

## Of Stem Cells and Gametes: Similarities and Differences

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**Abstract:** Fusion of a mammalian sperm cell with an oocyte will lead to the formation of a new organism. As this new organism develops, the cells that construct the organism gradually lose developmental competence and become differentiated, a process which is in part mediated *via* epigenetic modifications. These mechanisms include DNA methylation, histone tail modifications and association with Polycomb and Trithorax proteins. Several cells within the organism must however maintain or regain developmental competence while they are highly specialized. These are the primordial germ cells that form the gametes; the oocytes and sperm cells. In this review different epigenetic modifying mechanisms will be discussed as they occur in developing embryos. In addition, aspects of nuclear reprogramming that are likely to occur *via* removal of epigenetic modifications are important, and several epigenetic removal mechanisms are indeed also active in developing germ cells.

*In vivo*, a pluripotent cell has the capacity to form gametes, but *in vitro* terminal gametogenesis has proven to be difficult. Although development of pluripotent cells to cells with the characteristics of early germ cells has been unequivocally demonstrated, creating the correct culture milieu that enables further maturation of these cells has as yet been futile.

**Keywords:** Embryo, primordial germ cell, epigenetics, pluripotency, ES cell, methylation.

### CELLULAR POTENCY

After an oocyte has fused with a sperm cell, a new organism will be formed. During the development of this organism, the cells that construct the embryo will gradually lose developmental potential and gain more specialized functions. In this respect, differentiation equals a loss in cellular potency. One group of cells however has to maintain the capacity to form a new organism. This group encompasses the primordial germ cells (PGCs) that will give rise to the gametes later in development. Germ cells are unique cells since they are responsible for the continuity of genetic information across generations. They therefore have to maintain a certain level of cellular potency. In vertebrates, PGCs belong to the first embryonic lineage to be segregated, long before the gonads are recognizable. This specification event occurs outside the embryo at the border between the extraembryonic and embryonic region so that the PGCs can escape differentiation signals. Thereafter, the PGCs follow a complex migratory pattern to finally colonize the gonads [1].

Once in the gonads, male PGCs enter mitotic arrest (pre-spermatogonia cells) whereas female PGCs enter meiosis (oogonia). In males, the pre-spermatogonia cells start proliferating only after birth and give rise to spermatogonia which either self-renew or differentiate to cells that enter meiosis forming haploid sperm cells. Spermatogonia are therefore considered an adult stem cell population. The oogonia, on the other hand, undergo meiosis synchronously during embryonic development and arrest in the diplotene stage at birth. After puberty, oocytes periodically mature, resuming meiosis to arrest again at metaphase II after ovulation and complete meiosis only after fertilization [2]. Although the sperm and the oocyte are highly specialized cells, they also must maintain or regain a level of cellular naïveté.

Most differentiated cells retain an intact genome without alterations in their DNA sequences, but epigenetic mechanisms modify accessibility to parts of the genome leading to cell fate determination and cell function [3]. However, when the nucleus of a differentiated cell is introduced into the cytoplasm of a mature oocyte, that somatic nucleus can be reprogrammed to a totipotent state and the resulting cell can give rise to a new organism genetically identical to the somatic cell donor nucleus, a clone [4].

Interestingly, immature oocytes do not support reprogramming of somatic nuclei. Different pluripotent cell lines can be derived by long term culture of distinct cell populations: embryonic stem cells (ES cells) are derived from the inner cell mass of an embryonic day (E)3.5 mouse blastocyst, epiblast stem cells (EpiSCs) from an E5.5-E6.5 mouse epiblast, embryonal carcinoma cells (EC cells) from a mouse epiblast or germ cell-induced teratomas, embryonic germ cells (EG cells) from E8.5-E13.5 murine PGCs and adult spermatogonial stem cells (SSCs) from neonatal and adult spermatogonia [5-12].

Exactly how pluripotency is regulated and how germ cells are prevented from differentiation into somatic cells is unknown, but our biochemical understanding of these processes has increased considerably in the last few years. It has become clear that mechanisms that play an important role in maintaining potency of (primordial) germ cells are also active in stem cells, but that simultaneously many differences exist.

### EPIGENETIC REGULATION AS A DETERMINANT OF CELLULAR POTENCY

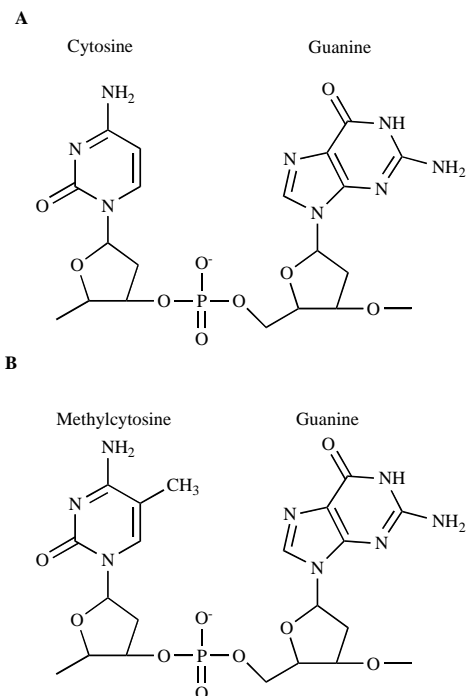
Although the genome in almost all cells of an organism remains intact, in differentiating cells it is partitioned into active and quiescent genes. This can be achieved by epigenetic modification of the chromatin (DNA and histones), which determines the accessibility of genes in a heritable fashion. There are several mechanisms by which chromatin can be epigenetically programmed, including DNA methylation at cytosine-phosphate-guanine (CpG) dinucleotides, covalent post-translational modification of the histone proteins or the use of histone variants in the nucleosome and the association with ATP-dependent complexes [13].

