Seminars in Cell & Developmental Biology 45 (2015) 104–113

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Refurbishing the germline epigenome: Out with the old, in with the new

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ARTICLE INFO

Article history:
Received 22 August 2015
Accepted 21 September 2015
Available online 24 October 2015

Keywords:
Germline
Epigenetics
Inheritance
Reprogramming
DNA methylation
Histone modification

ABSTRACT

Mammalian germline reprogramming involves the erasure and re-establishment of epigenetic information critical for germ cell function and inheritance in offspring. The bi-faceted nature of such reprogramming ensures germline repression of somatic programmes and the establishment of a carefully constructed epigenome essential for fertilisation and embryonic development in the next generation. While the majority of the germline epigenome is erased in preparation for embryonic development, certain genomic sequences remain resistant to this and may represent routes for transmission of epigenetic changes through the germline. Epigenetic reprogramming is regulated by highly conserved epigenetic modifiers, which function to establish, maintain and remove DNA methylation and chromatin modifications. In this review, we discuss recent findings from a considerable body of work illustrating the critical requirement of epigenetic modifiers that influence the epigenetic signature present in mature gametes, and have the potential to affect developmental outcomes in the offspring. We also briefly discuss the similarities of these mechanisms in the human germline and consider the potential for inheritance of epigenetically induced germline genetic errors that could impact on offspring phenotypes.

Contents

Abbreviations: AID, activation induced deaminase; BER, base excision repair; CGI, CpG island; DNMT, DNA methyltransferase; EED, embryonic ectoderm development; ERV, endogenous retrovirus; EZH, enhancer of zeste; GV, germinal vesicle; HDAC, histone deacetylase; IAP, intracisternal A-particle; LTR, long terminal repeat; PGCs, primordial germ cells; PRC, polycomb repressive complex; REs, repetitive elements; RNF, ring finger protein; TET, ten-eleven translocation; TDG, thymidine; DNA, glycosylase; 5-mC, 5-methylcytosine; 5-hmC, 5-hydroxymethylcytosine.

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http://dx.doi.org/10.1016/j.semcdb.2015.09.012
1084-9521/

1. Introduction

Germ cells transmit genetic and epigenetic information to the offspring. Although genetic inheritance is well understood and the DNA sequence provides the underlying material on which natural selection acts, the role of epigenetic inheritance in biology and evolution is relatively poorly understood. Moreover, epigenetic mechanisms perform fundamental roles in regulating genome stability and the possibility that altered germline epigenetics may mediate genetic changes that affect inheritance, remains poorly understood.

Epigenetic mechanisms regulate chromatin structure, allowing the DNA to be properly organised and compacted into the nucleus in a way that permits accurate gene transcription and gene silencing [1]. This is mediated primarily through DNA methylation and an array of chemical modifications to the histone proteins (histone modifications). These modifications are flexible in that they can be removed or added, providing a myriad of chromatin formats that profoundly affect developmental processes. However, epigenetic modifications are also heritable across cell generations and thereby provide cells with the ability to stabilise lineage identity and somatic gene expression profiles throughout life [2].

Similarly, heritability of some epigenetic modifications from the parent significantly affects behaviour, development and health in the offspring [3]. The best understood example of epigenetic inheritance is genomic imprinting, which involves differential methylation of genomic regions that confers allele or parent-specific gene expression important for offspring growth, development and behaviour. Germ cells perform a critical role in removing existing epigenetic information from the genome and establishing new epigenetic information in the forming gametes, a process known as *epigenetic reprogramming*. In most cases this process underpins normal outcomes in the offspring. However, the plasticity of epigenetic modifications also leaves the germline susceptible to environmental influences that potentially alter epigenetic state in the parental germline and may have consequences in the offspring.

In this review, we outline the role of the germline in regulating the epigenetic signature of the gametes and the potential for altered epigenetic states to be inherited by the next generation. Although most existing models focus on identifying epigenetic changes in the germline that alter outcomes in the offspring, we briefly raise the possibility that epigenetic change in the germline leads to secondary genetic changes that could be transmitted to the offspring. As most of what we know is derived from studies in mice, this review will primarily focus on the mouse model. However, recent advances in deriving human germline cells from pluripotent stem cells and from studies of *in vivo* germline development have significantly progressed our understanding of human germline epigenetics.

2. Epigenetic reprogramming in the germline

In the mouse, primordial germ cells (PGCs) are specified *via* BMP signalling in the posterior extra-embryonic mesoderm at embryonic day (E) 6.25 [4–6] (Fig. 1). The newly specified PGCs migrate from the base of the allantois, along the hindgut to reach the genital ridge by E11.0. Although there is a short period of mitotic arrest at E8.5, the PGCs are highly proliferative during migration and for around two days after colonising the gonad [7–9]. At ~E12.0 the bipotential gonad differentiates down the testis or ovarian pathway and germ cells initiate differentiation through sex-specific signalling from supporting somatic cells (Fig. 1) [10,11]. By E14.5 male germ cells exit the cell cycle and enter a period of mitotic arrest, which is not resumed until after birth [12]. In contrast,

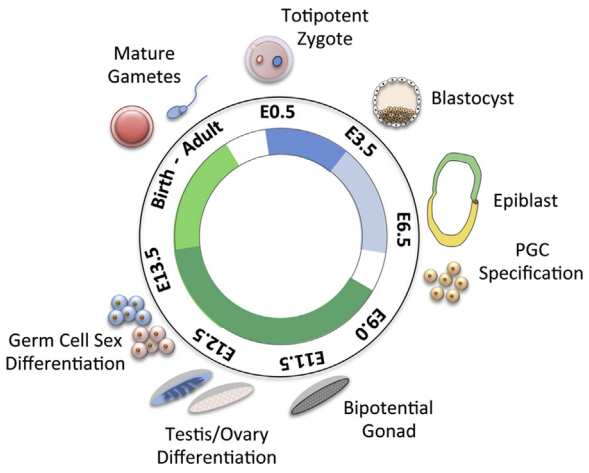

Fig. 1. Germline cycle and epigenetic reprogramming waves. Primordial germ cells (PGCs) are specified from the mouse epiblast at embryonic day (E)6.5. PGCs migrate and enter the bipotential gonad at E11.0, followed shortly after by gonad differentiation and germ cell sex determination. Germ cells subsequently follow sex-specific developmental trajectories; female germ cells enter meiosis and male germ cells enter mitotic arrest between E13.5–E14.5. In the adult, gametes mature through folliculogenesis in the ovary and spermatogenesis in the testis to produce mature gametes capable of fertilisation. After specification widespread epigenetic reprogramming occurs in the germline (wave 1: green bar) with an initial phase of epigenetic erasure (dark green) that removes parent-specific information, following by establishment of new sex-specific epigenetic information (light green). A second round of epigenetic reprogramming (wave 2: blue bar) occurs in the embryo (erasure/re-methylation: darker/lighter blue), but inherited epigenetic modifications survive this phase.

female germ cells cease to proliferate, enter meiosis and become arrested in prophase I by E14.5 [13], thus representing a finite gametogenic store for female individuals.

2.1. DNA methylation dynamics in the developing germline

During mammalian development there are two waves of extensive epigenetic reprogramming; the first occurring in the newly specified PGCs during migration and colonisation of the gonad, and the second taking place in the early preimplantation embryo shortly after fertilisation (Fig. 2). The first round of epigenetic reprogramming permits the removal of existing epigenetic information and establishment of an epigenome that is distinct to that of somatic lineages, a process that is essential for germline identity and function. Furthermore, epigenetic reprogramming in the germline establishes a chromatin signature that permits a return to totipotency (the ability to form all cell types in the body), which is essential for formation of a viable zygote, and that carries sex-specific epigenetic information critical for health and development in the offspring. Hence male and female germ cells represent specialised cell types that must acquire epigenetic patterning suitable for directing gametogenic function and the ability to support development.

