Epigenetics in Development

5

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Contents

Abstract

Epigenetic processes regulate cellular function at all stages of life. Epigenetic processes in their entirety are referred to as the epigenome, which include DNA methylation, non-coding RNAs, histone modifications, chromatin structure and accessibility. This multifaceted epigenome is highly dynamic across human

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development, requiring almost complete reprogramming at two developmental timepoints: during the development of germ cells, and also immediately after fertilization. Epigenetic modifications are also highly stable, for example, genomic imprinting must be protected from post-fertilization epigenetic reprogramming, and X-chromosome inactivation in females is crucial to balance gene dosage from the X chromosome. In this chapter, we describe the major epigenetic processes that occur throughout human development, from the DNA methylation erasures that occur in germ cells, to the epigenetic characteristics of differentiated cells that arise from previous lineage-specification events. Increasingly, advancing technologies, such as organoid systems and single-cell sequencing, are allowing the epigenome in development to be characterized in an unprecedented amount of detail, which has led to key insights into the epigenetics of not only normal human development, but also the developmental origins of disease.

Abbreviations

5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
CGI	CpG island
CpG	Cytosine (phosphate) guanine site
DMR	Differentially methylated region
DNAm	DNA methylation
ERVs	Endogenous retroviral
EWAS	Epigenome-wide association study
gDMRs	germline differentially methylated regions
ICM	Inner cell mass
ICR	Imprinting control region
mQTL	methylated quantitative trait loci
PGC	Primordial germ cells
PMD	Partially methylated domain
TE	Trophectoderm
XCI	X-chromosome inactivation
ZGA	Zygotic genome activation

5.1 Introduction

In development, tissues and cell types acquire increasing specificity that allows them to fulfill their biological functions. Molecularly, much of this functional specificity is achieved through expression of highly cell-specific gene pathways, coordinated by a multifaceted epigenome. The epigenome here refers to several distinct molecular processes including DNA methylation (DNAm), histones and their post-translational

modifications, non-histone chromatin proteins, higher-order chromatin and chromosome organization, and non-coding RNAs. Establishment of these epigenetic features requires precise timing and specificity during the normal development of mammalian cells, throughout every generation. Epigenetic resetting rapidly occurs after fertilization, and then gradual acquisition of epigenetic features occurs in a cell-specific manner, resulting in the variety of cell types that fulfill various biological niches. Most of our knowledge of developmental epigenetics comes from mouse studies, due to the difficulties in collecting and studying human tissues early in development, and it is assumed that similar processes occur in humans. However, important studies verifying (or disputing) these processes in humans are noted. Developmentally significant epigenetic processes are described as "hard-wired" in that they occur without requiring extrinsic signals. However, an understanding of these pre-determined events is essential to also understanding how extrinsic signals, such as from environment, can alter the developmental trajectory of cells into disease-associated states. In this chapter, we provide a foundation for understanding the epigenome in development, and as a consequence, an understanding of the factors that can cause human disease.

5.2 The Epigenomes of Germ Cells

Germ cell formation involves a series of epigenetic changes including erasure of previous marks, suppression of many genes, activation of genes specific to gametogenesis, and changes in chromatin that facilitate appropriate chromosome interactions during the process of meiosis. As a consequence, genome-wide, sperm and eggs in mammals are highly epigenetically distinct compared to other differentiated somatic cells. Sperm and egg epigenomes are also highly distinct from each other, owing to differences in both the timing and pattern of the epigenomic marks that are established during spermatogenesis and oogenesis.

5.2.1 Primordial Germ Cells

Primordial germ cells (PGCs) are formed from the epiblast cell layer in the gastrulating embryo through a series of epigenetic remodeling events and expression of specific genes [1]. Chromatin-remodeling events occur as the PGCs differentiate from other epiblast cells, which include structural changes such as heterochromatin decondensation, loss of chromocenters, and increase in nuclear size [2]. Early changes in histone modifications include the loss of H3K9me2, a repressive mark, which is replaced by H3K27me3 from the core component of the polycomb repressive complex 2 [2–4]. Repressive methylation is also added onto histone tails of H2A and H4 by nuclear protein complexes, which include Blimp1 and Prtmt5 [5]. Chromatin remodeling continues as the PGCs migrate and take residence in the genital ridges.