### METHYLATING THE DNA

Methylation of DNA at CpG sequences is mostly associated with transcriptional repression. It is widely used to ensure the silencing of repetitive DNA (constitutive heterochromatin), including satellite DNA and parasitic transposable elements; the transient silencing of specific genes or whole chromosomes (facultative heterochromatin); and the monoallelic expression of imprinted genes. It consists of the addition of a methyl group to the 5-position of a cytosine in a CpG dinucleotide (Fig. 1). The CpG methylation is symmetrical, i.e. occurring on both DNA strands and it directly inhibits the binding of specific transcription factors to DNA whereas it promotes the binding of methyl-CpG-binding proteins like MECP2, MBD1, MBD2, MBD3, MBD4 and Kaiso [13].

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Stretches of CpG sequences known as CpG islands present in the promoter sequences of many genes are predominantly unmethylated [14], whereas CpG islands in imprinted genes and genes on the inactive X-chromosome are frequently methylated [14, 15].



**Fig. (1).** Methylation of cytosine in a CpG dinucleotide. **A)** Unmethylated CpG dinucleotide and **B)** methylation of cytosine on the 5 position.

After fusion of the oocyte with the sperm cell, both maternal and paternal genomes undergo passive and active demethylation, respectively, excluding that of imprinted genes and some repetitive DNA sequences. At the blastocyst stage most methylation marks are removed and the genome, in particular that of the inner cell mass, is *de novo* methylated during implantation in mice [16].

The PGCs, during migration and in the gonads, undergo erasure of methylation, including that of imprinted genes and the inactive X chromosome, most likely by an active mechanism, as well as partial erasure in some repetitive DNA sequences [17, 18]. Once the genomes of PGCs have been demethylated, the male PGCs enter mitotic arrest and the female PGCs enter meiosis. Although these two processes (demethylation and cell-cycle arrest) may be uncoupled, there are advantages in stopping proliferation to keep chromosome stability in the germ cells. Re-establishment of the sex-specific methylation patterns occurs several days later in male spermatogonia and only after birth in the female oocytes. It is still unclear whether all the epigenetic information is reset in the germ line. However, nuclear transfer using nuclei of PGCs before the demethylation wave resulted in viable mice, whereas nuclear transfer with nuclei of demethylated PGCs resulted in embryonic lethality [19, 20]. Female ES cells show global genome hypomethylation, similar to the methylation status of the inner cell mass and this is in part due to the presence of two active X chromosomes [21]. Interestingly, several genes that are derepressed in germ cells are also demethylated in ES cells, suggesting common regulatory mechanisms for the maintenance of pluripotency in these cells [22].

Methylation of DNA occurs by DNA methyltransferases (DNMTs), of which DNMT3A and DNMT3B are largely responsible for establishment of *de novo* methylation patterns, and DNMT1 for their maintenance. DNMTs are expressed in many dividing cells, but expression in the germ line is driven by sex-specific promoters. Alternative splicing of the *Dnmt* genes frequently occurs in germ cells, but the function of the various splice variants is unclear

[23]. Absence of DNMTs in ES cells increased telomere recombination and telomere size, suggesting that DNA methylation is involved in regulation of telomere length and therefore maintenance of chromosome stability [24]. The ES mutant cells are viable but die when induced to differentiate. Mice knockout for *Dnmt1* and *Dnmt3b* are embryonic lethal and mice knockout for *Dnmt3a* die before reaching adulthood (reviewed by [13]). Deficiency in the methyl-CpG-binding domain (MBD) protein 3 results in embryonic lethality and *Mbd3* knockout ES cells show a restricted potential to differentiate [25]. It is clear that DNA methylation is of crucial importance for reprogramming the genome in embryonic development, gametogenesis and differentiation of ES cells.

