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Review
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Genetic and Epigenetic Regulators of Pluripotency

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Genetic and epigenetic mechanisms regulate the transition from the totipotent zygote to pluripotent primitive ectoderm cells in the inner cell mass of mouse blastocysts. These pluripotent cells can be propagated indefinitely in vitro, underpinned by a unique epigenetic state. Following implantation of the blastocyst, diverse epigenetic modifiers control differentiation of pluripotent epiblast cells into somatic cells, while specification of germ cells requires repression of the somatic program. Regenerating totipotency during development of germ cells entails re-expression of pluripotency-specific genes and extensive erasure of epigenetic modifications. Increasing knowledge of key underlying mechanisms heightens prospects for creating pluripotent cells directly from adult somatic cells.

Development and cell fate determination require close coordination between genetic and epigenetic programs. These are in turn regulated by signaling molecules, which together with interactions among neighboring cells induce appropriate transcriptional and epigenetic responses that are essential for cell fate determination. In addition, epigenetic mechanisms contribute to the repression of inappropriate developmental programs in time and space while ensuring heritability of existing or newly acquired phenotypic states. These extrinsic and intrinsic regulators determine the developmental origin and subsequent propagation of pluripotent states in vivo and in vitro.

Totipotency and pluripotency are two quite distinct epigenetic states with different developmental potentials. The zygote and to some extent early blastomeres are totipotent, as they are self-contained entities that can give rise to the whole organism. As these cells undergo cleavage divisions, they lack the capacity for self-renewal. Pluripotent cells are established from totipotent blastomeres within the inner cell mass (ICM) of blastocysts. As these cells cease cleavage divisions and acquire properties of normal cell division, they become responsive to external signals and acquire the capacity for self-renewal when cultured in vitro. Germ cells, while highly specialized, are unique because the end product of the lineage is the totipotent zygote. Furthermore, early germ cells repress the somatic program, and their epigenetic and transcriptional statuses share features that are compatible with pluripotency, although they cannot differentiate into diverse cell types. However, pluripotent stem cells can be derived in vitro from both the ICM and germ cells. Here we discuss the relationship between all of these different developmental states and their in vitro derivatives. We confine our discussion to chromatin- and DNA-based epigenetic changes and the transcription factors that contribute to their inherent states.

Pluripotent Cell Lineages and Their Derivatives

When development commences, the totipotent zygote contains key maternally inherited transcriptional and epigenetic factors that regulate early development. The switch from the zygotic to embryonic program occurs when transcription starts at the late zygote and at the two-cell stage (Solter et al., 2004). This is followed by preimplantation development involving about six cleavage divisions to form a blastocyst (Chazaud et al., 2006; Niwa et al., 2005; Yamanaka et al., 2006), a unique developmental stage in mammals without parallel in other organisms. Blastocysts consist of approximately 60 cells, with an ICM containing the pluripotent primitive ectoderm (PEct) cells, and the specialized outer trophectoderm cells that are required for implantation and development of the placenta. The ICM is the foundation of all somatic tissues and germ cells in adults. Following implantation, the ICM commences development to form the epiblast cells of the early egg cylinder, which are also pluripotent as judged by the expression of pluripotent cell-specific genes such as *Oct4*. These cells respond to signals from the surrounding extraembryonic tissues that direct differentiation and initiation of gastrulation (see Figure 1).

One of the earliest developmental events at the onset of gastrulation is the establishment of the founder germ cells (Surani et al., 2004). Germ cells are highly specialized cells established by a specific transcriptional program that includes repression of the somatic fate. Importantly, this is the only lineage that exhibits expression of pluripotency-specific genes after gastrulation. The transcriptional program involved in generating germ cells must also regulate the extensive epigenetic reprogramming of the genome, including genome-wide erasure of existing epigenetic modifications, which is evidently unique to this lineage and an essential step toward the eventual totipotent state.

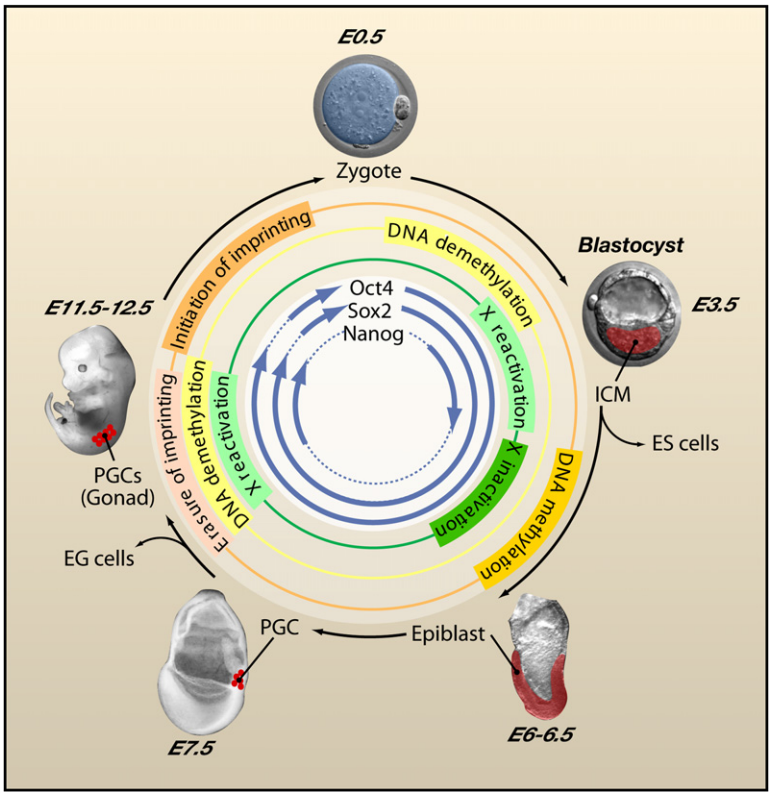

Figure 1. Genetic and Epigenetic Regulation of Pluripotency during Mouse Development

The totipotent zygote contains maternally inherited epigenetic modifiers and transcription factors, including Oct4, Sox2, and Ezh2. These, together with the embryonic transcripts, regulate development to the blastocyst stage, where the pluripotent cells are established in the inner cell mass (ICM). Deletion of *Oct4* and *nanog* compromises development of the ICM (Chambers et al., 2003; Mitsui et al., 2003; Nichols et al., 1998). In the postimplantation embryo, pluripotent epiblast cells are controlled by diverse repressive mechanisms during their differentiation into somatic and germ cell lineages (the latter of which undergo specification following repression of the somatic program). The early germ cells exhibit epigenetic and transcriptional states that are associated with pluripotency, and the ensuing epigenetic reprogramming within this lineage re-generates totipotency. The figure depicts the main epigenetic changes occurring during critical stages of development.

The ICM and primordial germ cells (PGCs) are in turn the precursors of pluripotent embryonic stem (ES) and embryonic germ (EG) cells, respectively, which are derived and maintained only in culture in vitro (Durcova-Hills et al., 2006; Matsui et al., 1992; Resnick et al., 1992; Ying et al., 2003). More recently, pluripotent stem cells have been derived from spermatogonial stem cells (Kanatsu-Shinohara et al., 2005). This suggests that the transcriptional network and epigenetic regulators capable of supporting pluripotency may be maintained during germ cell development. The ES cells can exhibit a perpetual pluripotent state in vitro, which may correspond to but is not identical to the transient pluripotent state of PEct cells in vivo. For example, specific cytokines promote the derivation and maintenance of ES cells. Leukemia inhibitory factor (LIF) and BMP4 are key factors that may not only modify PEct and evoke appropriate responses during the derivation of ES cells but also sustain pluripotency indefinitely in culture (Ying et al., 2003; Chambers and Smith, 2004). When released from the influence of these cytokines in vitro or following their introduction back into the blastocyst, ES cells undergo differentiation, just like PEct cells. These observations stress the transient nature of the pluripotency of PEct cells, as they progress quickly to the next developmental stage in vivo but can be maintained indefinitely as ES cells in vitro. Because ES (and EG) cells have no strict equivalents in vivo, theirs is a unique epigenetic state (Figure 1).