Genome-wide DNA demethylation is a major event characterising germline and preimplantation epigenetic reprogramming (Fig. 2). DNA methylation is a biochemical modification occurring primarily at cytosine residues within cytosine-guanidine (CpG) dinucleotides. DNA methylation has established roles in gene and retrotransposon silencing, genomic imprinting, X-chromosome inactivation and chromosomal stability [14–16]. Upon specification, the germline undergoes widespread global DNA demethylation [17], primarily at introns, intergenic regions and repeats, with more modest losses occurring within exons and promoters [18]. Germline DNA demethylation takes place in two discernible stages; the first prior to E9.5 when up to 70% of DNA methylation is lost, and another during the final stages of

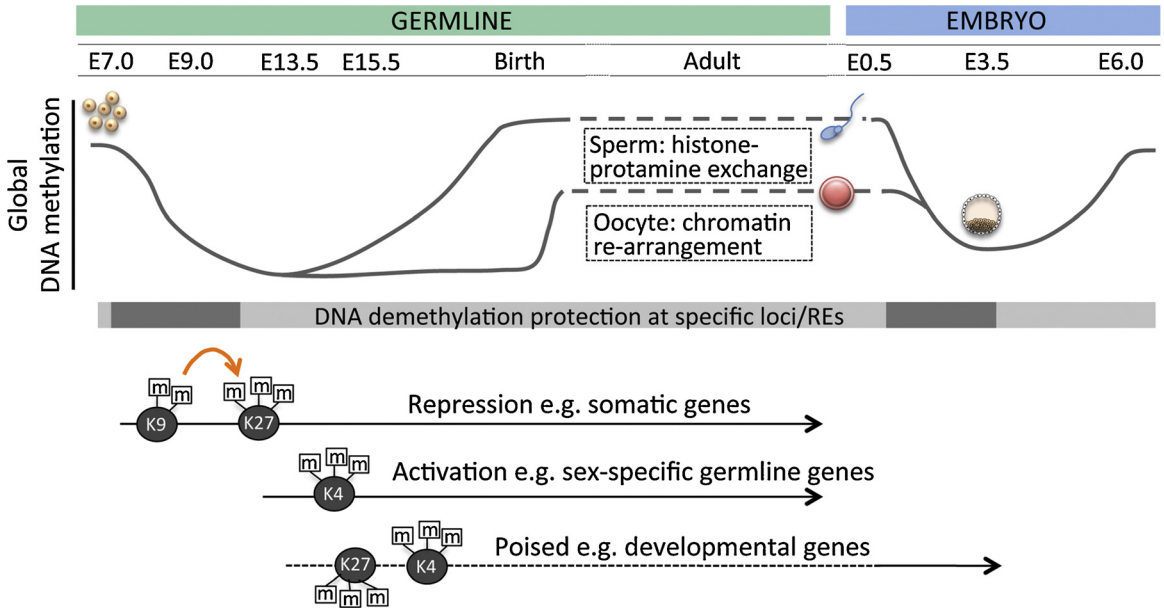

Fig. 2. Epigenetic reprogramming in the germline and early embryo. The mouse germline undergoes epigenetic remodelling to erase somatic epigenetic memory, to produce a gamete capable of re-establishing totipotency in the next generation and to provide gametes with sex-specific epigenetic information that is essential for offspring health and development. From E6.5 to E13.5, there is extensive global DNA demethylation in germ cells. Parental imprints are removed and the X-chromosome is reactivated in female germ cells. Significantly, a portion of genomic sequences are protected from complete DNA demethylation, including some repeat elements (REs). Sex-specific DNA methylation is re-established by birth in the male germline and postnatally in the female germline. Germline chromatin remodelling includes the removal, and replacement of, di-methylated histone (H)3 lysine (K)9 with tri-methylated H3K27 repressive marks at ~E8.0. Genes actively transcribed in germ cells, and critical for germ cell development and function may be marked with the 'active' histone modification, H3K4me3. Conversely, genes that are not expressed in the germline, but have essential functions in early embryo development may be 'poised' for transcription, containing bivalent domains enriched for both H3K4me3 and H3K27me3. After fertilisation, the embryo embarks on a second round of epigenetic reprogramming. Parental pronuclei in the early zygote undergo major chromatin remodelling and global DNA demethylation to basal levels in the E3.5 blastocyst. However, parental imprints and some REs are spared DNA demethylation at this time. Tissue-specific DNA methylation is re-established across emerging cell lineages. The germline of the next generation is specified at E6.5 and commences its first wave of epigenetic reprogramming, completing the cycle.

migration and gonad colonisation [19]. After PGCs enter the gonad, DNA methylation levels continue to fall, although at a slower rate than during migration, to ~14% and 7% in male and female E13.5 PGCs, respectively [19]. Importantly, this later stage completes demethylation of genomic imprints, which escape demethylation during preimplantation reprogramming, and CpG islands (CGIs) of X-chromosome linked germline expressed genes [20]. As a result E13.5 male and female PGCs possess similar minimal DNA methylation profiles, which allow subsequent establishment of paternal and maternal specific epigenetic information during sperm and oocyte development, respectively (Fig. 2).

Despite initial observations that the rapid decline in germline DNA methylation occurs through active mechanisms [18,21], there is now a strong argument that replication-dependent passive mechanisms contribute significantly to DNA demethylation [22]. In line with a passive mechanism, it is well established that the germline levels of *de novo* methyltransferases DNMT3A, DNMT3B and the co-factor DNMT3L are very low prior to E12.5 [17,22,23]. In addition, the transcription of *Uhrf1*, a recruitment factor required for the maintenance methyltransferase, DNMT1, is repressed in PGCs after specification [22,23] and there is minimal localisation of DNMT1 to replication foci in E10.5–E13.5 PGCs [22]. Furthermore, the timing of demethylation of a number of genomic imprints is consistent with the timing of replication-dependent passive demethylation based on PGC doubling time during the migratory period [22].

Nevertheless, during the later stages of epigenetic reprogramming (E9.5–E13.5) the methylcytosine dioxygenases ten-eleven translocation 1 (TET1) and TET2, and a number of base excision repair (BER) proteins are expressed in germ cells [21,22,24]. These proteins are capable of *active* DNA demethylation through conversion of 5-methylcytosine (5-mC) to the intermediary base 5-hydroxymethylcytosine (5-hmC). This is thought to provides substrates for secondary processes such as BER that remove 5-hmC, ultimately replacing it with unmethylated cytosine. Supporting a role for TET1/2-mediated 5-mC oxidation, 5-hmC is detected in germ cells during this wave of DNA demethylation. The loss of 5-mC between E9.5 and E10.5 is correlated with a global gain in 5-hmC levels, followed by depletion of 5-hmC [24,25]. Moreover, miRNA knock down of *Tet1* and *Tet2* in an *in vitro* model of PGC development impaired DNA demethylation, whereas overexpression of these proteins enhanced the removal of germline-specific DNA methylation [24].

Initial findings indicated that germline epigenetic reprogramming was unaffected in the absence of TET1, given the observation that *Tet1/2* deficiency did not negatively impact on germ cell differentiation *in vitro* [24], and that *Tet1*−/− mice produce viable and fertile offspring [26]. However, recent studies have revealed a critical role for TET1 in erasure of genomic imprints. Offspring generated from *Tet1* deficient fathers mated to wild type mothers present with a number of phenotypic abnormalities, including severe placental and foetal growth defects and a high incidence of embryonic death [27]. These developmental defects are attributed to a failure of maternal imprint erasure in the paternal genome, including DNA methylation at the maternally imprinted genes *Peg3* and *Peg10* [27,28]. Therefore, although global changes in DNA methylation are undetectable in E13.5 germ cells or sperm of *Tet1* knock out animals, TET1 is essential for driving active demethylation at specific loci [27,28].

2.2. Protecting specific loci from reprogramming is critical for the stability and integrity of the germline epigenome

Striking a balance between DNA demethylation and the retention of DNA methylation at repeat elements is critical. Adequate DNA methylation is required for the repression of repeat elements

in the germline, which have the potential to cause insertional mutagenesis if inappropriately activated, while sufficient demethylation must occur to complete reprogramming. Interestingly, normal germline development also involves protecting some sequences from DNA demethylation (Fig. 2). Although DNA methylation is normally removed from a number of repeat sequences including LINEs, SINEs and long terminal repeats (LTRs), some classes including LTR-endogenous retrovirus 1 (ERV1) and LTR-ERVK retrotransposons, which contain intracisternal A-particle (IAP) elements, are somewhat resistant to DNA demethylation [18–20,29]. In addition, CGIs close to IAP elements also retain higher DNA methylation levels [24] suggesting that they are protected from transcriptional activity. However, resistance to demethylation at CGIs and non-CGIs not proximal to IAPs is also observed [19,20,24].