## IMPRINTING

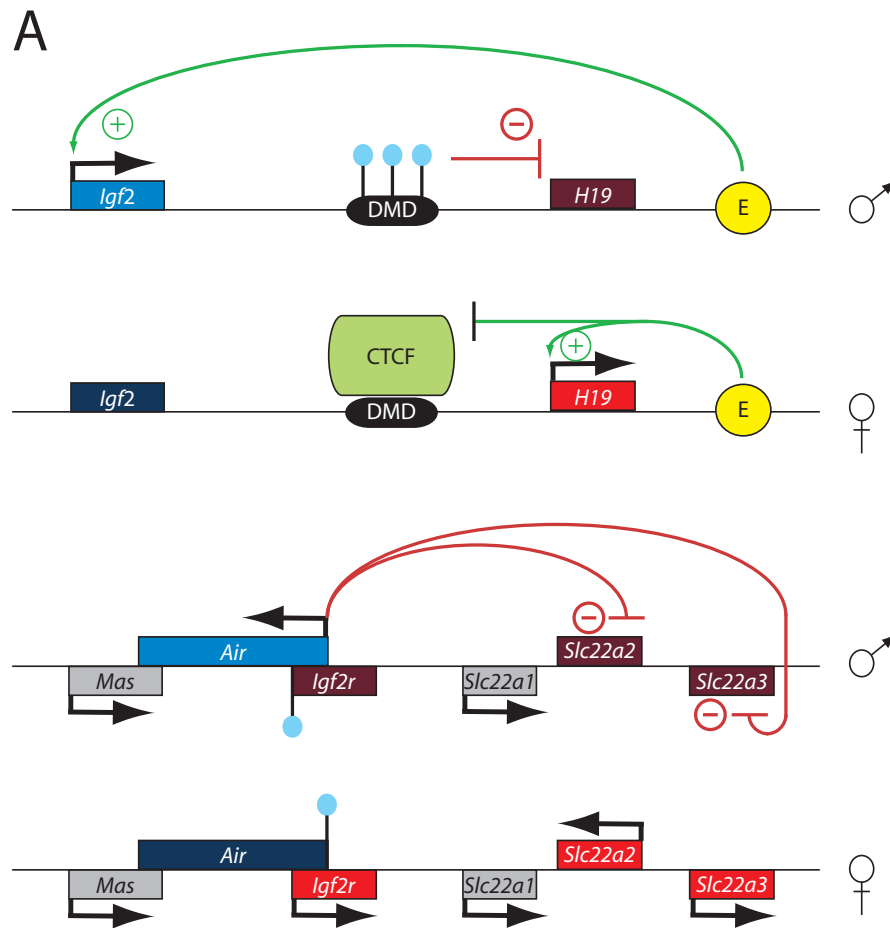
In viviparous mammals, and interestingly also in flowering plants, the epigenetic changes of a selected panel of genes are inherited to the next generation, depending on the parental origin of the allele. The discovery of imprinting demonstrated that the maternal and paternal genomes are functionally non-equivalent, even though they share equivalent genetic information [26, 27]. Therefore, embryos consisting of two fully imprinted maternal or paternal genomes cannot develop to term. These two kinds of embryos develop opposite phenotypic characteristics: the ones containing only maternal genes develop better embryos and the ones containing only paternal genes develop better placental tissues. Accordingly, the imprinted genes have various functions including embryonic and placental growth and suckling behaviour but why imprinting has evolved is still a matter of debate [28]. Imprinting occurs at close to 100 loci (at least in the mouse) and these appear in regional clusters on specific chromosomes rather than being distributed evenly throughout the genome [29].

Several mechanisms have been identified by which parental-allele-specific expression is achieved. Two examples are given here of two imprinted loci: (i) the enhancer-blocker model of the neighbouring but oppositely imprinted genes *H19* (maternally expressed) and *Igf2* (paternally expressed); and (ii) regulation of the maternally expressed *Igf2r/Slc22a2/Slc22a3* protein coding genes by the paternally expressed non-coding RNA *Air* (Fig. 2). A 1.6 kb differentially methylated domain (DMD) between the *Igf2* and *H19* genes functions as an insulator element. In the maternal allele, this domain is unmethylated, allowing the binding of the 11-zinc-finger protein CCCTC binding factor (CTCF), thereby blocking activation of the maternal copy of *Igf2* by the *H19* enhancer. In the paternal allele, the DMD is methylated, preventing the binding of CTCF and allowing the *H19* enhancer to activate the paternal *Igf2* [30].

The paternal-specific non-coding *Air* RNA of 108 kb is expressed from a promoter in the *Igf2r* gene on mouse chromosome 17. *Air* functions as a bidirectional silencer for the three maternally expressed imprinted protein coding genes *Igf2r*, *Slc22a2* and *Slc22a3*. The promoter region of the *Air* gene can be maternally methylated and is therefore silenced. On the paternal allele however this region is unmethylated, allowing transcription of *Air* RNA that can confer epigenetic silencing by a *cis*-acting mechanism [31] (Fig. 2). Although genomic imprinting is readily tractable, these examples show the levels of complexity that are present in the regulatory mechanisms of imprinted genes.

## HISTONE MODIFICATIONS

In most mammalian cells, the DNA inside a nucleus is wrapped (145-147 base pairs) twice around octamers of 2x histone H2A, H2B, H3 and H4 proteins forming the fundamental unit of chromatin, the nucleosome. The amino-terminal "tails" of the globular histone proteins protrude from the surface of the histone cores and pass over and between the gyres of the DNA superhelix [32]. Covalent modifications of the histone tails such as methylation, acetyla-



**Fig. (2).** Mechanisms of genomic imprinting. Two examples are given of the regulation of two imprinted loci. **A**) Regulation of the *H19* (maternally expressed) and *Igf2* (paternally expressed) genes. **B**) Regulation of the maternally expressed *Igf2r/Slc22a2/Slc22a3* genes. Light blue circles represent methylation of DNA. Red (expressed) and crimson (silenced) rectangles represent genes that are maternally expressed/paternally silenced; blue (expressed) and dark blue (silenced) rectangles represent genes that are paternally expressed/maternally silenced; grey rectangles represent genes that are not imprinted. See text for further details. DMD=differentially methylated domain; E=enhancer; CTCF= CCCTC binding factor.

tion, phosphorylation, sumoylation, ubiquitination, ADP ribosylation, deimination and proline isomerization can take place and have been described to occur on at least 60 different amino acid residues. In addition, methylation of lysines and arginines exists as three different forms: mono-, di- and trimethylation and mono-, asymmetric di- and symmetric dimethylation, respectively. Particularly methylation and acetylation of histone tail residues serve as epigenetic marks for transcriptional activity of genes (Fig. 3).

