. *Dev. Biol.* 178, 124–132.

Tanaka, S., Kunath, T., Hadjantonakis, A. K., Nagy, A. and Rossant, J. (1998). Promotion of trophoblast stem cell proliferation by FGF4. *Science* 282, 2072–2075.

Tesar, P. J., Chenoweth, J. G., Brook, F. A., Davies, T. J., Evans, E. P., Mack, D. L., Gardner, R. L. and McKay, R. D. G. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* 448, 196–199.

Tsakiridis, A., Huang, Y., Blin, G., Skylaki, S., Wymeersch, F., Osorno, R., Economou, C., Karagianni, E., Zhao, S., Lowell, S., et al. (2014). Distinct Wnt-driven primitive streak-like populations reflect in vivo lineage precursors. *Development* 141, 1209–1221.

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, 1315–1325.

Visel, A., Blow, M. J., Li, Z., Zhang, T., Akiyama, J. A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., et al. (2009). ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature* 457, 854–858.

Wan, H., Dingle, S., Xu, Y., Besnard, V., Kaestner, K. H., Ang, S.-L., Wert, S., Stahlman, M. T. and Whitsett, J. A. (2005). Compensatory roles of Foxa1 and Foxa2 during lung morphogenesis. *J. Biol. Chem.* 280, 13809–13816.

Wang, A., Yue, F., Li, Y., Xie, R., Harper, T., Patel, N. A., Muth, K., Palmer, J., Qiu, Y., Wang, J., et al. (2015). Epigenetic priming of enhancers predicts developmental competence of hESC-derived endodermal lineage intermediates. *Cell Stem Cell* 16, 386–399.

Wang, J., Zhuang, J., Iyer, S., Lin, X., Whitfield, T. W., Greven, M. C., Pierce, B. G., Dong, X., Kundaje, A., Cheng, Y., et al. (2012). Sequence features and chromatin structure around the genomic regions bound by 119 human transcription factors. *Genome Res.* 22, 1798–1812.

Weber, S., Eckert, D., Nettersheim, D., Gillis, A. J. M., Schäfer, S., Kuckenberg, P., Ehlermann, J., Werling, U., Biermann, K., Looijenga, L. H. J., et al. (2010). Critical function of AP-2 gamma/TCFAP2C in mouse embryonic germ cell maintenance. *Biol. Reprod.* 82, 214–223.

Werling, U. and Schorle, H. (2002). Transcription factor gene AP-2 gamma essential for early murine development. *Mol. Cell. Biol.* 22, 3149–3156.

Winnier, G., Blessing, M., Labosky, P. A. and Hogan, B. L. (1995). Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev.* 9, 2105–2116.

Yamaguchi, T. P., Harpal, K., Henkemeyer, M. and Rossant, J. (1994). fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev.* 8, 3032–3044.

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, 1016–1022.

Yamaji, M., Ueda, J., Hayashi, K., Ohta, H., Yabuta, Y., Kurimoto, K., Nakato, R., Yamada, Y., Shirahige, K. and Saitou, M. (2013). PRDM14 ensures naive

pluripotency through dual regulation of signaling and epigenetic pathways in mouse embryonic stem cells. *Cell Stem Cell* 12, 368–382.

Yang, S.-H., Kalkan, T., Morissroe, C., Marks, H., Stunnenberg, H., Smith, A. and Sharrocks, A. D. (2014). Otx2 and Oct4 drive early enhancer activation during embryonic stem cell transition from naive pluripotency. *Cell Rep* 7, 1968–1981.

Ying, Q.-L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. *Nature* 453, 519–523.

Yu, J., Angelin-Duclos, C., Greenwood, J., Liao, J. and Calame, K. (2000). Transcriptional repression by blimp-1 (PRDI-BF1) involves recruitment of histone deacetylase. *Mol. Cell. Biol.* 20, 2592–2603.

Zaret, K. S. and Carroll, J. S. (2011). Pioneer transcription factors: establishing competence for gene expression. *Genes Dev.* 25, 2227–2241.

Acknowledgements

We thank Erna Magnúsdóttir, Wolfram Gruhn and Toshihiro Kobayashi for helpful comments on the manuscript. U.G. is supported by the Isaac Newton Trust and the Leverhulme Trust Early Career Fellowship. M.A.S. is supported by a Wellcome Trust Investigator Award.