From Totipotency to Pluripotency

First, we consider the critical events that occur within the totipotent zygote and the origin of pluripotent cells during early development.

The Zygote: Creating a Template for Transcription

At fertilization, when the parental genomes come together in the oocyte cytoplasm to form the totipotent zygote, the paternal genome has a very different developmental history from the resident maternal genome and must acquire an appropriate epigenetic state to participate in development (Arney et al., 2001). Initially, the paternal genome is highly condensed, partly through its binding by protamines, which are rapidly replaced by histones. As this replacement occurs prior to S phase, a particular histone variant, H3.3, is selectively incorporated, probably by the histone chaperone Hira, into the paternal genome (Torres-Padilla et al., 2006; van der Heijden et al., 2005). Interestingly, the canonical histone H3.1 is absent from the paternal pronucleus before DNA replication (van der Heijden et al., 2005). This initial epigenetic asymmetry between the parental genomes is further manifested by differences in histone modifications and localization of numerous epigenetic modifiers such as Ezh2 (Erhardt et al., 2003).

The paternal pronucleus also features a specific pattern of histone modifications. While H3K4me1, H3K9me1, and H3K27me1 are detected at fertilization, H3K4me3, H3K9me2, and H3K27me3 become detectable only after

DNA replication (although H3K9me2 is detected very weakly) (Arney et al., 2001; Lepikhov and Walter, 2004; Santos et al., 2005; see also the Review by A. Groth et al., page 721 of this issue). The epigenetic status of the paternal pronucleus changes in other respects as well. There is extensive and rapid genome-wide DNA demethylation of the paternal genome (Mayer et al., 2000; Oswald et al., 2000). The molecular mechanism of this global DNA demethylation is currently unknown, but correct epigenetic configuration of the paternal chromatin is likely to be important given the fact that the maternal genome escapes this process. As histone methylation can direct DNA methylation, at least in particular genomic regions, the differences in histone modifications between parental pronuclei may explain the protection of the maternal genome from undergoing DNA demethylation. More recently, Stella was shown to be required for preventing DNA demethylation of the maternal genome; in Stella-deficient oocytes, the maternal genome is massively demethylated (Nakamura et al., 2007). However, as Stella is found in both maternal and paternal pronuclei, additional factors must cooperate to protect the maternal genome from DNA demethylation.

Switching from the Totipotent Zygote to the Embryonic Program

The zygote contains a number of key maternally inherited transcription factors, including some that are essential for pluripotency, such as Oct3/4 and Sox2, as well as epigenetic factors for histone modifications including Polycomb group (PcG) proteins such as Ezh2 and Eed, proteins of histone metabolism (Padi4), and chromatin remodelers such as Brg1 (see the Review by B. Schuettengruber et al., page 735 of this issue). As the key requirement at this stage of development is to convert the quiescent genome into a transcriptionally competent one, this must be accomplished by maternally inherited factors in the oocyte. Among the maternal factors whose function has been well defined is Brg1, a component of the SWI/SNF chromatin-remodeling complex (Bultman et al., 2006). Loss of Brg1 results in reduced transcription and arrest at the two-cell stage. Another example is Npm2, whose presence in the oocyte is essential for histone deacetylation and heterochromatin formation surrounding the nucleoli (Burns et al., 2003; Table 1).

From the late zygote to the two-cell stage, when the embryonic genome becomes activated, the epigenetic status of the parental genomes starts to become less distinct, with the exception of DNA methylation. The overall differences in DNA methylation persist for one to two cleavage divisions, followed by a passive and steady decline through preimplantation development (Mayer et al., 2000). This change is accompanied by a gradual increase in H3K9me2 (Santos et al., 2003; Yeo et al., 2005). Notably, examination of cloned mammalian embryos has revealed that the levels of methylated H3K9 in preimplantation embryos are important for further development (Santos et al., 2003). This provides further evidence for the importance of the chromatin configuration during this developmental stage.

The Origin of Pluripotent Cells

The early blastomeres, until about the eight-cell stage, are essentially identical and totipotent and retain considerable plasticity. However, individual blastomeres at the four-cell stage may have some bias in their contribution to the ICM and trophectoderm lineages in an unperturbed embryo (Zernicka-Goetz, 2005). Although the descendents of a single eight-cell-stage blastomere may give rise only to trophectoderm cells, no descendents of a single blastomere at this stage can give rise only to pluripotent PEct cells. At the eight-cell stage, each blastomere becomes polarized and divides either symmetrically to generate two polar outer cells (OCs) or asymmetrically to generate an apolar inner cell (IC) and a polar OC. Thus, between the eight- and 16-cell stage, the first distinct group of ICs and OCs are generated, which are the precursors of the pluripotent PEct cells in the ICM and the trophectoderm cells, respectively (Johnson and Ziomek, 1981; Yamanaka et al., 2006).

The ''permissive'' epigenetic state generated in the zygote allows a number of key transcription factors to play a critical role during development of the blastocyst. Among these factors are Oct4 and Cdx2, which are essential for development of the ICM and trophectoderm, respectively (Niwa et al., 2005). In the early morula stage, both of these factors are expressed in all blastomeres. In the late morula stage, when the IC and OC are formed, Oct4 is detected in the IC whereas Cdx2 is confined to the OC (Niwa et al., 2005). The ICM itself comprises the inner PEct and the outer primitive endoderm (PEnd). An additional feature of the ICs in a morula is the expression of Nanog, a homeodomain protein (Chambers et al., 2003; Mitsui et al., 2003). Recent studies show that the expression of *nanog* in early blastomeres may be regulated by the histone arginine methyltransferase Carm1 (Torres-Padilla et al., 2007), although the expression of Carm1 does not commit the cell to develop exclusively as an IC. The role of Nanog is apparently to promote development of PEct, as the ICM of the E3.5 blastocyst shows a mutually exclusive mosaic pattern of expression of Nanog and Gata6 in individual cells. (Expression of the latter is essential for the development of PEnd cells.) If Gata6 expression is eliminated from the ICM, all of the cells show expression of Nanog (Chazaud et al., 2006).

No distinct epigenetic differences between ICs and OCs in the morula have been reported, but we cannot exclude a possibility that such differences could dictate mutually exclusive expression of the key transcription factors described above. In any event, the ICs at the 16-cell stage are not yet fully committed and can develop into trophectoderm cells if extracted and exposed to the outside environment during subsequent development. It is likely, however, that once distinct cell fate decisions are made, appropriate gene- or locus-specific epigenetic modifications may ensure that the identities of the pluripotent PEct cells, as well as those of the trophectoderm and PEnd cells in the blastocyst, are maintained. The PEct cells in particular may be constrained from undergoing differentiation into extraembryonic tissues by epigenetic regulators.