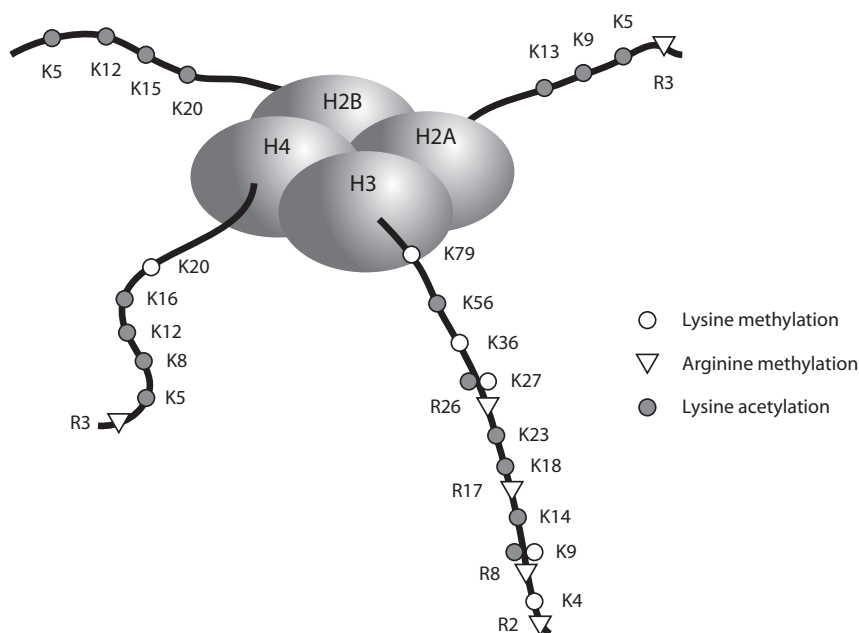
In mammals, constitutive heterochromatin is associated with low levels of acetylation and high levels of some methylation marks, including lysine 9, lysine 27 and lysine 20 on histone 3 (H3K9, H3K27, H3K20); whereas active euchromatin has high levels of acetylation and methylation marks on H3K4, H3K36 and H3K79 (reviewed by [33]). The modification of histones is accomplished by different enzymes, including histone acetyl transferases (HATs), histone deacetylases (HDACs), methyltransferases and demethylases. Histone modifications are therefore thought to be dynamic. Intriguingly, an arginine demethylating enzyme has not yet been identified. It has been hypothesized that the histone tail modifications form a 'histone code' that is identified by other proteins and modulate higher order chromatin structures [34].

Recently in mouse ES cells the existence of so-called "bivalent domains" has been described [35]. These bivalent domains consist of the opposing chromatin marks H3K27me (silencing) and H3K4me (activating) in the same chromatin region. In ES cells bivalent domains were observed in the loci of transcription factors

expressed at low levels. This was interpreted as a means to keep transcription on hold, but already half way to activation of repression in case of ES differentiation to a specific lineage. In support of this, it has been reported that the majority of protein encoding genes in human ES cells, including transcriptionally inactive genes, have H3K4me, H3K9ac and H3K14ac rich promoter regions. These modifications were particularly found in nucleosomes that appeared just downstream of transcription start sites, however the coexistence with H3K27me and therefore the presence of "bivalent domains" in human ES cells was not analysed [36].

By sequencing chromatin immunoprecipitated DNA, genome-wide chromatin-state maps of pluripotent mouse ES and differentiated cells were made [37]. Availability of such maps of cells with distinct functions and throughout development, including cell abnormalities, will be of great value to help understand many aspects of cellular state. During PGC development, the levels of the "permissive" H3K4me and H3K9ac are consistently high, whereas the levels of the "repressive" H3K27me increase steadily until the PGCs enter the gonads; in the gonads the levels of both marks are transiently high [38] and may reflect the existence of "bivalent domains" and the ability of PGCs to dedifferentiate into EG cells.

Although histone modifications occur at selected residues, because of the multiple combinations it is difficult to predict or explain how certain modifications affect transcriptional regulation and thereby cell function. Many fundamental aspects of histone tail modifications are indeed unexplained. Are histone tail modifica-



**Fig. (3).** Histone tail modifications. Schematic illustration of histone core proteins and the residues that can be target for acetylation or methylation. Only half of the histone octamer is shown.

tions identical in both proteins of each of the pairs within the nucleosome core? How is the modification of histone tails regulated? If there indeed is a ‘histone code’ that contains vital information for cells, how is it deciphered and translated to downstream partners? Knowledge regarding these principles will profoundly increase our understanding of basic aspects of cell function and pluripotency.