Figure Legends

Fig. 1: PGC specification *in vivo* and *in vitro*

(A) BMP4 signalling from the ExE specifies PGCs in the posterior epiblast of post-implantation embryos at ~E6.5. Nodal signalling induces the expression of *Bmp4* in the ExE, which in turn induces the expression of *Wnt3* in the epiblast. WNT3 maintains *Nodal* expression. It was shown that BMP and WNT signalling are required for the induction of PGCs. The AVE is source for inhibitory signals against posteriorisation of the anterior embryo. ExE: Extraembryonic ectoderm, (A)VE: (anterior) visceral endoderm; Epi: Epiblast; EM: embryonic mesoderm; PGCs: primordial germ cells; ExM: extraembryonic mesoderm.

(B) Post-implantation epiblasts separated from the extraembryonic tissues respond to BMP2 or BMP4 and large numbers of cells acquire PGC-like fate in *ex vivo* culture. In the presence of the AVE, the addition of both BMP4 and BMP8b but not of BMP4 alone is sufficient for the induction of PGCLCs. PGCLCs: PGC-like cells.

(C) In mouse, ESCs (mESCs) are differentiated into EpiLCs in the presence of bFGF and Activin A. Day2 EpiLCs respond to BMP4 and give rise to mouse PGCLCs (mPGCLCs). Similarly, the induced expression of BLIMP1/PRDM14/AP2ɣ or T

results in mPGCLC induction. The direct induction of human PGCLCs (hPGCLCs) with BMP2 or BMP4 from human ESCs (hESCs) cultured in conventional medium is very inefficient. In contrast, hESCs in 4i conditions reversibly gain and retain competence for hPGCLCs, and acquire germ cell fate in response to BMP2 or BMP4. Unlike in the mouse, the induced expression of SOX17 results in the upregulation of germ cell markers.

(D) The specification of PGCs in mice relies on BMP4, which induces the expression of WNT3. WNT3 induces the expression of T. T upregulates the expression of BLIMP1 and PRDM14. BLIMP1 induces AP2ɣ. Together, these transcription factors drive the PGC fate.

Fig. 2: Enhancer setup

Transcriptional enhancers show a combinatorial enrichment of histone modifications. The enrichment of H3K4me1 at enhancers could result in an open chromatin structure, avoid repressor binding or DNA methylation and thus could act as a 'placeholder' for transcription factor binding. Upon signalling, transcription factors can engage these enhancer elements to activate transcription of target genes. The activation of an enhancer element is marked by the enrichment of H3K27ac, which could be catalysed by p300, which is also bound by many enhancers.

Fig. 3: Preformation of enhancer-promoter contacts

(A) In ESCs, RING1B-bound promoters are in contact with poised enhancers that are enriched for H3K4me1, H3K27me3 and p300.

(B) In *Ring1A/B* knockout cells, these enhancers loose H3K27me3 but gain H3K27ac, which results in the upregulation of associated target gene expression. Of note, p300, which can catalyse the acetylation of H3K27, appears to be inhibited at poised enhancers. Since the loss of *Ring1A/B* results in enrichment of H3K27ac, perhaps the PRC1 complex is involved in the inhibition of p300 histone acetyltransferase activity.