Table 1. Epigenetic Modifiers that Are Critical for Pre- and Early Postimplantation Development in the Mouse

Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
Histone Modifications					
Glp/Ehmt1	HMTase	Severe growth retardation and lethality at E9.5; reduction of H3K9me1 and H3K9me2 in embryos	ND	yes	Tachibana et al. (2005)
G9a/Ehmt2	HMTase	Loss of H3K9 methylation in euchromatin; developmental and growth arrest at E8.5	yes	yes	Tachibana et al. (2002)
Eset/ SETDB1	HMTase	Peri-implantation lethality (between E3.5 and E5.5); defects in ICM outgrowth	yes	no	Dodge et al. (2004)
Suv39h1 Suv39h2	HMTase	Double knockout shows loss of H3K9 methylation in heterochromatin; polyploidy in MEF cells; chromosome pairing defects during spermatogenesis; male sterility and death of some double-mutant embryos at E14.5	ND	yes	Peters et al. (2001)
Ezh2/ Enx-1	HMTase PRC2 complex	Growth defect of the primitive ectoderm; peri-implantation lethality	yes	no	O'Carroll et al. (2001)
Mll/All-1	HMTase	Skeletal abnormalities; Hox gene misregulation (loss of H3K4me1 and aberrant DNA methylation); other morphogenetic defects by E10.5; embryonic lethality; truncation in exon 5 leads to early developmental arrest prior to two-cell stage	ND	ES viable (defective gene expression)	Glaser et al. (2006); Yagi et al. (1998); Yu et al. (1995)
Meisetz	HMTase	Meiotic defect causing sterility	no	ND	Hayashi et al. (2005)
PRMT1	Arg MTase	Early postimplantation lethality before gastrulation	ND	yes	Pawlak et al. (2000)
Blimp1/ PRDM1	PR/SET domain protein	Patterning defects; loss of germ cell precursors	no	yes	Ohinata et al. (2005); Vincent et al. (2005)
Gcn5	HAT	Lethal at E7.5–E8.5; patterning defects	yes	ND	Xu et al. (2000)
HDAC1	HDAC	Defects in proliferation; delayed development; embryonic lethality by E10.5	yes	yes (ES cells defective)	Lagger et al. (2002)
Polycomb					
Eed	PRC2/3 complex	Defective gastrulation; failure to maintain inactive X in trophoblast cells	yes	yes	Shumacher et al. (1996)
Suz12	PRC2/3 complex	Early postimplantation lethality; gastrulation defects	yes	ND	Pasini et al. (2004)
YY1	PRC2/3 interaction	Defects in epiblast cell growth/survival; peri-implantation lethality	yes	no	Donohoe et al. (1999)
Ring1b/ Rnf2	Ubiquitin ligase PRC1 complex	Gastrulation defects; lethality by E9.5	yes	ES viable	Voncken et al. (2003)
DNA Methylation					
Dnmt1	DNA MTase	Genome-wide demethylation; developmental arrest at E8.5	yes	yes	Li et al. (1992)
Dnmt3a	DNA MTase	Malfunction of gut; spermatogenesis defects; postnatal lethality (~4 weeks of age)	yes	yes	Okano et al. (1999)
Dnmt3b	DNA MTase	Demethylation of minor satellite DNA; mild neural tube defects; embryonic lethality at E14.5–E18.5	yes	yes	Okano et al. (1999)

Table 1. *Continued*

Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
Dnmt3L	DNA MTase (no enzymatic function)	Failure to establish maternal methylation imprints in oocytes; male sterility due to spermatogenesis defects	yes	ND	Bourc'his et al. (2001); Hata et al. (2002)
MBD Proteins					
Mbd3	Chromatin-remodeling NuRD complex	Normal implantation; developmental arrest at E6.5 or earlier	yes	no (ES viable)	Hendrich et al. (2001); Kaji et al. (2006)
Chromatin-Remodeling/Histone Chaperones					
Brg1	SWI/SNF	Growth defects of primitive ectoderm and trophectoderm; peri-implantation lethality; oocyte depletion causes zygotic arrest	yes	no	Bultman et al. (2000); Bultman et al. (2006)
Snf5/Ini1/ Smarcb1	SWI/SNF	Peri-implantation lethality	ND	no	Klochendler-Yeivin et al. (2000)
Lsh/Hells/ PASG	SWI/SNF	Global demethylation of genomic DNA at E13.5; role in meiotic chromosome synapsis and retrotransposon silencing in female germline; postnatal lethality	yes	ND	Geiman and Muegge (2000); Sun et al. (2004); De La Fuente et al., 2006
Srg3/ Smarcc1	SWI/SNF	Lethality around implantation; defective ICM outgrowth	ND	no	Kim et al. (2001)
ATRX	SWI/SNF	Male-specific embryonic lethality by E9.5 due to defect in formation of extraembryonic trophoblast and X inactivation	yes	ND	Garrick et al. (2006)
CAF-1	Histone chaperone	Early preimplantation lethality; arrest at 16-cell stage; defects in constitutive heterochromatin	yes	no	Houlard et al. (2006)
HIRA	Histone chaperone	Gastrulation defects; embryonic lethality by E10.5	yes	yes	Roberts et al. (2002)
Nasp	Histone chaperone	Preimplantation lethality at blastocyst stage	yes	no	Richardson et al. (2006)
Npm2	Histone chaperone	Defective nucleolar structure; loss of heterochromatin and acetylated histone H3; early preimplantation lethality (most embryos arrested at two-cell stage)	yes	no	Burns et al. (2003)
miRNA Metabolism					
Ago2	miRNA processing	Lethal at E9.5	ND	ND	Liu et al. (2004)
Dicer	miRNA processing	Postimplantation lethality before gastrulation	yes	no (ES viable)	Bernstein et al. (2003)

The role of key epigenetic modifiers has been established by genetic experiments. Deletion of many of these genes also causes a failure of the ICM to give rise to ES cells in vitro, suggesting a direct role for these factors in the establishment or maintenance of pluripotency. ND, not determined; E, embryonic day; MBD, methylcytosine binding domain; HAT, histone acetyltransferase; HMTase, histone methyltransferase; DNA MTase, DNA methyltransferase; ES viable, viability of ES cells when the second allele or both alleles are deleted from established ES cells in vitro.

Nevertheless, at the blastocyst stage, there are clear epigenetic differences between the ICM and trophectoderm cells. This is evident from the analysis of X inactivation in these tissues, which may be indicative of other differences between them. In female embryos, the "imprinted" paternal X chromosome is preferentially inactivated during preimplantation development. The initial event involves expression of the noncoding RNA *Xist* from the paternal X chromosome, which is followed by histone modifications including loss of H3K4me2 and

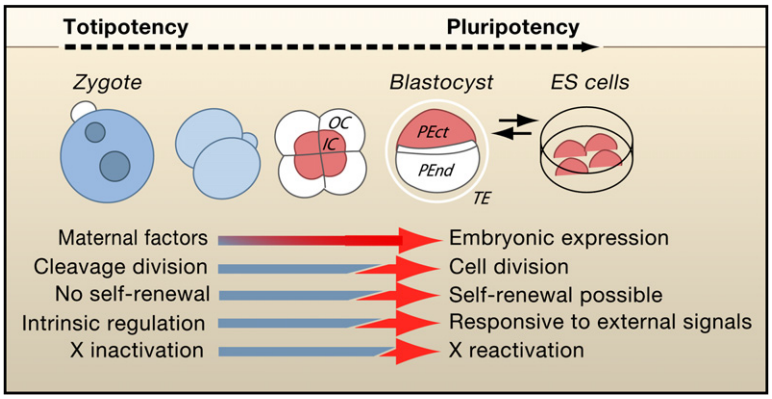

Figure 2. The Transition from Totipotency to Pluripotency

The zygote contains maternally inherited factors that, together with the embryonic transcripts, regulate cleavage divisions. At the morula stage, two distinct cell populations, inner cells (ICs) and outer cells (OCs), are formed. The ICs are the precursors of the pluripotent primitive ectoderm cells (PEct) within the ICM. The ICM also contains the outer layer of primitive endoderm cells (PEnd). Cleavage divisions are replaced by cell divisions as the primitive ectoderm cells within the ICM undergo final epigenetic reprogramming to generate pluripotent cells. These cells can be propagated indefinitely under appropriate conditions as pluripotent ES cells in vitro, where they exhibit a unique epigenetic state, and can differentiate into all of the diverse cell types upon reintroduction into host blastocysts.