### POLYCOMB/TRITHORAX PROTEINS WRITING ON THE CHROMATIN

Once the genome is programmed in differentiating cells, it is important that the epigenetic state of a cell is properly maintained. In mammals, Polycomb group (PcG) and Trithorax group (trxG) proteins are largely responsible for these processes. They form large clusters and can bind near the promoter or can cover a transcriptional unit thereby controlling gene expression. PcG protein binding will mostly lead to transcriptional repression although it is not excluded that genes maintain expression after PcG binding [39]. Polycomb and Trithorax proteins not only set, but also maintain the “repressive” H3K27me [40] and the “permissive” H3K4me marks [41], respectively. It is thought that these two types of complexes play antagonistic roles, regulating gene expression *via* PcG response elements (PREs) and are therefore responsible for some degree of cellular memory [39, 42]. The identification of histone demethylating enzymes suggests that these mechanisms are more dynamic than previously thought [43].

The PcG proteins function in three distinct complexes, Polycomb repressive complex 1 (PRC1) that recognizes H3K27me and acts as a maintenance complex, PRC2 that acts as an initiation complex *via* association with HDACs and DNMTs and the recently identified PhoRC that, at least in *Drosophila*, has sequence-specific DNA-binding activity and might tether PRC1 and PRC2 complexes to DNA [44]. Relatively few of their target genes in mammals are known and little is known about how mammalian target genes recruit the complexes. Presumably, proteins of PRC1 (containing RING1A, BMI1, HPC1-3, HPG1-3, SCMH1-2) and PRC2 (containing four core components: EZH2, EED, SUZ12, RbAp46/48) co-occupy promoter areas of target genes, most of which are transcription factors that need to be kept silent to maintain pluripotency, but are activated during differentiation [40, 42, 45].

The trxG proteins function as transcriptional activators, and they too are recruited to gene promoter regions. Two large subgroups can be distinguished in the trxG group; the SET-domain factors and the ATP-dependent chromatin remodelling complexes. The SET domain, also present in some of the PCR2 core proteins, is responsible for methyltransferase activity towards specific lysine residues. The mammalian SET-domain protein MLL1 plays an important role in the regulation of Hox genes during development [46].

The second class of trxG factors include components of the ATP-dependent chromatin remodelling multisubunit complexes SWI/SNF (derived from ‘switching defective’ and ‘sucrose non-fermentor’ in *Saccharomyces cerevisiae*) and the imitation SWI (ISWI). Both complexes use energy from ATP hydrolysis to create structural changes in the nucleosome, including dissociation of DNA-histone contacts that result in DNA looping; the translocation of the nucleosome along DNA (sliding); or the exchange of histone variants. Both changes make DNA more accessible to transcriptional regulators. The ISWI complex can bind H3K4me facilitating transcriptional activation. Although the role of ISWI in mammalian cells is unclear, it does play a role maintaining self-renewal and the meiotic block in *Xenopus* and *Drosophila* germ cells [42, 47]. The nucleosome remodelling and histone deacetylation (NuRD) complex, another complex belonging to the ATP-dependent chromatin remodellers, but not to the trxG family, has recently been shown to be involved in maintaining pluripotency of ES cells, *via* MBD3 [25].

In early development, the PcG proteins EZH2, EED (PRC2 proteins) and RING1B (a PRC1 protein) are important for early differentiation and correct Hox gene expression boundaries [48-50]. Indeed also in ES cells, these proteins are important for maintaining pluripotency, most likely by repressing differentiation genes or contributing to the maintenance of bivalent domains together with trxG complexes. Not surprisingly, abnormalities in the expression of some members of the PcG or trxG group are associated with several types of cancer [51, 52].

The whole complex nature of PcG and trxG protein functioning is currently not fully elucidated. The exact composition of the various complexes for instance and the specific functions of the different proteins within the complexes largely remain to be established.

More knowledge will not only help to understand fundamental aspects of disease, but may also help to advance studies on stem cells and germ cells.