The mechanisms that underpin retention of DNA methylation in these circumstances are poorly understood, but dysregulation of these processes could lead to abnormal DNA methylation signatures that are subsequently transmitted to the offspring [30,31]. Recent work has identified a role of the histone methyltransferase, SETDB1, in mediating H3K9me3 deposition at partially methylated retrotransposons, including LTR-ERVs and IAPs [32]. Indeed, deletion of germline *Setdb1* results in loss of DNA methylation and the repressive histone modification H3K27me3, and reactivation of some ERV sequences in a sex-dependent manner. This is associated with loss of male germ cells and post-natal gonadal defects in both sexes [32]. Despite the central role of SETDB1 in these processes, it is very likely that additional repressive mechanisms also contribute to silencing of repetitive elements in PGCs.

While appropriate removal of DNA methylation and probably other epigenetic modifications is essential during germline reprogramming, re-establishment of appropriate sex-specific modifications is also critical. This is best understood for DNA methylation and is mediated by the *de novo* DNA methyltransferases (DNMT3A, DNMT3B and DNMT3L), which are essential for the sex-specific re-establishment of DNA methylation at genomic imprints, single-copy genes and repeat elements in germ cells. In the male germline this occurs from E15.5, while in females it is mediated after birth, during oocyte growth. Perturbing these remethylation pathways results in the activation of repeat elements, meiotic defects and failed gametogenesis (reviewed in [33]). The highly conserved PIWI proteins also mediate retrotransposon silencing in germ cells and deletion in the mouse confers similar defects to disruption of DNMT activity [33].

2.3. Chromatin remodelling in germ cells and maturing gametes

The remodelling of the germline epigenome also occurs at the chromatin level and includes extensive reorganisation of histone modifications, exchange of histone linker proteins and the mobilisation of histone chaperone proteins. Coupled with substantial loss of DNA methylation at E8.0, germ cells exchange the repressive histone mark H3K9me2 with another repressive but more plastic mark, H3K27me3, at euchromatic regions (Fig. 2) [8,17]. In addition, further chromatin reorganisation has been observed in germ cells coincident with their entry into the gonad, although the extent of these changes is debated [34]. However, recent mapping of histone modifications during germline development has identified a cohort of genes co-enriched with both *repressive* H3K27me3 and *active* H3K4me3. These include developmental genes that are not expressed in male or female germ cells, which maintain these bivalent marks from foetal stages [35–38] until post-meiotic stages in adult animals [36,39]. The presence of these modifications in mature sperm suggests that they perform a critical role in regulating gene expression during activation of totipotency and developmental competence in the zygote, and during preimplantation development in the offspring.

Dynamic control of germline H3K27me3 has been functionally associated with early germ cell development and the re-establishment of pluripotency. For example, the PR domain containing 14, PRDM14, protein is a transcriptional regulator exclusively expressed in male and female germ cells until ~E13.5-E14.5 [5]. Deletion of *Prdm14* results in loss of germ cells in developing ovaries and testes due to impaired germ cell specification and activation of pluripotency factors including *Sox2* [5]. These defects are associated with an inability to repress the H3K9me2 methyltransferase, *Ehmt1*, resulting in the failed exchange of H3K9me2 for H3K27me3 between E8.5 and E9.5 [5]. Another example is the JmjC-domain containing demethylase, UTX. UTX displaces the H3K27 methylating polycomb repressive complex 2 (PRC2), actively demethylates H3K27me3 and associates with H3K4me3 methyltransferases, to activate genes required for development [40–42]. E12.5 germ cells deficient for *Utx* contain abnormally high levels of H3K27me3 and lower germ cell pluripotency [43]. This inability to return to ground state pluripotency in the absence of epigenetic regulators, such as PRDM14 and UTX, indicates that H3K27me3 reprogramming is essential for normal germ cell function and the establishment of the germline epigenome.

Many questions remain regarding the establishment of epigenetic marks during PGC development, their genomic distribution and the precise role that they may have at specific loci. Although it is established that certain epigenetic modifiers are required for spermatogenesis and oogenesis, there is limited understanding of how these modifiers might alter epigenetic information in the germline and how particular modifications are targeted to specific genomic regions in germ cells. Establishing the roles for specific epigenetic modifiers, and the potential interactions between epigenetic modifications, will be vital to understanding how epigenetic signatures established in the germline may be affected by drugs, diet or other environmental influences, and how epigenetic changes in the germline alter phenotypes in offspring.

3. Transmission of epigenetic information to the offspring

While reprogramming occurs during PGC development and in the foetal germ cells, at least some of this information must then be retained through meiosis and the final stages of gametogenesis before being transmitted to the offspring. During the late stages of gametogenesis the oocyte and sperm undergo extensive chromatin remodelling and nuclear condensation, which is required to silence germline transcriptional programmes and acquire a developmentally competent nuclear state capable of supporting early embryogenesis.

3.1. Chromatin remodelling in the oocyte

During oocyte growth, chromatin is reorganised from a decondensed configuration during the transcriptionally active period in diplotene-stage oocytes, to a condensed configuration in mature oocytes [44,45]. Histone variants present during oocyte growth include the oocyte-specific H1 linker histone variant H1foo [46,47], the H2A variant macro H2A [48] and the replication independent variant H3.3 [49], suggesting that there is dynamic remodelling of maternal chromatin during this time. Genetic studies have demonstrated the essential requirement for the oocyte-specific histone H1 linker proteins for embryonic development [50]. H1foo is necessary for maturation of germinal vesicle (GV) stage oocytes and is required for meiotic resumption, suggesting a role in cell cycle [51]. siRNA depletion of H3.3 in oocytes reveals an essential role of this maternally derived factor for preimplantation reprogramming and for the reactivation of pluripotency programmes in the zygote [52]. In addition, maternal H3.3 and its chaperone protein HIRA is

required for male pronuclear formation and is essential for mouse development beyond the zygote stage [53–55].

Early oocyte growth is associated with histone hyperacetylation permitting active transcription required for oocyte development [56]. As meiotic competence is acquired mid-way through oocyte growth, transcriptional programmes are inactivated and the chromatin condenses allowing the acquisition of developmental competence [57]. This is associated with rapid deacetylation, catalysed by the class I histone deacetylases HDAC1 and HDAC2, deletion of which leads to developmental arrest of secondary-stage follicles and sterility [58]. Moreover, pharmaceutical inhibition of HDACs in GV-stage oocytes results in disordered chromatin decondensation, disrupted binding of the centromeric heterochromatin binding protein ATRX, and meiotic defects [59].

Modifying HDAC1/2 levels in the growing oocyte can also exert secondary epigenetic effects beyond increased histone acetylation levels. This is illustrated by the increased activity of the histone demethylase, *Kdm5b*, and reduced H3K4 methylation in *Hdac1/Hdac2* mutants that is associated with genome-wide gene repression [58]. Furthermore, the dominant catalyst of H3K4 trimethylation, MLL2, is required for global transcriptional silencing associated with the onset of meiotic resumption [60]. Class II histone deacetylase activity is also implicated in oocyte chromatin reorganisation, with over-expression of the predominantly cytoplasmic HDAC6 in GV-stage oocytes leading to premature chromatin compaction [61].

3.2. Chromatin remodelling in sperm

During spermiogenesis, extensive chromatin remodelling in round spermatids is associated with the resumption of meiosis, nuclear condensation and the acquisition of developmental competence to produce mature spermatozoa. Unlike the nucleosomal inheritance of the maternal genome, the paternal genome undergoes genome-wide histone-protamine exchange [62], which is required for packaging the genome into the sperm head and significantly affects epigenetic programming in the zygote [63]. While protamines are rapidly replaced with maternally derived histones after fertilisation [64], approximately 1% and 10% of histones are retained in mouse and human sperm, respectively [62,65] and therefore could transmit epigenetic information that mediates paternal epigenetic inheritance.