H3K4me3 and the gain of H3K9me2 and H3K27me3, as well as the ubiquitination of H2A (Heard, 2004; see also the Review by P.K. Yang and K.I. Kuroda, page 777 of this issue). Notably, all of the cells of blastocysts initially show epigenetic marks that are consistent with the inactivated paternal X chromosome. In the late blastocyst, however, the epigenetic marks associated with the inactive paternal X chromosome are preferentially erased in the PEct cells, where both X chromosomes become potentially active (Mak et al., 2004; Okamoto et al., 2004). The paternal X chromosome stays imprinted and inactivated only in the extraembryonic trophectoderm and PEnd cells. The erasure of the imprint on the paternal X chromosome occurs in the ICM, where the pluripotent PEct cells reside, and this event may signify the establishment of the pluripotent state. Subsequently, there is random X inactivation in the developing embryo when PEct cells commence differentiation (Figure 2).

Profound epigenetic differences between the ICM and trophectoderm cells appear not only at the level of histone modifications but also at the level of DNA methylation. Following passive DNA demethylation, which is characteristic for preimplantation development, the ICM of the blastocyst starts to reacquire DNA methylation marks, which is coupled with the restricted expression of Dnmt3b in the ICM (Watanabe et al., 2002). By contrast, trophectoderm cells stay relatively hypomethylated. These differences are also reflected in the mechanisms used for the maintenance of genomic imprints, which involves modifications of histones and DNA methylation in the placenta and the embryo, respectively (Lewis et al., 2004). This is perhaps an evolutionary adaptation, given that the placenta exists for a relatively short duration compared to the embryo, which develops into an adult.

The ICM "Niche" and Establishing the Pluripotent State

Significant epigenetic events such as the erasure of the epigenetic marks associated with the paternal inactive X chromosome occur specifically in PEct cells within the ICM, which may be indicative of other epigenetic reprogramming events. It is possible that X reactivation observed in the PEct cells is a consequence of epigenetic reprogramming, which may be essential in these cells for them to acquire pluripotency. Currently, little is known about the precise mechanisms that trigger these epigenetic changes in PEct cells and what other epigenetic changes occur in these cells that could be critical for pluripotency.

It is possible that the ICM provides a "niche" where signaling molecules from the surrounding cells may regulate the erasure of some of the epigenetic modifications as PEct cells acquire pluripotency. The reactivation of the inactive paternal X chromosome includes uplifting of chromatin marks, such as H3K27me3, which is introduced by Ezh2, a member of the PcG complex (Mak et al., 2004; Okamoto et al., 2004). It is possible that the underlying mechanism could have some similarities with the molecular processes that are associated with the transdetermination phenomenon in *Drosophila.* In the imaginal discs, signaling molecules drive the transdetermination process that involves uplifting of the repression of the existing phenotypic identity exerted by the PcG proteins (Lee et al., 2005; Maurange et al., 2006). This results in increased developmental plasticity and establishment of a new cellular identity. If such a model applies in the case of ICM, we may envisage that certain signaling molecules may trigger epigenetic reprogramming of PEct cells, including erasure of paternal X inactivation during the establishment of pluripotency in PEct cells. Reprogramming in the ICM may then include general disruption of heterochromatic gene silencing, leading to an increased genomic plasticity. Importantly, these reprogramming processes occur in the presence of transcription factors such as Nanog that are expressed specifically in the ICM (Chambers et al., 2003; Mitsui et al., 2003), which are necessary for the acquisition and the maintenance of pluripotency. While PEct cells undergo epigenetic changes associated with pluripotency, there are also important changes in the nature of the cell cycle and division. Notably, cleavage divisions that accompany early

development and result in progressive reduction in cell size are replaced by normal cell divisions accompanied by cell growth.

The Zygote and ICM—Two Distinct Epigenetic Entities

Further insights into the epigenetic mechanisms that regulate pluripotency come from examining the fate of somatic nuclei in cloning experiments, which may be informative regarding how the ICM niche may play a role in conferring pluripotency. Experiments have demonstrated that when a female (XX) somatic nucleus is transplanted into the oocyte, the original inactive X (Xi) is preferentially inactivated in the extraembryonic tissues (Bao et al., 2005; Eggan et al., 2000). However, as is the case for the imprinted paternal X chromosome in normal embryos, this persisting epigenetic memory associated with the Xi is erased in the ICM, which is followed by random X inactivation during subsequent development. This analysis also suggests that the oocyte may have some potential for DNA demethylation but that the histone modification mark, H3K27me3, which is imposed by the PcG protein Ezh2 on the Xi, cannot be erased in the zygote and during early preimplantation development. This mark is only erased later in the ICM, thus leading to subsequent random X chromosome inactivation in the embryo (Bao et al., 2005; Eggan et al., 2000). These studies emphasize the fact that the oocyte and the ICM have distinct potentials for modifying epigenetic information, and the specific features of the ICM may be critical for the generation of pluripotent cells in the ICM.

The impression from at least some studies involving nuclear transplantation using somatic cells as donors is that the development of the trophectoderm in blastocysts is relatively less affected compared to the ICM (Kishigami et al., 2006). This can be judged by the highly variable expression of many markers of pluripotency, such as *Oct4* (Bortvin et al., 2003), whereas the expression of *Cdx2* in the trophectoderm is relatively stable. By contrast, development following transplantation of nuclei from ES cells is significantly better. One possibility is that the ES cell chromatin is more responsive to the maternally inherited transcription (and epigenetic) factors present in the oocyte. Gene silencing in ES cells is governed by an unusual combination of histone modifications on individual loci, which include the repressive H3K27me3 that coexists with the H3K4 modification usually associated with active genes. Similar epigenetic marks are also detected on the maternal pronuclei (Erhardt et al., 2003; Lepikhov and Walter, 2004; Santos et al., 2005). By contrast, the H3K9me2 repressive mark and the exclusion of H3K4 methylation ensure gene silencing in somatic nuclei. This epigenetic state makes somatic nuclei less responsive to the transcription factors in the oocyte, thus contributing to the difficulty in reprogramming them to pluripotency.

Pluripotency: Preimplantation to Postimplantation

Following implantation of blastocysts, the pluripotent epiblast cells exhibit an exceptionally rapid cell cycle (O'Farrell et al., 2004), and they respond to the signals from the surrounding extraembryonic tissues. The pluripotent primitive ectoderm cells in the ICM undergo changes and form epiblast cells, which initially retain pluripotency (Figure 1). These epiblast cells, however, differ transcriptionally from the primitive ectoderm cells. For example, the pluripotency-associated gene *nanog* is rapidly downregulated in the epiblast, as are *stella*/*PGC7* and *Rex1* (Chambers et al., 2003; Payer et al., 2003; Sato et al., 2002). However, other genes such as *Fgf5* and *Prce* are upregulated in the epiblast cells. Nevertheless, in many respects, the epiblast cells are pluripotent and will generate all of the somatic tissues as well as germ cells. Notably, just as ES cells are derived from PEct cells, it is possible to generate pluripotent stem cells mimicking epiblast cells, which have different characteristics from ES cells (Rathjen et al., 1999). We anticipate that these pluripotent stem cells from postimplantation embryos may have a relatively stable epigenetic state compared to ES cells derived from the ICM since these cells are formed at the time when a new and possibly more stable epigenetic state is being established (see below).