### THE FIRST DIFFERENTIATION STEPS OF THE EMBRYO

When an early mammalian embryo cleaves it will form a clump of cells called a morula ( $\pm 16$  cells). It is at this stage that the first morphological differences between the individual cells can be observed. The majority of the outer cells become epithelialized and form the trophectoderm lineage that will only give rise to so-called extra-embryonic structures such as the placenta thus supporting the growth of the foetus in the uterine environment, whereas most of the inner cells generate a pluripotent group of cells called the inner cell mass (ICM) that will later form the epiblast. When ICM cells are cultured *in vitro* under the proper conditions they can give rise to pluripotent ES cells. *In vivo*, the choice to become either a cell from the trophectoderm or from the ICM is sealed, at least in the mouse, by a reciprocal inhibition between two transcription factors, Caudal related homeobox 2 (CDX2) and the transcription factor OCT3/4, a member of the POU (PIT/OCT/UNC) class of homeodomain proteins. The expression of OCT3/4 is restricted to early embryos and the germline whereas pluripotent ES cells also express OCT3/4 [53-56]. Initially, *Cdx2* and *Oct3/4* are both expressed in embryonic cells. As the embryo develops, an imbalance is created so that *Cdx2* is predominantly expressed in the trophectoderm, thereby blocking and eventually limiting *Oct3/4* expression [57]. Indeed trophectoderm-like cells from *Cdx2* null embryos do express *Oct3/4* [58] and overexpression of *Cdx2* in ES cells induces trophectoderm differentiation [57]. How the imbalance occurs is unclear, but cell polarity or cell size could be decisive [59]. The next differentiation event to occur in the embryo is the formation of the primitive endoderm (PE) lineage. In the mouse, the PE can already be morphologically distinguished from the ICM at E4.5, and blastocyst injection experiments demonstrated that lineage restriction had already occurred [60]. Lineage segregation seems to occur from as early as E3.5 by complementary expression of the homeodomain factor NANOG and the zinc finger transcription factor GATA6, with cells expressing NANOG becoming pluripotent epiblast cells and cells expressing GATA6 giving rise to PE [61]. In the majority of the embryos, the expression of either NANOG or GATA6 does not seem to depend on either blastomere history or position in the embryo [61]. However, already at the second cleavage division, at least in the mouse, the orientation of the division might determine cellular potency, and this seems to depend on H3R2me, H3R17me, and H3R26me. When the sequential second cleavage divisions are meridional and equatorial, the first dividing 2-cell blastomere contributes to the largest proportion of the pluripotent cells of the ICM [62]. After such divisions of unequal orientation, in tetrahedral four cell-stage embryos, higher levels of arginine methylation of histone H3 predict a larger contribution to pluripotent ICM cells. This was found to be correlated with higher levels of *Nanog* and *Sox2* expression, indicating that the promoters of both *Nanog* and *Sox2* are regulated by H3 arginine methylation [63]. Interestingly, in the large majority (about 80%) of mouse embryos, the second cleavage divisions are of similar orientation, either meridional-meridional or equatorial-equatorial, and no differences in cell fate are observed. Indeed, this is accompanied by equal levels of H3 methylation in the blastomeres, confirming that H3 arginine methylation predicts cell fate [63], but it is not fully understood why such predisposition is only observed in a minority of embryos, or whether other changes in chromatin structure are important for cellular fate.

Epiblast cells continue to express *Oct3/4*, but expression rapidly decreases, from anterior to posterior, when gastrulation commences, although *Oct3/4* continues to be expressed until E9 in the neural groove and unsegmented pre-somite mesoderm [64]. In addition, *Oct3/4* transcription remains active in the PGCs. Expression of *Oct3/4* is regulated by cis-acting elements:

*Oct3/4* is regulated by cis-acting elements: expression in cells of the ICM, pluripotent ES cells and in the PGCs is activated by a distal enhancer element. Expression in the epiblast and tissues derived thereof is on the other hand driven by a proximal enhancer [64]. In the developing embryo and differentiating ES cells, *Oct3/4* expression is repressed by an interaction of the orphan receptor germ cell nuclear factor (GCNF) with the proximal promoter of *Oct3/4* [65]. GCNF binds the DR0 domain of the proximal promoter by its DNA binding domain (DBD) thereby recruiting the nuclear receptor corepressors methylated CpG binding domain, MBD3 and binding of this factor to unmethylated CpG dinucleotides. When repression of the *Oct3/4* gene is initiated, *de novo* DNA methylation is triggered and MBD2 is recruited leading to gene silencing [66].

Embryonal carcinoma (EC) cells are derived from terato(carcino)mas and have characteristics very similar to those of ES cells. When fibroblasts were exposed to extracts from EC cells, specific regions of the *Oct3/4* locus were partially demethylated, particularly the proximal promoter and the distal enhancer, which was accompanied by transcriptional activation. In addition, treatment with EC extracts promoted acetylation and demethylation of H3K9 in fibroblasts [67]. These results indicate that stem cell extracts can remodel chromatin of differentiated cells such that epigenetic marks are removed and promoters of genes important for pluripotency become active.

### SPECIFICATION OF THE GERM-CELL LINEAGE

Cell position plays an important role in the determination of the germ cells in mammals. Bone morphogenetic protein (BMP) signals secreted from adjacent extra-embryonic tissues (extra-embryonic ectoderm, formed from the trophectoderm and visceral endoderm from the PE lineage) instruct cells of the proximal epiblast to become competent to form primordial germ cells [68-70], but these cells are not yet lineage restricted. At about E7.25 a group of approximately 40 cells in the extra-embryonic mesoderm at the posterior part of the primitive streak has been identified as the founder pool of cells that are germ lineage restricted [71]. It is hypothesized that even before the founder population of PGCs has been established, allocation towards the germ lineage has started. B-lymphocyte-induced maturation protein-1 (*Blimp1*) encodes a transcriptional repressor with a proline-rich region, a SET-domain, a DNA-binding domain comprised of five Krüppel-type zinc fingers, and a carboxyl-terminal acidic region [72, 73]. *Blimp1* may repress transcriptional activity by preventing binding of transcription factors to promoter sequences [72]. In addition, *Blimp1* can bind chromatin-modifying enzymes such as histone deacetylases [74], the histone H3 methyltransferase G9a [75] and the co-repressor Groucho [76]. In PGCs, *Blimp1* interacts with the arginine histone methyltransferase Prmt5, leading to symmetrical addition of two methyl groups to the guanidine nitrogen atoms of arginine 3 on histones H2A and H4 [77, 78]. *Blimp1*-deficient embryos form a cluster of only 20 PGC-like cells, but these cells fail to migrate away from the original cluster as normal PGCs do and fail to repress the default somatic pathway [79, 80]. Indeed, region-specific homeobox genes such as *Hoxa1* and *Hoxb1* are expressed in these mutant cells [80].