Figure 1

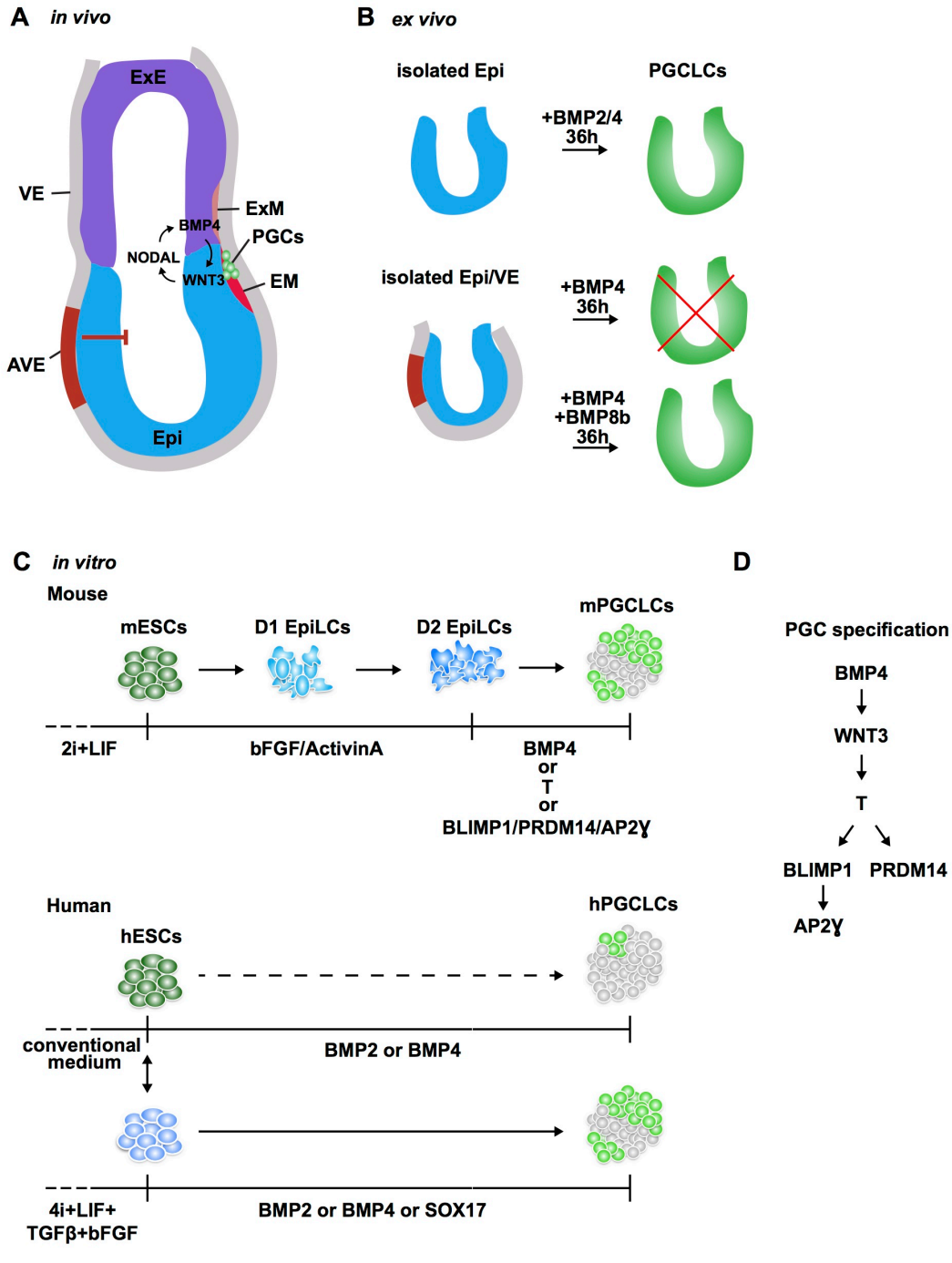

A in vivo
ExE
VE
ExM
BMP4
PGCs
NODAL
WNT3
EM
AVE
Epi
B ex vivo
isolated Epi
PGCLCs
+BMP2/4
36h
isolated Epi/VE
+BMP4
36h
+BMP4
+BMP8b
36h
C in vitro
Mouse
mESCs
D1 EpiLCs
D2 EpiLCs
mPGCLCs
2i+LIF
bFGF/ActivinA
BMP4
or
T
or
BLIMP1/PRDM14/AP2γ
Human
hESCs
hPGCLCs
conventional
medium
BMP2 or BMP4
4i+LIF+
TGFβ+bFGF
BMP2 or BMP4 or SOX17
D
PGC specification
BMP4
WNT3
T
BLIMP1
PRDM14
AP2γ