The epigenetic mechanism operating at the interface between preimplantation and postimplantation development is perhaps most relevant to the understanding of how ES cells are maintained in an undifferentiated state and how the early stages of their differentiation may be regulated. This period is particularly sensitive to epigenetic regulation, perhaps due to the necessity to maintain pluripotency in the primitive ectoderm and subsequently in the epiblast cells in preparation for their differentiation into specific cell types, while preventing them from differentiating into extraembryonic lineages such as the trophectoderm cells. It is noteworthy that development up to the blastocyst stage, which involves cleavage divisions and is partly regulated by maternally inherited transcriptional and epigenetic modifiers, is relatively less prone to perturbation. By contrast, the transition to the postimplantation development is driven by epigenetic and genetic regulators, which are transcribed by the embryo itself. Thus, whereas preimplantation development primarily involves both erasure and maintenance of epigenetic modifications, postimplantation development primarily involves establishment of new epigenetic modifications. In light of these observations, it is not surprising that some of the key histone methyltransferases, such as Ezh2, Eset, G9a, and members of the NuRD complex, have their critical effects on development during the transition from pre- to postimplantation development. For example, oocytes depleted of all maternally inherited Ezh2 can develop as parthenogenetic blastocysts, but *Ezh2* null blastocysts cannot develop much further following implantation (Erhardt et al., 2003). The significance of some key epigenetic modifiers on early development is summarized in Table 1.

In summary, in the zygote at the initiation of development, the primary events are centered around the creation of permissive chromatin for the maternal factors to initiate

embryonic transcriptional program. The transition from the totipotent zygote to the development of pluripotent PEct cells is one of the key outcomes of early development. Transcription factors including Oct4, Sox2, Nanog, and Cdx2 are important for this development to the blastocyst stage. By contrast, a common feature during postimplantation development is the influence of diverse repressive mechanisms involving histone methyltransferases, transcription repressors, miRNAs, and Dnmts, which regulate the transition from PEct to epiblast cells. Their role may be not simply to sustain pluripotency in postimplantation epiblasts when *nanog*, *stella/PGC7*, and other similar genes are downregulated but also to prevent premature ectopic expression of lineage-specific genes. Considering all of the data mentioned above, early development requires a critical balance between the activities of transcriptional and epigenetic factors.

Germ Cells versus Soma

Germ cell lineage generates the totipotent state and provides the enduring link between all generations. Extensive epigenetic modifications occur in this lineage, which is crucial for the development of gametes and totipotency. Unlike somatic cells, germ cells show expression of some key pluripotency-specific genes, although they are highly specialized cells and cannot contribute to chimeras if introduced into blastocysts. Nevertheless, it is possible to derive pluripotent stem cells (EG cells) directly from early PGCs (Matsui et al., 1992; Resnick et al., 1992). Notably, germ cells have distinct characteristics that differ from both epiblast and somatic cells. Specification of germ cells generally occurs in very early embryos, but in mice, this event is deferred until postimplantation development. PGCs originate from a few pluripotent proximal epiblast cells, while the remaining cells acquire a somatic fate. These proximal epiblast cells acquire competence to form germ cells and show expression of *fragilis* at E6.25 (Saitou et al., 2002). Evidently, all competent cells are initially destined toward a somatic fate as judged by the initiation of the expression of genes such as *Hoxb1*. However, cells that undergo specification into PGCs exhibit repression of the somatic program and subsequently show expression of some of the key pluripotency-specific genes (Ohinata et al., 2005; Surani et al., 2004; Yabuta et al., 2006).

Repression of the somatic program during germ cell specification is observed in many model organisms, although the molecular mechanisms are not conserved among different species. In *C. elegans*, repression of the somatic transcriptional program is achieved through Pie-1, an RNA-binding protein that interferes with elongation and/or RNA processing in the P blastomere precursor of germ cells (Mello et al., 1996; Seydoux and Dunn, 1997). Taking into account the high levels of H3K4me2 and low levels of phosphorylation of the RNA polymerase II (Schaner et al., 2003; Seydoux and Dunn, 1997), it seems that, although the epigenetic status is permissive for gene expression, the repression machinery extinguishes mRNAs by both transcriptional and posttranscriptional mechanisms. In *Drosophila*, neither H3K4 methylation nor phosphorylation of the RNA polymerase II is observed in germ cell precursors, the pole cells. This transcriptional quiescence is instead dependent upon the polar granule component (Pgc). In *pgc* mutant pole cells, H3K4 methylation and phosphorylation of the RNA polymerase II are elevated, and, more specifically, a variety of genes are ectopically expressed (Deshpande et al., 2004; Martinho et al., 2004; Nakamura et al., 1996). Thus, in *Drosophila*, global repressive histone modifications contribute to the quiescence in the germ cells.

In mice, the proximal pluripotent epiblast cells from which PGCs arise are transcriptionally active. These cells respond to signals from the extraembryonic tissues and can undergo differentiation to form either germ cells or somatic cells (Surani et al., 2004). Therefore, in cells that are destined to form germ cells, there is a need to impose repression of the somatic program and ensure that they retain or reacquire the pluripotent character, which is the background against which subsequent critical epigenetic modifications occur in early germ cells. Recent studies have shown that the transcriptional repressor Blimp1 is the crucial molecular determinant of the germ cell lineage in mice (Ohinata et al., 2005; Vincent et al., 2005).

Blimp1: Repression of the Somatic Program during PGC Specification

Analyses of candidate genes in founder PGCs and somatic neighbors showed that *Blimp1* (or *Prdm1*) is expressed in the founder PGCs but not in the somatic neighbors (see Figure 3). Blimp1 is a transcriptional repressor with an N-terminal PR/SET domain, a proline-rich region, five C_2H_2 zinc fingers, and a C-terminal acidic domain. *Blimp1* was first identified as the key gene involved in the specification of plasma cells from B cells, partly through its repression of the B cell-specific genetic program. It is noteworthy that *Blimp1* expression is detected in many tissues and in many organisms. A variety of functions are attributed to Blimp1 protein, but it seems that it is only in mice (and perhaps other mammals) that it has a role in germ specification. The diverse roles of this highly conserved gene suggest that it must have acquired new enhancers or regulatory elements for its role in germ cell specification in mammals.

Blimp1 is first detected in about six cells among the proximal pluripotent epiblast cells at E6.25 of development, prior to the onset of gastrulation (Ohinata et al., 2005). This number increases progressively to approximately 28 cells by the late streak stage and approximately 40 specified PGCs at E7.25 of development. Genetic lineage tracing experiments confirmed that all the early Blimp1-positive cells are lineage-restricted PGC precursor cells (Ohinata et al., 2005). Deletion of *Blimp1* results in aberrant development of founder PGCs as they cease proliferation and form a tight cluster, unlike in normal embryos, where they begin to migrate away from the cluster. More significantly, these mutant cells show inconsistent repression of the somatic program as judged by the