Although testis-specific histone variants, including TH2B and H1t are not retained during histone-protamine exchange, others including H2AL1/2 [66], H2A.Bbd [67,68], H1t2 [69], Hils1 [70] and H2A.Lap1 [71] are specifically incorporated during histone-protamine exchange. However, with the exception of H2AL1/2, which is removed soon after fertilisation and presumably is not inherited [72], the potential for testis-specific histone variants to contribute to paternal epigenetic inheritance is not known. Despite this, it is clear that some of these variants are essential for normal spermiogenesis since deletion of *Hlt2* results in defective spermatids, delayed chromatin condensation and reduced fertility [69].

Histone hyperacetylation is a further hallmark of spermatid chromatin remodelling [73] and may be important for histone-protamine exchange and nuclear condensation. In sperm, acetylated histones are recognised by the testis-specific bromodomain protein, BRDT [74], which is essential for normal nuclear condensation and spermatid development in mice [75]. Modifiers of histone methylation are also critical for normal spermiogenesis, including the H3K9me1/2 demethylase, JMJDA1 [76], and the histone methyltransferase, Mll5 [77].

Of particular interest in epigenetic inheritance is the repressive polycomb group complex, PRC2, first identified in Drosophila for its role in repressing homeotic genes [78]. PRC2 catalyses the trimethylation of H3K27 at developmental genes such as the *Hox* genes [65,79,80]. In the male germline, conditional deletion of the PRC2 component Embryonic ectoderm development (*Eed*) results in complete infertility due failed meiosis [81], revealing a role of PRC2 in male germline development. Genome-wide studies of chromatin state in mature sperm reveal that H3K27me3, H3K4me3 and DNA methylation are found on distinct sequences indicating that these epigenetic modifications provide a specific epigenetic signature in male gametes [65,80]. Moreover, these modifications may guide the histone-protamine exchange process with DNA methylation marked for protamination, H3K4me3 enriched sequences marked for exchange of canonical histones H3.1 and H3.2 for the H3.3 variant, and enrichment of H3K27me3 corresponding with the retention of canonical H3.1 [82]. Of particular interest, H3K27me3 is enriched at the promoters of a range of developmental genes that are repressed during preimplantation development in the offspring [82]. These data provide important insights into a chromatin signature that is established during male germline development and presumably transmitted to offspring in whom it may mediate paternal epigenetic inheritance.

3.3. Do epigenetically induced genetic alterations contribute to inheritance?

It is clear that both the maternal and paternal gametes transmit complex epigenetic information to the offspring. Moreover, numerous reports in animal models and human cohorts indicate that environmental impacts on the parent can contribute to behavioural, endocrine and metabolic defects in offspring/children (reviewed in [3,83]). These outcomes are often attributed to epigenetic changes in the germline that are transmitted to the offspring, although in most cases the potential underlying epigenetic defect remains unknown and a definitive link to epigenetic inheritance remains to be confirmed [84,85]. It is also possible that epigenetic defects may induce genetic changes in the germline and have an indirect affect on the offspring [86]. In such a case, although the primary lesion may be epigenetic, this may lead to a genetic change(s) in the germline that is transmitted to and affects phenotype in the offspring (Fig. 3).

The most obvious genetic changes that could occur involve derepression and insertional mutagenesis of repetitive sequences, such as ERVs. Indeed, a number of examples demonstrate the mobilisation of repetitive elements in the germline, including blockages in the piRNA pathway and H3K9 methylation [32,33]. However, it is possible that other sequences may also be involved, resulting in copy number variants such as gene duplications or deletions, although evidence for this is currently lacking. Clearly the distinction between epigenetic and genetic inheritance is challenging to assess in a human cohort, and also in animal studies of mixed genetic backgrounds, although would be possible in rodent studies using inbred animals. Despite these challenges, this question of whether epigenetic changes in the germline are directly transmitted to the offspring, whether they alter the primary genetic sequence and are then transmitted, or a combination of the two is critical. Resolution of these points will provide significant insight into the balance between epigenetic and genetic inheritance, their respective affects on offspring, and for determining how environmental influences inducing epigenetic change might affect genetic diversity.

4. Epigenetic reprogramming in the preimplantation embryo

Notwithstanding these qualifications, it is clear that epigenetic inheritance is essential for offspring development, with genomic imprints providing the classical example. Mechanisms

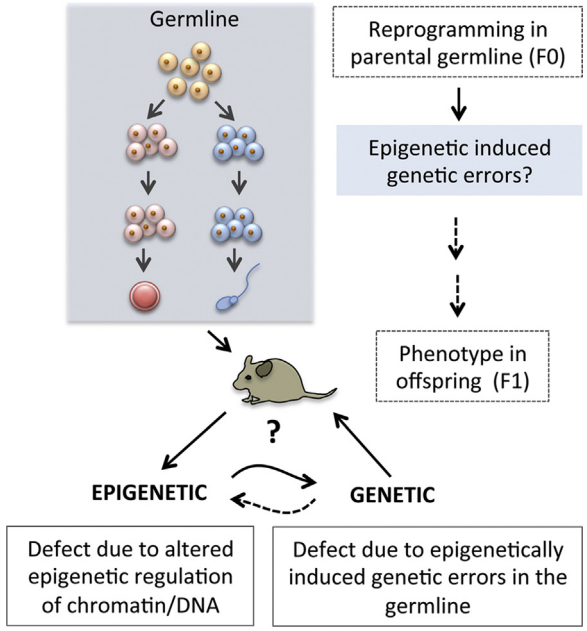

Fig. 3. Epigenetic inheritance: epigenetic vs genetic effects? How epigenetic modifications to DNA and chromatin are inherited by successive generations is enigmatic, and the subject of recent investigations in maturing gametes and the early embryo. Evidently, a germline epigenetic error can result in a sub-optimal phenotype in offspring. In some cases, this phenotype remains stable across generations. However, since epigenetic modifications significantly affect genome stability, another possible explanation for the propagation of an undesirable phenotype through successive generations is that an epigenetic defect in the germline might cause a genetic error. This could include a copy number variant (insertion/deletion) or base substitution that is then transmitted to, and impacts on the offspring. Epigenetic inheritance may therefore involve either of these possibilities or a combination of the two.

have evolved to facilitate transmission of these effects to the somatic tissues, and have been a major focus of research that has progressed significantly in recent years. Preimplantation development involves substantial reorganisation of the highly differentiated and non-equivalent parental genomes after fertilisation, to facilitate totipotency and support embryonic development. This includes extensive epigenetic remodelling of histone modifications at pericentric chromatin, genome-wide DNA demethylation and zygotic gene activation. In the zygote, the parental genomes are transcriptionally inactive and early development relies on maternal factors that are supplied by the oocyte before the initiation of zygotic transcription [87]. Although maternal factors effectively drive the early stages of development in the zygote and preimplantation embryo, it is clear that germline chromatin possesses qualities that are also required to support normal embryogenesis. How germline chromatin states are prepared for initiating development in the zygote is a topic of ongoing scrutiny, particularly with respect to the epigenetic modifications that are transmitted to the next generation and the epigenetic modifiers that are involved.

4.1. DNA methylation dynamics in the preimplantation embryo

Zygotic and preimplantation reprogramming must achieve two goals to support development. Firstly, the chromatin must be reorganised in order to support the acquisition of a naïve pluripotent state capable of supporting early developmental programmes. Secondly, this stage of reprogramming must protect parent-specific epigenetic information transmitted by the gametes and which is required to support development and health in later life (*e.g.* genomic imprints). The first goal is achieved through genome-wide DNA demethylation of the asymmetric parental methylomes, inherited from the egg and sperm [88,89]. After fertilisation, the diverse parental methylomes represented by hypermethylated sperm and intermediately methylated oocytes [90,91], are re-set and DNA methylation levels are globally reduced to their lowest levels in the inner cell mass at E3.5 (Fig. 2) [92]. Despite these global changes in DNA methylation, the second goal is achieved by protecting some DNA sequences from demethylation in the early embryo, such as imprints [93–95] and certain repeat elements including LTRs and IAPs [29], which remain methylated to preserve genomic integrity.