Figure 2

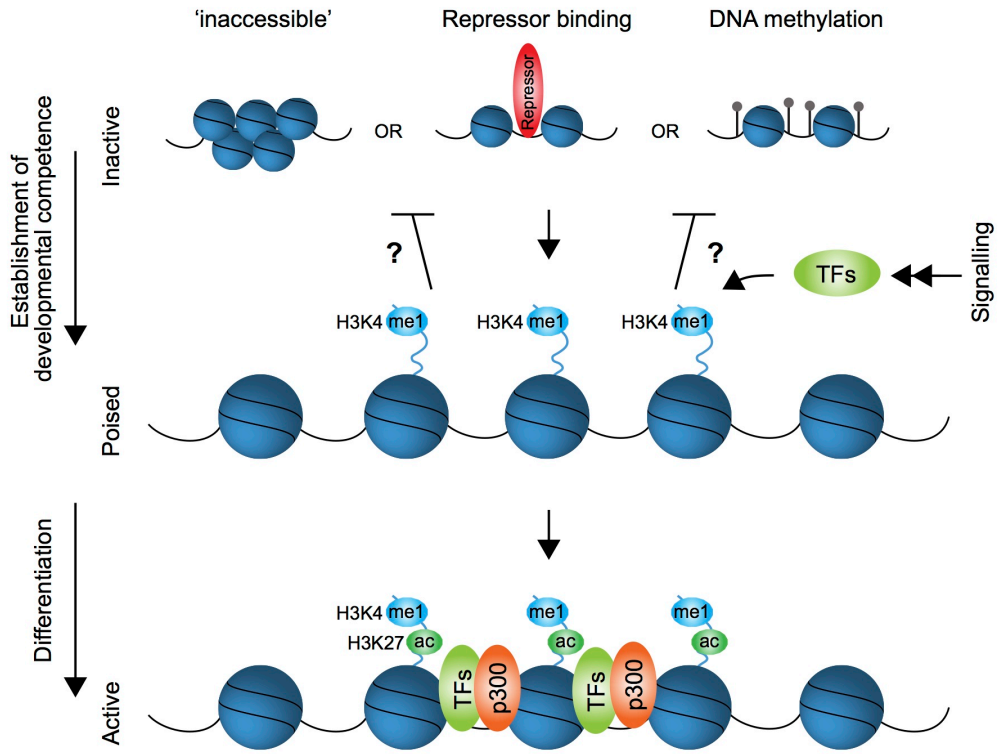
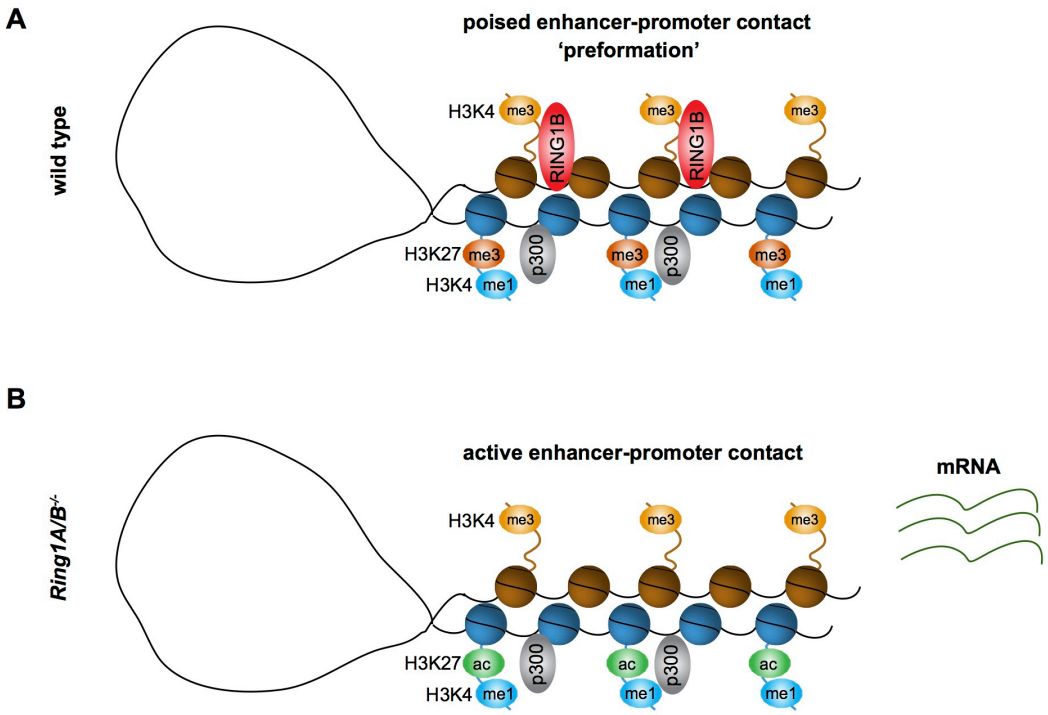

'inaccessible'
Repressor binding
DNA methylation
Repressor
OR
OR
Establishment of developmental competence
Inactive
?
?
TFs
Signalling
H3K4 me1
H3K4 me1
H3K4 me1
Poised
Differentiation
H3K4 me1
H3K27 ac
me1
ac
me1
ac
TFs
p300
TFs
p300
Active

Figure 3


A
poised enhancer-promoter contact
'preformation'
wild type
H3K4 me3
RING1B
me3
RING1B
me3
H3K27 me3
H3K4 me1
p300
me3
me1
p300
me3
me1
B
active enhancer-promoter contact
mRNA
Ring1A/B-/-
H3K4 me3
me3
me3
H3K27 ac
H3K4 me1
p300
ac
me1
p300
ac
me1