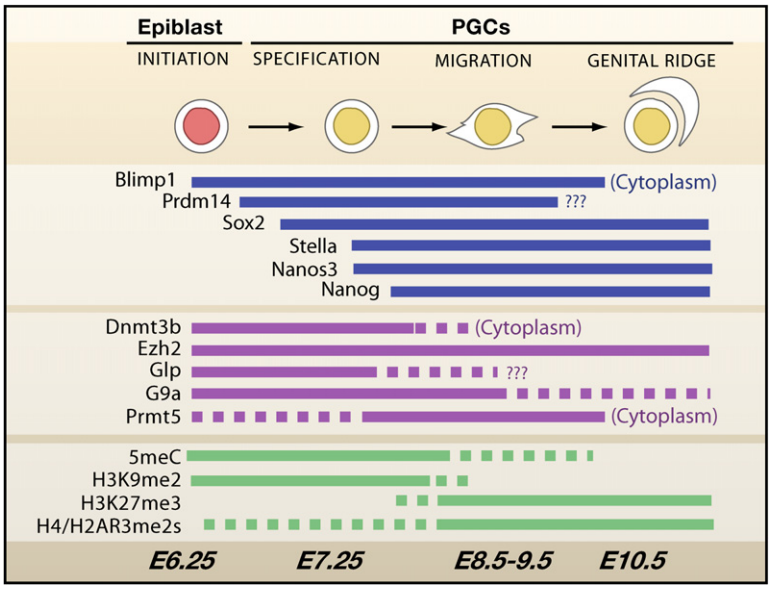

Figure 3. Epigenetic Regulation of Germ Cell Specification

Primordial germ cells (PGCs) are specified from pluripotent epiblast cells. The Blimp1/Prmt5 complex potentially represses the somatic program during specification and subsequently maintains this lineage. A number of key epigenetic modifications (for example, an increase in H3K27me3 and loss of H3K9me2) that are consistent with pluripotency are observed, together with the re-expression of pluripotency-specific genes such as *nanog*. More extensive erasure of epigenetic modifications follows at E11.5 in PGCs in the developing gonads when Blimp1/Prmt5 exits from the nucleus.

aberrant expression of *Hoxb1*. The mutant cells also fail to show expression of *stella* as well as *Sox2* consistently, the latter being a key gene that is associated with pluripotency. Recently, Prdm14, another member of the PRDM family, has been detected very early and specifically in PGC precursors, which suggests that this factor may also have a role in PGC specification (Yabuta et al., 2006).

The Blimp1/Prmt5 Complex in the Germ Cell Lineage

Blimp1 has the potential to form many different types of repressive complexes. For example, it apparently forms a complex with a histone modifier, G9a, as well as with Groucho and Hdac2, and other complexes may also form in a context-dependent manner. Recent studies have indicated that Blimp1 can form a novel complex with Prmt5, an arginine-specific histone methyltransferase, which mediates symmetrical dimethylation of arginine 3 on histone H2A and/or H4 tails (H2A/H4R3me2s) (Ancelin et al., 2006). Blimp1 and Prmt5 are coexpressed in the mouse germ cell lineage, and this is associated with high levels of the H2A/H4R3me2s modification in germ cells. It is important to note that Prmt5 also has the potential to methylate other protein substrates outside the nucleus, such as spliceosomal Sm proteins. Interestingly, in flies, mutation in the Prmt5 homolog, Capsuleen/dart5, has a striking effect on germ cells. The males are sterile, as the *Capsuleen/dart5* mutation affects development of spermatocytes, whereas in females, *Capsuleen/dart5* mutation affects specification of germ cells (Gonsalvez et al., 2006; Anne et al., 2007). In mice, the presence of Prmt5 in the nucleus of PGCs may be critical through its role in histone H2A/H4R3me2s modification at the earliest stages of the formation of PGC precursors and germ cell specification, but its precise role is currently under investigation (W.W. Tee, personal communication). Nevertheless, a number of putative targets have been identified for the Blimp1/Prmt5 complex in the nucleus, among which is *Dhx38*, an RNA helicase whose homolog has a role also in the *C. elegans* germline. In the mouse PGCs, *Dhx38* is repressed until E12.5, and this repression is associated with H2A/H4R3me2s modification of the locus. It is likely that the Blimp1/Prmt5 complex has a continuing role in the mouse germ cell lineage as a repressive complex with many other targets. One of the potential roles of this complex may also be to maintain the early germ cell lineage at a time when the epigenetic status of early PGCs is relatively plastic and they show expression of some key genes that are detected generally in pluripotent cells (see below).

Epigenetic Modifications and Lineage-Specific Character of Founder PGCs

A key feature of the specification of the germ cell lineage is that it reacquires and retains some of the essential characteristics of pluripotency (Figure 3). Some of the initial epigenetic changes in the founder PGCs are probably necessary for this generation of a unique pluripotent-like state, which is rapidly lost from the majority of the cells that differentiate into somatic cells.

Shortly after specification, a genome-wide change in histone modifications occurs in newly specified PGCs. There is loss of H3K9me2 in the PGCs, while the H3K27me3 mark becomes more prominent (Seki et al., 2005; K. Ancelin, P.H., and M.A.S., unpublished data). It has been observed that early PGCs downregulate *Ehmt1* (*Glp*) after E7.25, which is not observed in the neighboring somatic cells (Yabuta et al., 2006). By contrast, expression of *Ehmt2* (*G9a*) remains unchanged in both somatic cells and PGCs. It is known that G9a and GLP form a complex that is essential for H3K9me2 methylation (Tachibana et al., 2005), indicating that the downregulation of *Glp* may allow loss of H3K9me2 to proceed in PGCs. Thus, while mutation in *G9a* is early embryonic lethal, this does not affect PGC specification (K. Ancelin,

P.H., and M.A.S., unpublished data). Alternatively, the erasure of H3K9me2 may also involve a histone demethylase, such as Lsd1, or possibly other mechanisms. At the same time, the elevation of H3K27me3 is associated with high expression of Ezh2 in PGCs, which may be a key characteristic of pluripotency since many somatic genes are repressed by H3K27me3 in pluripotent stem cells (Bernstein et al., 2006; Boyer et al., 2006b). It will be important to determine whether a similar mechanism also contributes to the repression of somatic genes in PGCs. In addition, *Dnmt3b* is downregulated specifically in PGCs after E7.25, and, while observed later in PGCs, the Dnmt3b protein is located in the cytoplasm (Hajkova et al., 2002). This may allow DNA demethylation to proceed once PGCs enter the gonads (see below). It will be important to investigate the interactions between diverse epigenetic factors to clarify which factors regulate the epigenetic status of specific genomic targets in germ cells, such as the gene-encoding regions, repetitive elements, and imprinting control elements (see Figure 3).

As the germ cells increase in number, they start to migrate into the developing gonads, which they reach around E10.5. Soon afterwards, PGCs undergo a wave of genome-wide DNA demethylation, which is accompanied also by the profound changes in chromatin configuration and erasure of some histone modification marks (P.H., K. Ancelin, and M.A.S., unpublished data). This DNA demethylation affects single-copy genes as well as genomic imprints and repetitive elements, and the kinetics of this epigenetic event is indicative of an active process. The female PGCs, which initially show random X chromosome inactivation as seen in somatic cells, subsequently show reactivation of the Xi, most likely as a part of the global epigenetic reprogramming process.

Evidence suggests that the entry of PGCs into the genital ridge may be important for the onset of the reprogramming process (Tam et al., 1994). It is possible that signaling molecules from these somatic cells may regulate this major epigenetic event, which bears some similarity to the epigenetic reprogramming event in the ICM of blastocysts discussed above, and the importance of the appropriate developmental niche. There is reactivation of the X chromosome and transient loss of Polycomb chromatin marks in both instances. However, while the erasure of chromatin modifications in the ICM is perhaps important for generating pluripotency, the extensive erasure of epigenetic modifications in the gonadal PGCs is an important step toward the eventual regeneration of totipotency. In both cases, such extensive epigenetic modifications are a route to significantly alter the phenotypic and developmental state of cells. PGCs also show similarity to the events in the zygote, including extensive erasure of DNA methylation, which allows the transition from the zygotic to the embryonic developmental program. It seems that the PGCs may employ a combination of epigenetic mechanisms and the expression of key molecules that are seen in both the zygote and the ICM. This combination of the epigenetic mechanisms enabling erasure of both DNA methylation and histone modifications makes the germline a unique epigenetic entity.