Historically, it was considered that the zygotic paternal genome is actively demethylated before the first mitotic division [96,97], and was linked to the rapid DNA methylation loss shortly after protamine-histone exchange in the zygote [98]. A recent flurry of papers has put these initial assumptions to the test and concluded that the vast majority of DNA demethylation occurring in the zygotic paternal (and maternal) genome occurs through replication-dependent DNA demethylation [92,99–101]. Nevertheless, evidence suggests that this process is facilitated by active DNA demethylation, catalysed by the oxidation of 5-mC to 5-hmC by TET3 [90,99,101–103]. Furthermore, in contrast to the notion that active DNA demethylation only occurs in the paternal genome, recent evidence suggests that TET3 actively demethylates a significant number of maternal loci in the maternal genome [92,101] both in the zygote [99,101] and after the two-cell stage [92]. It also appears that in the zygote, maternal demethylation at repetitive elements is counter-balanced by *de novo* methylation [104].

TET3 is expressed in oocytes of primary follicles and during oocyte growth and maturation. In the zygote, TET3 is enriched in the male pronucleus as 5-mC levels decrease and 5-hmC levels rise [102,105]. Moreover, conditional deletion of *Tet3* in the female germline blocks the formation of 5-hmC in the male pronucleus and impairs paternal genomic DNA demethylation after fertilisation [105,106]. This is accompanied by reduced fecundity and increased mid-gestation developmental failure [105], despite apparently normal preimplantation development and zygotic gene activation [101,105]. However, recent data suggest that TET3 activity may be more significant at selected loci than on a global level [90,101,104]. Enhanced whole genome bisulfite sequencing of *Tet3* depleted zygotes indicate that TET3 is required for DNA demethylation of intergenic and gene body regions, as well as non-CGI containing promoters [90]. Furthermore, this work demonstrates that at the majority of loci, DNA demethylation is not impaired by deficient TET3 activity, suggesting that alternative or passive pathways are primarily involved in DNA demethylation during early reprogramming [90].

Indeed, very recently, the role of 5-hmC in coordinating DNA demethylation of the paternal pronuclei has been challenged by data showing that the sub-lethal phenotype present in maternal *Tet3* deficient embryos is not caused by defective 5-mC oxidation [107]. Moreover, the majority of DNA demethylation during this wave of epigenetic reprogramming is replication-dependent, as demonstrated by the inability of TET3 to normally demethylate the parental genomes when DNA replication is blocked [101]. Interestingly, at a subset of CGI-associated promoters, TET3 protects the sequence from *de novo* DNA methylation rather than catalysing demethylation [90]. Preventing DNA methylation at these targets may permit their transcriptional activation during early embryogenesis, highlighting alternative roles for TET3 and demonstrating the complexity of loci specific epigenetic control during preimplantation reprogramming.

Although the mechanisms that facilitate DNA demethylation in the pre-mitotic zygote are not fully elucidated, they may be required to give the process a 'head-start' prior to replication-dependent DNA methylation. This would ensure that the genome is sufficiently depleted of DNA methylation marks, and the correct transcriptional programmes are activated for developmental

competence. This is further complicated by the requirement to facilitate transmission of essential epigenetic modifications that must be protected from reprogramming during preimplantation development.

4.2. Differential regulation of chromatin state within the parental pronuclei

At the most fundamental level, constitutive heterochromatin performs crucial roles in chromosomal segregation and the maintenance of genome integrity [108]. This is complemented by facultative heterochromatin, which is required for the maintenance of cell identity between mitotic divisions, ensuring the correct repression of certain gene subsets [109]. In the early zygote, H3K9me3 is maintained in constitutive heterochromatin at maternal pericentric regions by the maternally derived H3K9 specific methyltransferase, SUV39h [110], and the heterochromatic adaptor protein, HP1β [111]. In contrast, SUV39h is absent from the male pronucleus and paternal pericentric heterochromatin is enriched with H3K27me3, catalysed by maternally supplied PRC1 components including ring finger protein 2 (RNF2) [112]. Recent data also show that chromobox homolog 2, CBX2, targets catalytically active PRC1 to paternal constitutive heterochromatin, and that HP1β present on maternal pericentric heterochromatin prevents this interaction [113].

At euchromatic regions, a number of developmentally associated genes are bivalently marked with H3K27me3 and H3K4me3. The repression of these genes involves cooperation of PRC1 and PRC2, where PRC2 recruits PRC1 and gene silencing is mediated by RNF2-induced monoubiquitination of H2A lysine 119 (H2AK119u1) [114,115]. However, PRC1-mediated H3K27me3 enrichment at paternal pericentric heterochromatin is not dependent on PRC2 activity in the zygote [112]. On the other hand, absence of the maternal PRC1 protein, RNF2, leads to increased de-repression of paternal but not maternal major satellites [112]. Notably, depletion of *Suv39h* (loss of H3K9me3) results in the accumulation of maternal PRC1 components, and H3K27me3, on pericentric heterochromatin of both parental genomes, indicating a degree of hierarchy between H3K9me3 and H3K27me3 [112].

While complete deletion of *Rnf2* is embryonic lethal [116], maternal deficiency of RNF2 does not disrupt embryonic development [117,118]. However, this is explained by functional redundancy with the RNF2 paralogue RING1 in the oocyte [117,119], and removal of maternal RNF2 and RING1 stalls embryonic development beyond the two-cell stage. Moreover, this phenotype is not rescued through microinjection of *Rnf2* mRNA into the early zygote, indicating the necessity of PRC1 (RNF2 and RING1) during oocyte growth for normal embryonic development [117]. Indeed, more than 2500 transcripts with roles in development and lineage specification are dysregulated in GV oocytes in which both *Rnf2* and *Ring1* are deleted during oocyte growth [117]. Furthermore, sophisticated chromosome transfer studies demonstrate that both PRC1 defined cytoplasmic factors and PRC1 mediated patterning of the maternal genome, contribute to early embryogenesis [117]. Thus PRC1 is a crucial chromatin modulator that maintains a repressive state at developmental and lineage specifying genes during oogenesis, and directs inheritance of cytoplasmic programmes and germline chromatin patterns by the zygote that are required for early developmental competence.

5. Conservation of epigenetic mechanisms in the human germline

There is a strong imperative to determine how heritability of epigenetic changes affects human health outcomes. To understand this there is a necessity to determine how the human germline develops, reprogrammes epigenetic information and how these processes may be affected by external influences. Clearly, studying the role of germline epigenome inheritance in humans is challenging. However, recent advances in unravelling early germline programming events will enable future investigations into the role of highly conserved epigenetic modifiers in human germ cell biology.

Human PGCs are specified during week 2 of development and migrate along the hindgut to enter the genital ridge on week 5 [120]. Sex-determination occurs during weeks 6–8 and human germ cells, now termed gonocytes, are enclosed within testis cords from around week 7 [121,122]. Oogonia begin to enter meiosis by week 9 in females, while pre-spermatogonia enter mitotic arrest by week 18 in males [122]. Week 2–9 human germ cells are developmentally aligned with E6.5–E13.5 mouse germ cells, equivalent to the main period during which DNA methylation is removed from the developing germline [120,123].

Epigenetic reprogramming occurs in human germ cells during week 4–9 of development [120], with the lowest levels of global DNA methylation observed by weeks 10–11 of gestation [123,124]. Remethylation of the male germ cell genome is thought to occur sometime later, possibly not until after 19 weeks, and may occur later than in the female germline [123,125]. Recent studies employing immunofluorescence and whole genome bisulfite sequencing indicate that human germ cells are globally hypomethylated as early as week 7 [120]. Like the mouse, DNA demethylation in human germ cells is associated with the repression of the *de novo* methyltransferases DNMT3A and DNMT3B, and the functionally associated protein UHRF1 at week 7 of development, indicating that DNA methylation may be at least partially depleted through passive mechanisms [120,124]. Conversely, TET1 levels are increased in human first trimester germ cells, in combination with strong enrichment of the BER pathway, suggesting that active DNA demethylation processes may also be involved [120,123,125].