### GAMETOGENESIS, REGAINING TOTIPOTENCY

After entering the gonads, the somatic tissue of the gonads undergoes sex determination and will actually start differentiating to either testis or ovary tissue. It is the somatic gonadal environment that determines whether the PGCs will develop into oocytes or sperm, independent of their sex chromosome constitution [81], explaining the phenomenon known as sex-reversal. Male germ cells undergo mitotic arrest until birth and female germ cells initiate meiosis slowly to arrest at the diplotene stage around birth.

Male and female germ cells develop into phenotypically very different cells. The male cells develop into one of the best-established adult populations of stem cells, the spermatogonia. These cells are able to self-renew and differentiate to sperm (unipotent), ensuring an unlimited source of sperm throughout the male's life. Although spermatogonia are not pluripotent, they can be made pluripotent by *in vitro* culture of both newborn pre-spermatogonia and adult spermatogonia [6, 12].

To date, there is no clear evidence of a self-renewing female stem cell population although this subject has been heavily debated [82-85]. Once female germ cells enter meiosis they will never divide mitotically again, but each oocyte will give rise to a single oocyte and two polar bodies. However, after maturation, the oocyte has a remarkable and unique feature; it can reprogram any nucleus to a totipotent state, able to give rise to a complete organism. In this regard, this highly specialized cell does share important similarities with ES cells, despite the fact that reprogramming by ES cells does not result in the creation of a new organism. The factors that are responsible for reprogramming remain to be elucidated, but 4 transcription factors play an important role in this process.

### PLURIPOTENCY

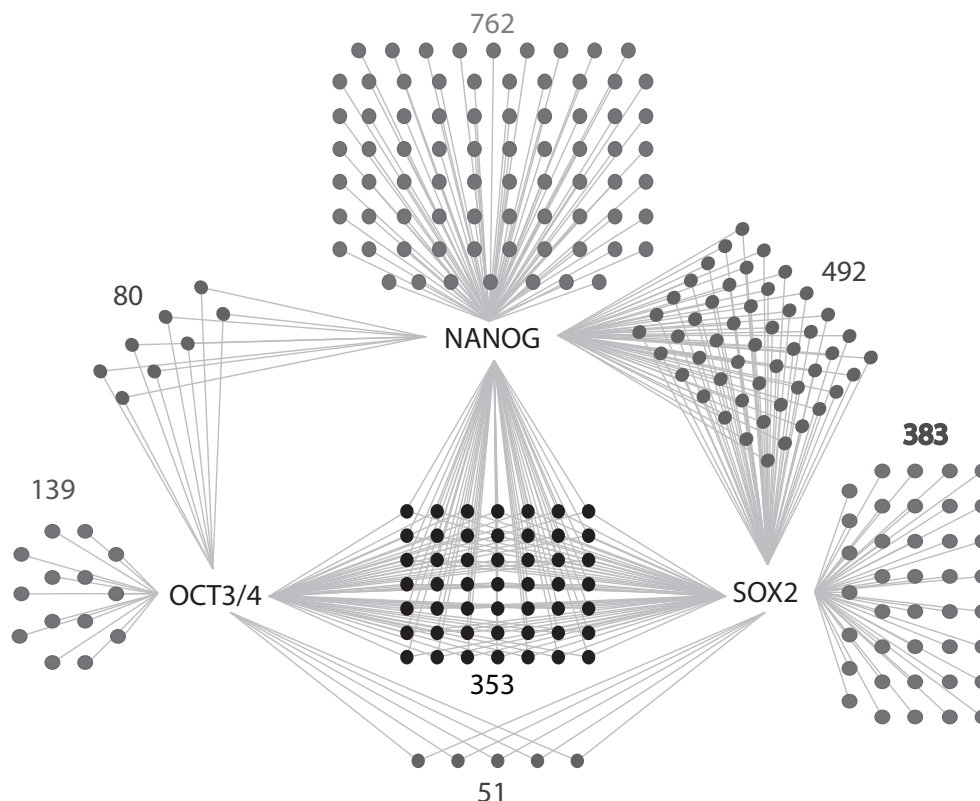
In the developing embryo, pluripotency only exists transiently and is lost before gastrulation commences. Only in the germ cells cellular potency has to be maintained or regained. Likewise, ES cells derived from pluripotent cells have to be cultured at such conditions that differentiation is inhibited and pluripotency is maintained.