The epigenetic modifications that occur at the establishment of the germ cell lineage discussed above are accompanied by the expression of some of the key pluripotency-associated genes, such as *Sox2*, which precedes expression of *stella*, a marker of specified founder PGCs. Also, *nanog*, which is downregulated in the epiblast immediately after implantation of the embryo, shows increased transcription in founder PGCs, and the protein is detected shortly thereafter (Yamaguchi et al., 2005). *Oct4*, on the other hand, is continually expressed throughout in PGC precursors and following their specification onward. Thus, the three key pluripotency-associated genes, *Oct4*, *Sox2*, and *nanog*, are detected in the early PGCs. However, other markers of pluripotency are detected later in the germ cell lineage. For example, Esg1 is not detected until E8.5, while Dppa2 and Dppa4 are not detected until E10.5 or slightly later. Therefore, PGCs have their own specific characteristics with respect to the expression of pluripotency-specific genes.

While the PGCs exhibit expression of some pluripotency-specific genes, there is also the expression of lineage-specific genes. Among these is *fragilis*. In addition, there is the expression of *Nanos3*, a mouse homolog of an RNA-binding protein that is essential for the development of PGCs following their specification. Dnd, which has a role in RNA metabolism, is also detected in slightly more advanced PGCs. Expression of other genes such as the mouse homologs of *vasa* (*mvh*) and *Dazl* is detected later with the entry of PGCs into the developing gonads. Expression of these genes emphasizes that PGCs have unique lineage-specific characteristics, which coexist with the expression of pluripotency-specific genes.

Genetic/Epigenetic Regulation of Pluripotent Stem Cells

As discussed above, the pluripotent PEct cells of the ICM and the early PGCs can both give rise to pluripotent stem cells (ES and EG cells, respectively) in culture. Once established, the pluripotent ES and EG cells can, albeit under strict culture conditions, perpetuate their undifferentiated pluripotent state indefinitely. The ES and EG cells are thus in vitro derivatives without strict counterparts in vivo.

From ICM to Pluripotent ES Cells

Currently, little is known about how pluripotent PEct cells in the ICM are transformed into pluripotent ES cells or about potential involvement of any epigenetic regulators in this process. However, it is likely that in the blastocyst, the PEct cells may have already undergone the crucial stages of epigenetic reprogramming, including the erasure of epigenetic modifications associated with the Xi chromosome and possibly even the overall derepression of the genome within the ICM niche as described above. The pluripotent ES cells thus seem to capture a state that may occur only transiently in vivo, since the PEct cells progress rapidly through development to form the epiblast cells.

The derivation of mouse ES cells is usually carried out in the presence of LIF and FCS or BMP4 (Ying et al., 2003). These conditions must allow for the selection of cells that ultimately form the pluripotent ES cells. One of the effects of LIF is to induce Stat3 phosphorylation that has the potential to activate transcription of target genes. However, the derivation of human ES cells, which are similar but not identical to mouse ES cells, requires the presence of Fgf2. The reason for the differences between these stem cells is unclear. Detailed analysis of transcription and epigenetic regulation of ES cells gives further insights into the nature of the pluripotent state. The transcription factors Oct4, Nanog, and Sox2, which are present in the ICM, are critical for maintaining pluripotency of stem cells. The interaction between Oct4 and Cdx2, which is central to cell fate decision between ICM and trophectoderm, is also evident since induced expression of Cdx2 in ES cells causes their differentiation into trophectoderm cells (Niwa et al., 2005). It is likely that *Cdx2* is normally repressed in mouse ES cells by an unknown epigenetic mechanism since they are highly refractory to differentiation into extraembryonic tissues. The role of Nanog in this context is to prevent the ES cells from acquiring the PEnd fate, which can be overcome by expression of *Gata4* and *Gata6* in ES cells. These two genes are normally repressed in ES cells (Chazaud et al., 2006).

Although pluripotent mouse ES cells seldom differentiate into extraembryonic tissues, they are poised to differentiate into all of the somatic cells found in the embryo itself. The genome-wide analysis of the key transcription factors Oct4, Sox2, and Nanog and some of the epigenetic regulators implicated in the plasticity of ES cells has been reported recently (Boyer et al., 2006a; Loh et al., 2006). These factors have a large number of targets that are both transcriptionally active and repressed, although the comparisons between the targets in mouse and human show only a slight overlap. The reasons for this are unclear, but it could be due to real differences between mouse and human ES cells or a reflection of differences in technical approaches between the two studies. In the human ES cells, the factors were associated with many genes needed for development of both extraembryonic and embryonic tissues, suggesting their direct role in regulating pluripotency. In both types of ES cells, genes encoding for some of the histone modifiers such as *Jarid2* and *Smarcad1* as well as *Rif1* were detected (Boyer et al., 2006a). Some targets such as *Esrrb* were found to be targets only in mouse ES cells.

The pluripotency of ES cells is underpinned by an unusual state of their chromatin. Recent studies have demonstrated that ES cell chromatin is in a highly dynamic state, with an apparently transient association of chromatin structural proteins, which is reflected in the relatively decondensed chromatin of ES cells (see the Review by T. Misteli, page 787 of this issue). This distinct state of chromatin in ES cells is complemented by a unique epigenetic mechanism to sustain pluripotency. A large number of genes that are important for development have the H3K27me3 repressive mark, which is imposed by Ezh2. However, at the same time, these sites also have the H3K4me3 mark, which is associated with active genes (Bernstein et al., 2006; Boyer et al., 2006b; see also the Reviews by B. Li et al. and B.E. Bernstein et al., pages 707 and 669 of this issue). The presence of these dual and opposing epigenetic marks on certain genes suggests that they are poised to be released from repression as soon as the ES cells start to undergo differentiation. These dual marks are also associated with the highly conserved noncoding elements, suggesting evolutionary conservation of these DNA regions. More importantly, many of the sites with these epigenetic marks are also bound by Oct4, Sox2, and Nanog, the key factors associated with pluripotency. The target loci of these transcription factors associated with the repressive chromatin mark also replicate early in S phase in ES cells (Azuara et al., 2006), which also suggests that, in pluripotent stem cells, these loci are poised to be activated. Consistently, two members of the PRC2 complex, Eed and Suz12, are also detected at these loci and may be responsible for repression of these target genes, a repression that is apparently relieved in *Eed* mutant ES cells (Boyer et al., 2006b; Loh et al., 2006).

Mutations in several key epigenetic regulators, including *Ezh2*, *Eset*, *MBD3*, and *Dicer*, abolish the ability to generate pluripotent ES cells from the ICM (see Table 1). Notably, however, some of these proteins, such as Mbd3 and Dicer, can be deleted after the ES cells are established, suggesting that different sets of molecular factors are necessary for the derivation and maintenance of the pluripotent state. It should be noted, however, that in both cases, the mutant ES cells are defective in their potential to undergo differentiation into diverse cell types, which shows that their pluripotency is indeed affected.

From PGCs to Pluripotent EG Cells

PGCs exhibit lineage-specific characteristics as well as many characteristics similar to pluripotent cells. Importantly, it is also possible to generate pluripotent stem cells (EG cells) from PGCs starting from approximately E8.5, which coincides with epigenetic modifications following PGC specification and the expression of some key pluripotency-specific genes (Durcova-Hills et al., 2006). The derivation of EG cells from PGCs is possible until about E11.5, when the extensive epigenetic reprogramming of the genome occurs, followed by progressive downregulation of some pluripotency-specific genes. The precise mechanism involved in PGC-to-EG dedifferentiation is as yet unknown, except that the presence of Fgf2 as well as LIF and stem cell factor (SCF) is needed for this transformation. In vivo, PGCs can also undergo transformation into teratocarcinoma cells, and pluripotent EC cells can be derived from them. A number of mutations are known to accelerate this process, including those in *Dnd*, *Pten*, and *Pgct1*, among others.