In common with the mouse, human germ cell retrotransposon sequences including LINE and SINE elements, and particularly, evolutionarily young L1, ERVK and Alu elements show site-specific resistance to DNA demethylation [120,123,125]. This is of importance to the hypothesis that genomic sequences escaping DNA demethylation contribute to epigenetic inheritance. For example, a number of sequences classed as DNA demethylation 'escapees' that are not associated with repeat elements, are enriched for H3K9me3 and are found at gene regions involved in brain development and obesity [120].

Rapid progress is being made in the study of the human germline epigenome with the recent production of germ cells using *in vitro* approaches [120,123,124,126], and research in human foetal germ cells [120,123–126]. However, the processes underpinning epigenetic reprogramming in the human germline and transmission of epigenetic information to the offspring remain poorly understood. Better understanding of these processes is essential as the human population faces many new environmental challenges with the potential for enduring consequences [83,127].

6. Conclusions

Over the past two decades, and particularly in the last five years, significant progress has been made in unravelling how the mammalian germline is epigenetically reprogrammed to influence: (1) germline development, gamete formation and fertility, and (2) transmission of epigenetic information that permits a return to totipotency and directs subsequent development in the offspring. Numerous genetic studies have focused on the role of epigenetic modifiers during oogenesis and spermatogenesis, and the consequences of their depletion on offspring. While these studies make

important contributions to our understanding of the potential for both maternal and paternal epigenetic inheritance to influence the offspring, the possibility that epigenetically induced genetic errors could contribute should be also considered. Recent developments in our knowledge of the human germline epigenome are predicted to result in exciting advances in the understanding of how influences on the epigenome may impact on both evolutionary processes and health and well-being in the human population.

Conflict of interest statement

The authors have no conflicts of interest relating to this work.

Acknowledgments

We extend our apologies to the authors of studies related to this review but were not included due to length limitations. We thank Dr Jessica Stringer and Ms Lexie Prokopuk for review and comment during the preparation of this manuscript. This work was supported by NHMRC Project Grant 1043939 awarded to PW, the Hudson Institute for Medical Research and Monash University, and the Victorian Government Operational Infrastructure Support Program.

References

[1] Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 2007;447:425–32.
[2] Hemberger M, Dean W, Reik W. Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. Nat. Rev. Mol. Cell Biol 2009;10:526–37.
[3] Trerotola M, Relli V, Simeone P, et al. Epigenetic inheritance and the missing heritability. Hum. Genomics 2015;9:17.
[4] Okamura D, Hayashi K, Matsui Y. Mouse epiblasts change responsiveness to BMP4 signal required for PGC formation through functions of extraembryonic ectoderm. Mol. Reprod. Dev 2005;70:20–9.
[5] Yamaji M, Seki Y, Kurimoto K, et al. Critical function of Prdm14 for the establishment of the germ cell lineage in mice. Nat. Genet 2008;40:1016–22.
[6] Ying Y, Qi X, Zhao GQ. Induction of primordial germ cells from murine epiblasts by synergistic action of BMP4 and BMP8B signaling pathways. Proc. Natl. Acad. Sci. U. S. A 2001;98:7858–62.
[7] Miles DC, Western PS. Germ cell sex and cell cycle. Histol. Histopathol 2012;27:445–57.
[8] Seki Y, Yamaji M, Yabuta Y, et al. Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development 2007;134:2627–38.
[9] Tam PP, Snow MH. Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. J. Embryol. Exp. Morphol 1981;64:133–47.
[10] Kocer A, Reichmann J, Best D, et al. Germ cell sex determination in mammals. Mol. Hum. Reprod 2009;15:205–13.
[11] Spiller CM, Bowles J. Sex determination in mammalian germ cells. Asian J. Androl 2015;17:427–32.
[12] Western PS, Miles DC, van den Bergen JA, et al. Dynamic regulation of mitotic arrest in fetal male germ cells. Stem Cells 2008;26:339–47.
[13] Western P. Foetal germ cells: striking the balance between pluripotency and differentiation. Int. J. Dev. Biol 2009;53:393–409.
[14] Bourc'his D, Xu GL, Lin CS, et al. Dnmt3L and the establishment of maternal genomic imprints. Science 2001;294:2536–9.
[15] Dodge JE, Okano M, Dick F, et al. Inactivation of Dnmt3b in mouse embryonic fibroblasts results in DNA hypomethylation, chromosomal instability, and spontaneous immortalization. J. Biol. Chem 2005;280:17986–91.
[16] Kaneda M, Sado T, Hata K, et al. Role of de novo DNA methyltransferases in initiation of genomic imprinting and X-chromosome inactivation. Cold Spring Harb. Symp. Quant. Biol 2004;69:125–9.
[17] Seki Y, Hayashi K, Itoh K, et al. Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev. Biol 2005;278:440–58.
[18] Popp C, Dean W, Feng S, et al. Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. Nature 2010;463:1101–5.
[19] Seisenberger S, Andrews S, Krueger F, et al. The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. Mol. Cell 2012;48:849–62.
[20] Guibert S, Forne T, Weber M. Global profiling of DNA methylation erasure in mouse primordial germ cells. Genome Res 2012;22:633–41.
[21] Hajkova P, Jeffries SJ, Lee C, et al. Genome-wide reprogramming in the mouse germ line entails the base excision repair pathway. Science 2010;329:78–82.
[22] Kagiwada S, Kurimoto K, Hirota T, et al. Replication-coupled passive DNA demethylation for the erasure of genome imprints in mice. EMBO J 2013;32:340–53.
[23] Kurimoto K, Yabuta Y, Ohinata Y, et al. Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. Genes Dev 2008;22:1617–35.
[24] Hackett JA, Sengupta R, Zylicz JJ, et al. Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. Science 2013;339:448–52.
[25] Yamaguchi S, Hong K, Liu R, et al. Dynamics of 5-methylcytosine and 5-hydroxymethylcytosine during germ cell reprogramming. Cell Res 2013;23:329–39.
[26] Dawlaty MM, Ganz K, Powell BE, et al. Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. Cell Stem Cell 2011;9:166–75.
[27] Yamaguchi S, Shen L, Liu Y, et al. Role of Tet1 in erasure of genomic imprinting. Nature 2013;504:460–4.
[28] Yamaguchi S, Hong K, Liu R, et al. Tet1 controls meiosis by regulating meiotic gene expression. Nature 2012;492:443–7.
[29] Lane N, Dean W, Erhardt S, et al. Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. Genesis 2003;35:88–93.
[30] Daxinger L, Whitelaw E. Transgenerational epigenetic inheritance: more questions than answers. Genome Res 2010;20:1623–8.
[31] Hackett JA, Surani MA. Beyond DNA: programming and inheritance of parental methylomes. Cell 2013;153:737–9.
[32] Liu S, Brind'Amour J, Karimi MM, et al. Setdb1 is required for germline development and silencing of H3K9me3-marked endogenous retroviruses in primordial germ cells. Genes Dev 2014;28:2041–55.
[33] Zamudio N, Bourc'his D. Transposable elements in the mammalian germline: a comfortable niche or a deadly trap? Heredity (Edinb) 2010;105:92–104.
[34] Stringer JM, Barrand S, Western P. Fine-tuning evolution: germ-line epigenetics and inheritance. Reproduction 2013;146:R37–48.
[35] Mochizuki K, Tachibana M, Saitou M, et al. Implication of DNA demethylation and bivalent histone modification for selective gene regulation in mouse primordial germ cells. PLoS ONE 2012;7:e46036.
[36] Lesch BJ, Dokshin GA, Young RA, et al. A set of genes critical to development is epigenetically poised in mouse germ cells from fetal stages through completion of meiosis. Proc. Natl. Acad. Sci. U. S. A 2013;110:16061–6.
[37] Ng JH, Kumar V, Muratani M, et al. In vivo epigenomic profiling of germ cells reveals germ cell molecular signatures. Dev. Cell 2013;24:324–33.
[38] Sachs M, Onodera C, Blaschke K, et al. Bivalent chromatin marks developmental regulatory genes in the mouse embryonic germline in vivo. Cell Rep 2013;3:1777–84.
[39] Hammoud SS, Low DH, Yi C, et al. Chromatin and transcription transitions of mammalian adult germline stem cells and spermatogenesis. Cell Stem Cell 2014;15:239–53.
[40] Agger K, Cloos PA, Christensen J, et al. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 2007;449:731–4.
[41] Hong S, Cho YW, Yu LR, et al. Identification of JmjC domain-containing UTX and JMJD3 as histone H3 lysine 27 demethylases. Proc. Natl. Acad. Sci. U. S. A 2007;104:18439–44.
[42] Issaeva I, Zonis Y, Rozovskaia T, et al. Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. Mol. Cell. Biol 2007;27:1889–903.
[43] Mansour AA, Gafni O, Weinberger L, et al. The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. Nature 2012;488:409–13.
[44] Wiekowski M, Miranda M, Nothias JY, et al. Changes in histone synthesis and modification at the beginning of mouse development correlate with the establishment of chromatin mediated repression of transcription. J. Cell Sci 1997;110(Pt 10):1147–58.
[45] Zuccotti M, Piccinelli A, Giorgi Rossi P, et al. Chromatin organization during mouse oocyte growth. Mol. Reprod. Dev 1995;41:479–85.
[46] Tanaka M, Hennebold JD, Macfarlane J, et al. A mammalian oocyte-specific linker histone gene H1oo: homology with the genes for the oocyte-specific cleavage stage histone (cs-H1) of sea urchin and the B4/H1M histone of the frog. Development 2001;128:655–64.
[47] Tanaka M, Kihara M, Hennebold JD, et al. H1FOO is coupled to the initiation of oocytic growth. Biol. Reprod 2005;72:135–42.
[48] Chang CC, Ma Y, Jacobs S, et al. A maternal store of macroH2A is removed from pronuclei prior to onset of somatic macroH2A expression in preimplantation embryos. Dev. Biol 2005;278:367–80.
[49] Akiyama T, Suzuki O, Matsuda J, et al. Dynamic replacement of histone H3 variants reprograms epigenetic marks in early mouse embryos. PLoS Genet 2011;7:e1002279.
[50] Fan Y, Nikitina T, Morin-Kensicki EM, et al. H1 linker histones are essential for mouse development and affect nucleosome spacing in vivo. Mol. Cell. Biol 2003;23:4559–72.
[51] Furuya M, Tanaka M, Teranishi T, et al. H1foo is indispensable for meiotic maturation of the mouse oocyte. J. Reprod. Dev 2007;53:895–902.