The nucleus of a differentiated somatic cell that has lost cellular potency can be reprogrammed by the cytoplasm of an unfertilized egg, but also by ES and EG cells [4, 86-88]. Remarkably, somatic cells can also be reprogrammed to an ES cell-like state by retrovirus-mediated introduction of the transcription factors OCT3/4,

SOX2, c-MYC and KLF4 [89]. Particularly when these induced-pluripotent cells are further selected by expression of *Nanog* or *Oct3/4* they exhibit DNA methylation, gene expression and chromatin state similar to those of ES cells. In addition, these cells have germline competence [90, 91]. Although with only 4 transcription factors, pluripotency seems to be a simple biological process, chromatin immunoprecipitation (ChIP) combined with DNA micro arrays (ChIP-CHIP) revealed that in human ES cells OCT3/4, SOX2 and NANOG together bind to the promoter regions of 2260 genes, 1303 of which are actively transcribed genes and 959 of which are inactive genes, creating a complex gene network that can be regulated by these factors (Fig. 4) [92]. Furthermore, in undifferentiated ES cells, OCT3/4, SOX2 and NANOG occupied repressed genes of which many encode developmentally important homeodomain proteins [92]. Human fibroblasts can also be reprogrammed to a pluripotent state by the introduction of 4 transcription factors, of which Oct4 and Sox2 seem to be critical and two other factors appear to be more redundant [93, 94].

### FROM GERM CELLS TO STEM CELLS AND BACK AGAIN

During culture *in vitro*, PGCs can be turned to pluripotent stem cells, called EG cells and share all the characteristics of ES cells. On the other hand, ES cells can develop into cells of all tissues in the body, including germ cells. Indeed, the *in vitro* derivation of both oocyte-like and sperm-like cells from mouse ES cells have been described [95-99] but no living progeny has been generated from *in vitro*-derived oocytes from ES cells. The oocyte-like cells seem to be able to develop to the blastocyst-stage, by spontaneous parthenogenetic activation, but, to date, clear evidence that the genetic material of such an oocyte-like cell is able to fuse with that of a sperm cell, or that cytoplasm of these cells is able to reprogram a somatic nucleus is lacking.



**Fig. (4).** Target genes of NANOG, OCT3/4 and SOX2 and their interactions. Each dot represents 10 genes to which the transcription factors bind at the promoter region. The numbers represent the total numbers of gene promoter sequences bound by the (combination of) transcription factors [92].

Sperm-like cells have been generated from ES cells and have been successfully used to fertilize wildtype oocytes [99], resulting in the generation of live-born pups. However, due to abnormalities in the imprinting status of those cells, the pups died shortly after birth, throwing doubts on the functionality of the *in vitro*-derived sperm from ES cells. The pups obtained displayed either overgrowth or growth-retardation, in line with the observed imprinting abnormalities.

Surprisingly, oocyte-like cell derivation using human ES cells has not been reported yet, although PGC-like cells expressing the correct markers have been identified by several groups after culture of hES cells [100, 101].

In conclusion, it seems to be relatively easy to obtain PGCs from ES cells and stem cells (EG cells) from PGCs. Those cells share the expression of many transcription factors and have sufficient epigenetic plasticity to be able to interchange identity. However, the factors necessary to keep EG and ES cells in the pluripotent state are not identical and therefore those two pluripotent cell lines are strictly spoken not equivalent. The generation of functional gametes from ES cells seems to be much more challenging. The female and male gametes mature in a very specific niche and the right molecular environment seems to be capital for the establishment of epigenetic modifications that specify their functionality.

Epigenetic regulation, important for cellular potency, is a vast but largely unexplored territory of which many aspects are unclear. The many combinations of DNA methylation and histone modification alone can account for an almost unlimited number of varieties. Only with computer-aided high-throughput screening methods are we beginning to get a better understanding of these processes. It is evident however, that the cellular potency of ES cells and that of (primordial) germ cells is highly dependent on these mechanisms. Further characterization of these cells will help to gain a better understanding of the principal aspects of cellular potency and differentiation.

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## ABBREVIATIONS

ATP	= Adenosine triphosphate
BMP	= Bone morphogenetic protein
CDX	= Caudal related homeobox
ChIP	= Chromatin immunoprecipitation
CpG	= Cytosine-phosphate-guanine
DBD	= DNA binding domain
DMD	= Differentially methylated domain
DNA	= Deoxyribonucleic acid
DNMT	= DNA methyltransferase
E	= Embryonic day
EC cells	= Embryonal carcinoma cells
EG cells	= Embryonic germ cells
Epi SCs	= Epiblast stem cells
ES cells	= Embryonic stem cells
GCNF	= Germ cell nuclear factor
HAT	= Histone acetyltransferase

HDAC	= Histone deacetylase
ICM	= Inner cell mass
ISWI	= Imitation SWI
K	= Lysine
MBD	= Methyl-CpG-binding domain
NuRD	= Nucleosome remodelling and histone deacetylation
PcG	= Polycomb group
PE	= Primitive endoderm
POU	= PIT/OCT/UNC
PGC	= Primordial germ cell
PR	= Positive regulatory
PRC1	= Polycomb repressive complex 1
PRDI-BF1	= Positive regulatory domain I-binding factor 1
PRE	= Polycomb response element
R	= Arginine
SET	= Suv39, Enhancer of Zeste, Trithorax
SNF	= Sucrose nonfermentor
SSC	= Spermatogonial stem cell
SWI	= Switching defective
trxG	= Trithorax group

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