The potential to derive EG cells from PGCs at E8.5–E11.5 coincides with the presence of a specific chromatin signature, including enrichment of H3K27me3 and H3K4me3 (Seki et al., 2005; P.H., K. Ancelin, and

M.A.S., unpublished data), which may be required for obtaining pluripotency during the derivation of EG cells. It is similarly striking that both PEct cells of the ICM and PGCs possess the ability to dramatically alter their chromatin, which suggests that this feature may be important for their ability to give rise to pluripotent stem cells. It is also particularly noteworthy that during the derivation of EG cells from PGCs, there is extensive epigenetic reprogramming of the genome as seen in the gonadal PGCs described above (Tada et al., 1998), including extensive DNA demethylation and the erasure of the parental imprints, even when EG cells are derived from E8.5 PGCs. In vivo, this event is timed to occur only when the PGCs enter into the genital ridges. EG cells are in most respects very similar to ES cells, except for the erasure of the epigenetic modifications associated with genomic imprints.

It is most likely that Blimp1 is downregulated during the derivation of EG cells from PGCs, since Blimp1 protein is not detectable in EG cells, or indeed in any other type of pluripotent stem cells. This downregulation of Blimp1 may cause phenotypic changes in the germ cell lineage. It is likely that during the EG cell derivation, a number of targets of the Blimp1/Prmt5 complex are derepressed, such as *Dhx38* (Ancelin et al., 2006). Ectopic expression of Blimp1 in pluripotent EC cells leads again to the repression of *Dhx38*. It will be of interest to determine whether EC cells and indeed all pluripotent stem cells acquire a PGC character upon expression of Blimp1.

Reprogramming and Rederived Pluripotency

In principle, any adult cell can be reprogrammed to reacquire pluripotency, albeit at a very low frequency. For an optimum response, reprogramming of somatic cells requires erasure of the existing epigenetic modifications and reinitiation of a pluripotency-specific transcriptional network in the reprogrammed cells. Much of the evidence for this comes from nuclear transplantation into oocytes. Additionally, pluripotent stem cells themselves also have the potential to reprogram somatic cells in ES/EG-somatic cell hybrids (Tada et al., 1997, 2001).

The mechanism that confers pluripotency on somatic nuclei in ES/EG-somatic cell hybrids has yet to be fully investigated. It is possible that ES cells, which have the key transcription factors necessary for pluripotency, also have the potential to erase some of the histone modifications from somatic nuclei in ES-somatic hybrids, a property likely to be inherited from the PEct cells of the ICM. Similarly, somatic nuclei in EG-somatic hybrids also acquire characteristics of pluripotency. However, in the latter there is also extensive erasure of genomic imprints and DNA demethylation, which is not observed in ES-somatic cell hybrids (Tada et al., 1997, 2001). EG cells must acquire this additional property from PGCs. Somatic cells hybridized with ES cells exhibit dramatic changes in histone modifications, which include enrichment of H3K4me2/3 and H3K27me3 in gene loci that are normally repressed in ES cells, while gene loci that are normally expressed in ES cells are reset predominantly with an H3K4me3 epigenetic mark (Kimura et al., 2004). Thus, the overall epigenetic status of the somatic nucleus is reset to resemble the ES cell state. Similarly, the inactive X chromosome in the somatic nucleus is reactivated in hybrid cells (Tada et al., 2001). Recent studies have also shown that the frequency of reprogramming of somatic nuclei in hybrids with ES cells is significantly enhanced, either directly or indirectly, following increased expression of Nanog in ES cells (Silva et al., 2006). However, it is known that remodeling of the somatic nucleus can occur in the absence of Nanog, as seen with somatic nuclei transplanted into oocytes.

Recent studies have shown that somatic cells can be rendered pluripotent and made to resemble ES cells by the introduction of four transcription factors, Oct4, Sox2, Klf4, and c-Myc, albeit at a very low frequency (Takahashi and Yamanaka, 2006). The first two are the core transcription factors of pluripotency, while Klf4 and c-Myc are involved in self-renewal of ES cells. Klf4 can also augment the levels of Oct4 and is implicated in transactivation of *lefty* (Nakatake et al., 2006). The detection of endogenous *nanog* expression in these reprogrammed cells probably follows activation by Oct4 and Sox2. Thus, the four factors may alter the existing transcriptional network quite extensively since the reprogrammed cells show both the capacity for self-renewal and extensive differentiation in chimeric fetuses, but their contribution to the germline needs further investigation. A detailed microarray analysis of these pluripotent cells shows that their gene expression pattern is similar but not identical to normal ES cells. Some of the key genes, including *Sox2* and *Oct4*, are not expressed from the endogenous copies to the extent seen in normal ES cells, and these cells must thus rely on the constitutive expression of introduced transgenes.

Following transplantation into the oocyte, somatic nuclei undergo epigenetic modifications, as exemplified by the initial increase in the size of the nucleus indicating an alteration of the chromatin template, while being exposed to the maternally inherited transcription factors. More importantly, ES cells derived from the resulting blastocysts are almost identical to the ES cells derived from normal embryos (Brambrink et al., 2006). Their transcriptional profile and developmental potential are indistinguishable from normal ES cells, probably because the donor somatic nucleus was exposed to the appropriate epigenetic modifications in the oocyte and subsequently in the ICM. Transcription factors acting on such epigenetically modified nuclei during development may allow almost complete reprogramming of somatic nuclei to pluripotency, albeit at a low frequency. By contrast, ES cells generated in vitro from somatic cells by the introduction of transcription factors alone as described above (Takahashi and Yamanaka, 2006) are similar but not identical to the normal ES cells with only partial epigenetic reprogramming as judged by the methylated status of *Oct4* in these cells. These studies suggest that a combination of epigenetic and genetic programs acting in concert is necessary for successful induction of complete pluripotency. However,

it is important to bear in mind that the environmental factors and signaling molecules may also have a significant role in effective reprogramming.

Perspective

The transition from the totipotent zygote leading to the establishment of pluripotent PEct cells within the ICM involves an interaction between genetic and epigenetic modulators. The early blastomeres rely more on intrinsic regulators, including maternal factors that drive largely stereotypical responses. The cessation of cleavage divisions and the resumption of normal cell divisions are probably accompanied by epigenetic reprogramming events in PEct cells in the ICM, including X reactivation and possible general derepression of the epigenome, as these cells acquire plasticity and responsiveness to extrinsic signals. The early totipotent blastomeres have no capacity for self-renewal, which is a property acquired later by the pluripotent cells within the ICM. The concomitant responsiveness to extrinsic signals acquired by these cells makes it possible to derive self-renewing pluripotent ES cells in vitro. These cells exhibit a unique epigenetic state that ensures their ability to differentiate into somatic cells and germ cells, except that differentiation into extraembryonic tissues is largely repressed.

Pluripotency is also initially maintained in early postimplantation epiblast cells, which undergo very rapid cell divisions. At this stage, many epigenetic modifiers and repressors are used to ensure rigorous control over differentiation into diverse cell types. Germ cell specification, which involves repression of the somatic program as the cells acquire characteristics that are associated with pluripotency, also occurs at this time. These early germ cells can in turn be used to generate pluripotent stem cells. Subsequently, there is extensive epigenetic reprogramming of the genome, notably the extensive erasure of the epigenetic modifications that are essential for eventually generating totipotency. Knowledge gained from development of the pluripotent state in vivo involving an interaction between the transcriptional network and epigenetic mechanisms may provide important insights that will aid in generating pluripotent cells directly from any somatic cell.

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