[52] Wen D, Banaszynski LA, Liu Y, et al. Histone variant H3.3 is an essential maternal factor for oocyte reprogramming. Proc. Natl. Acad. Sci. U. S. A 2014;111:7325–30.
[53] Lin CJ, Koh FM, Wong P, et al. Hira-mediated H3.3 incorporation is required for DNA replication and ribosomal RNA transcription in the mouse zygote. Dev. Cell 2014;30:268–79.
[54] Santenard A, Ziegler-Birling C, Koch M, et al. Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. Nat. Cell Biol 2010;12:853–62.
[55] Torres-Padilla ME, Bannister AJ, Hurd PJ, et al. Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos. Int. J. Dev. Biol 2006;50:455–61.
[56] Kim JM, Liu H, Tazaki M, et al. Changes in histone acetylation during mouse oocyte meiosis. J. Cell Biol 2003;162:37–46.
[57] De La Fuente R, Eppig JJ. Transcriptional activity of the mouse oocyte genome: companion granulosa cells modulate transcription and chromatin remodeling. Dev. Biol 2001;229:224–36.
[58] Ma P, Pan H, Montgomery RL, et al. Compensatory functions of histone deacetylase 1 (HDAC1) and HDAC2 regulate transcription and apoptosis during mouse oocyte development. Proc. Natl. Acad. Sci. U. S. A 2012;109:E481–9.
[59] De La Fuente R, Viveiros MM, Wigglesworth K. ATRX, a member of the SNF2 family of helicase/ATPases, is required for chromosome alignment and meiotic spindle organization in metaphase II stage mouse oocytes. Dev. Biol 2004;272:1–14.
[60] Andreu-Vieyra CV, Chen R, Agno JE, et al. MLL2 is required in oocytes for bulk histone 3 lysine 4 trimethylation and transcriptional silencing. PLoS Biol 2010:8.
[61] Verdel A, Seigneurin-Berny D, Faure AK, et al. HDAC6-induced premature chromatin compaction in mouse oocytes and fertilised eggs. Zygote 2003;11:323–8.
[62] Gatewood JM, Cook GR, Balhorn R, et al. Sequence-specific packaging of DNA in human sperm chromatin. Science 1987;236:962–4.
[63] Kishigami S, Van Thuan N, Hikichi T, et al. Epigenetic abnormalities of the mouse paternal zygotic genome associated with microinsemination of round spermatids. Dev. Biol 2006;289:195–205.
[64] Gao S, Chung YG, Parseghian MH, et al. Rapid H1 linker histone transitions following fertilization or somatic cell nuclear transfer: evidence for a uniform developmental program in mice. Dev. Biol 2004;266:62–75.
[65] Brykczynska U, Hisano M, Erkek S, et al. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. Nat. Struct. Mol. Biol 2010;17:679–87.
[66] Govin J, Escoffier E, Rousseaux S, et al. Pericentric heterochromatin reprogramming by new histone variants during mouse spermiogenesis. J. Cell Biol 2007;176:283–94.
[67] Chadwick BP, Willard HF. A novel chromatin protein, distantly related to histone H2A, is largely excluded from the inactive X chromosome. J. Cell Biol 2001;152:375–84.
[68] Ishibashi T, Li A, Eirin-Lopez JM, et al. H2A.Bbd: an X-chromosome-encoded histone involved in mammalian spermiogenesis. Nucleic Acids Res 2010;38:1780–9.
[69] Martianov I, Brancorsini S, Catena R, et al. Polar nuclear localization of H1T2, a histone H1 variant, required for spermatid elongation and DNA condensation during spermiogenesis. Proc. Natl. Acad. Sci. U. S. A 2005;102:2808–13.
[70] Yan W, Ma L, Burns KH, et al. HILS1 is a spermatid-specific linker histone H1-like protein implicated in chromatin remodeling during mammalian spermiogenesis. Proc. Natl. Acad. Sci. U. S. A 2003;100:10546–51.
[71] Soboleva TA, Nekrasov M, Pahwa A, et al. A unique H2A histone variant occupies the transcriptional start site of active genes. Nat. Struct. Mol. Biol 2012;19:25–30.
[72] Wu F, Caron C, De Robertis C, et al. Testis-specific histone variants H2AL1/2 rapidly disappear from paternal heterochromatin after fertilization. J. Reprod. Dev 2008;54:413–7.
[73] Hazzouri M, Pivot-Pajot C, Faure AK, et al. Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone deacetylases. Eur. J. Cell Biol 2000;79:950–60.
[74] Pivot-Pajot C, Caron C, Govin J, et al. Acetylation-dependent chromatin reorganization by BRDT, a testis-specific bromodomain-containing protein. Mol. Cell. Biol 2003;23:5354–65.
[75] Shang E, Nickerson HD, Wen D, et al. The first bromodomain of Brdt, a testis-specific member of the BET sub-family of double-bromodomain-containing proteins, is essential for male germ cell differentiation. Development 2007;134:3507–15.
[76] Liu Z, Zhou S, Liao L, et al. Jmjd1a demethylase-regulated histone modification is essential for cAMP-response element modulator-regulated gene expression and spermatogenesis. J. Biol. Chem 2010;285:2758–70.
[77] Yap DB, Walker DC, Prentice LM, et al. Mll5 is required for normal spermatogenesis. PLoS ONE 2011;6:e27127.
[78] Franke A, DeCamillis M, Zink D, et al. Polycomb and polyhomeotic are constituents of a multimeric protein complex in chromatin of *Drosophila melanogaster*. EMBO J 1992;11:2941–50.
[79] Boyer LA, Plath K, Zeitlinger J, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006;441:349–53.
[80] Hammoud SS, Nix DA, Zhang H, et al. Distinctive chromatin in human sperm packages genes for embryo development. Nature 2009;460:473–8.
[81] Mu W, Starmer J, Fedoriw AM, et al. Repression of the soma-specific transcriptome by Polycomb-repressive complex 2 promotes male germ cell development. Genes Dev 2014;28:2056–69.
[82] Erkek S, Hisano M, Liang CY, et al. Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. Nat. Struct. Mol. Biol 2013;20:868–75.
[83] Hogg K, Price EM, Hanna CW, et al. Prenatal and perinatal environmental influences on the human fetal and placental epigenome. Clin. Pharmacol. Ther 2012;92:716–26.
[84] Isbel L, Whitelaw E. Commentary: Far-reaching hypothesis or a step too far: the inheritance of acquired characteristics. Int. J. Epidemiol 2015;44:1109–12.
[85] Daxinger L, Whitelaw E. Understanding transgenerational epigenetic inheritance via the gametes in mammals. Nat. Rev. Genet 2012;13:153–62.
[86] Miura A, Yonebayashi S, Watanabe K, et al. Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis. Nature 2001;411:212–4.
[87] Tadros W, Lipshitz HD. The maternal-to-zygotic transition: a play in two acts. Development 2009;136:3033–42.
[88] Ci W, Liu J. Programming and inheritance of parental DNA methylomes in vertebrates. Physiology (Bethesda) 2015;30:63–8.
[89] Surani MA, Hayashi K, Hajkova P. Genetic and epigenetic regulators of pluripotency. Cell 2007;128:747–62.
[90] Peat JR, Dean W, Clark SJ, et al. Genome-wide bisulfite sequencing in zygotes identifies demethylation targets and maps the contribution of TET3 oxidation. Cell Rep 2014;9:1990–2000.
[91] Smallwood SA, Tomizawa S, Krueger F, et al. Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat. Genet 2011;43:811–4.
[92] Wang L, Zhang J, Duan J, et al. Programming and inheritance of parental DNA methylomes in mammals. Cell 2014;157:979–91.
[93] Cirio MC, Ratnam S, Ding F, et al. Preimplantation expression of the somatic form of Dnmt1 suggests a role in the inheritance of genomic imprints. BMC Dev Biol 2008;8:9.
[94] Hirasawa R, Chiba H, Kaneda M, et al. Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. Genes Dev 2008;22:1607–16.
[95] McGraw S, Zhang JX, Farag M, et al. Transient DNMT1 suppression reveals hidden heritable marks in the genome. Nucleic Acids Res 2015;43:1485–97.
[96] Mayer W, Niveleau A, Walter J, et al. Demethylation of the zygotic paternal genome. Nature 2000;403:501–2.
[97] Oswald J, Engemann S, Lane N, et al. Active demethylation of the paternal genome in the mouse zygote. Curr. Biol 2000;10:475–8.
[98] Dean W, Santos F, Reik W. Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer. Semin. Cell Dev. Biol 2003;14:93–100.
[99] Guo F, Li X, Liang D, et al. Active and passive demethylation of male and female pronuclear DNA in the mammalian zygote. Cell Stem Cell 2014;15:447–58.
[100] Inoue A, Zhang Y. Replication-dependent loss of 5-hydroxymethylcytosine in mouse preimplantation embryos. Science 2011;334:194.
[101] Shen L, Inoue A, He J, et al. Tet3 and DNA replication mediate demethylation of both the maternal and paternal genomes in mouse zygotes. Cell Stem Cell 2014;15:459–70.
[102] Iqbal K, Jin SG, Pfeifer GP, et al. Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. Proc. Natl. Acad. Sci. U. S. A 2011;108:3642–7.
[103] Wossidlo M, Nakamura T, Lepikhov K, et al. 5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. Nat. Commun 2011;2:241.
[104] Arand J, Wossidlo M, Lepikhov K, et al. Selective impairment of methylation maintenance is the major cause of DNA methylation reprogramming in the early embryo. Epigenet. Chromatin 2015;8:1.
[105] Gu TP, Guo F, Yang H, et al. The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature 2011;477:606–10.
[106] Santos F, Peat J, Burgess H, et al. Active demethylation in mouse zygotes involves cytosine deamination and base excision repair. Epigenet. Chromatin 2013;6:39.
[107] Inoue A, Shen L, Matoba S, et al. Haploinsufficiency, but not defective paternal 5mC oxidation, accounts for the developmental defects of maternal Tet3 knockouts. Cell Rep 2015;10:463–70.
[108] Melcher M, Schmid M, Aagaard L, et al. Structure-function analysis of SUV39H1 reveals a dominant role in heterochromatin organization, chromosome segregation, and mitotic progression. Mol. Cell. Biol 2000;20:3728–41.
[109] Delcuve GP, Rastegar M, Davie JR. Epigenetic control. J. Cell. Physiol 2009;219:243–50.
[110] Peters AH, O'Carroll D, Scherthan H, et al. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell 2001;107:323–37.
[111] Martin C, Beaujean N, Brochard V, et al. Genome restructuring in mouse embryos during reprogramming and early development. Dev. Biol 2006;292:317–32.

[112] Puschendorf M, Terranova R, Boutsma E, et al. PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. Nat. Genet 2008;40:411–20.
[113] Tardat M, Albert M, Kunzmann R, et al. Cbx2 targets PRC1 to constitutive heterochromatin in mouse zygotes in a parent-of-origin-dependent manner. Mol. Cell 2015;58:157–71.
[114] Stock JK, Giadrossi S, Casanova M, et al. Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. Nat. Cell Biol 2007;9:1428–35.
[115] van der Stoop P, Boutsma EA, Hulsman D, et al. Ubiquitin E3 ligase Ring1b/Rnf2 of polycomb repressive complex 1 contributes to stable maintenance of mouse embryonic stem cells. PLoS ONE 2008;3: e2235.
[116] Voncken JW, Roelen BA, Roefs M, et al. Rnf2 (Ring1b) deficiency causes gastrulation arrest and cell cycle inhibition. Proc. Natl. Acad. Sci. U. S. A 2003;100:2468–73.
[117] Posfai E, Kunzmann R, Brochard V, et al. Polycomb function during oogenesis is required for mouse embryonic development. Genes Dev 2012;26: 920–32.
[118] Terranova R, Yokobayashi S, Stadler MB, et al. Polycomb group proteins Ezh2 and Rnf2 direct genomic contraction and imprinted repression in early mouse embryos. Dev. Cell 2008;15:668–79.
[119] Endoh M, Endo TA, Endoh T, et al. Polycomb group proteins Ring1A/B are functionally linked to the core transcriptional regulatory circuitry to maintain ES cell identity. Development 2008;135:1513–24.
[120] Tang WW, Dietmann S, Irie N, et al. A unique gene regulatory network resets the human germline epigenome for development. Cell 2015;161:1453–67.
[121] Bendsen E, Byskov AG, Laursen SB, et al. Number of germ cells and somatic cells in human fetal testes during the first weeks after sex differentiation. Hum. Reprod 2003;18:13–8.
[122] Gaskell TL, Esnal A, Robinson LL, et al. Immunohistochemical profiling of germ cells within the human fetal testis: identification of three subpopulations. Biol. Reprod 2004;71:2012–21.
[123] Guo F, Yan L, Guo H, et al. The transcriptome and DNA methylome landscapes of human primordial germ cells. Cell 2015;161:1437–52.
[124] Gkountela S, Li Z, Vincent JJ, et al. The ontogeny of cKIT+ human primordial germ cells proves to be a resource for human germ line reprogramming, imprint erasure and in vitro differentiation. Nat. Cell Biol 2013;15:113–22.
[125] Gkountela S, Zhang KX, Shafiq TA, et al. DNA demethylation dynamics in the human prenatal germline. Cell 2015;161:1425–36.
[126] Irie N, Weinberger L, Tang WW, et al. SOX17 is a critical specifier of human primordial germ cell fate. Cell 2015;160:253–68.
[127] Casati L, Sendra R, Sibilia V, et al. Endocrine disrupters: the new players able to affect the epigenome. Front. Cell Dev. Biol 2015;3:37.