Genome-wide erasure of DNAm in PGCs also begins during the migration to the genital ridge. DNA methylation is first lost by an active process where the actions of

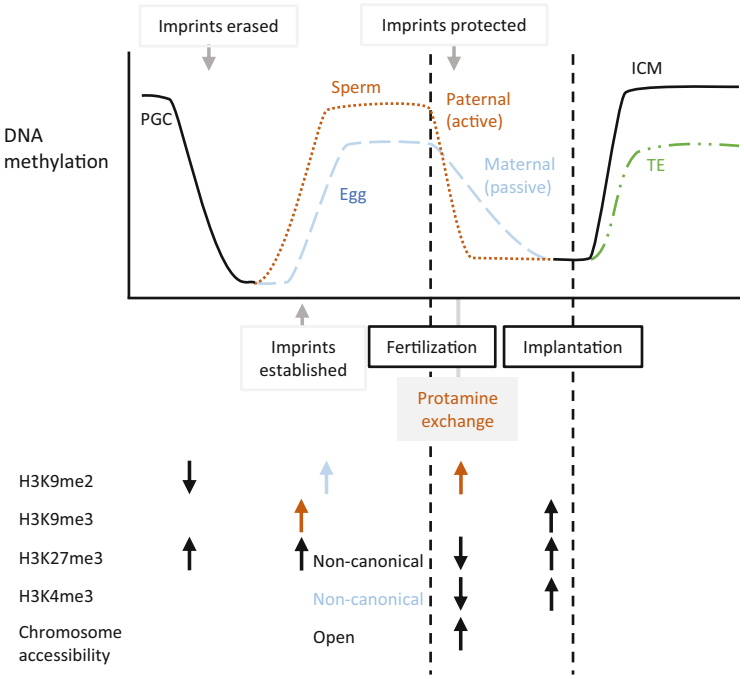

Fig. 5.1 Schematic of epigenetic reprogramming during development. The first major genome-wide DNA methylation erasure occurs in primordial germ cells (PGCs), and includes erasure of prior gametic imprints and replacement of H3K9me2 with H3K27me3. H3K27me3 undergoes further reconfiguration during migration to the genital ridge. H3K9me2 is reestablished in growing oocytes and H3K9me3 in growing spermatocytes. Concomitantly, DNA methylation is reestablished, but earlier and to a greater extent in sperm as compared to oocytes (egg). After fertilization, the asymmetric paternal and maternal epigenomes begin reprogramming. H3K4me3, which is distributed in a non-canonical pattern in the growing oocytes, is erased and a non-canonical distribution begins to be established. The paternal genome undergoes active demethylation, and protamines are replaced by histones. The maternal genome also undergoes demethylation, but more gradually through passive replication-dependent mechanisms. Gamete-specific differentially methylated regions (i.e., genomic imprints) and most repetitive sequences are protected during post-fertilization epigenetic reprogramming. At the blastocyst stage, there is the first lineage-specification event of inner cell mass (ICM) and trophectoderm (TE). By the blastocyst stage, most canonical patterns of histone modifications are established, such as bivalent promoters marked by H3K4me3/H3K27me3 at developmental genes. After implantation, DNA methylation is established in both ICM and TE, but in TE remains lower, a difference that is retained through development

TET enzymes convert 5'mC to 5'hmC, and then passively lost through successive replication events owing to the absence of normal DNAm maintenance activity (Fig. 5.1) [6, 7]. After migration to the genital ridge and completion of genome-wide demethylation, PGCs achieve a nearly completely demethylated genome [8, 9]. However, a subset of stable marks such as DNAm at imprinted loci and on the inactive X chromosome are removed only after migration to the genital ridge is complete, which may involve additional repression of *Dnmt3a*, *Dnmt3b*, and *Uhrgf1*

[9]. By the end of this process, genomic DNAm drops from around 71–80% to 7–14%, and PGCs enter either spermatogenesis or oogenesis depending on the sex of the embryo [9, 10].

5.2.2 Spermatogenesis

In male embryos, sperm progenitors (prospermatogonia) undergo alterations to DNAm starting before birth and continuing until completion of meiotic pachytene after puberty [11, 12]. Sperm DNA becomes highly remethylated owing largely to the activity of DNMT3A and DNMT3L [13]. DNMT3B is involved in methylating imprinted genes and repetitive sequences in sperm, but is inactive in oocytes [14, 15]. PIWI-interacting small RNAs are also responsible for establishing DNAm, which involve Dnmt3c, and H3K9me3 to suppress evolutionarily young copies of retrotransposons [16–18]. Another unique aspect of spermatogenesis is that initially, the sperm DNA is tightly wrapped around histones, but the majority are replaced first by non-canonical histone variants, then by transition proteins, and finally by protamines in the haploid stage after maturation, which allows DNA to be tightly packaged inside the sperm head [12, 19, 20]. However, approximately 1% of sperm histones are not replaced by protamines, and are instead retained in the mature sperm DNA specifically at gene promoters [21], suggesting a possible mechanism for transgenerational inheritance of paternal histone marks that can contribute to gene regulation in early development.

5.2.3 Oogenesis

In female embryos, after PGCs migrate to the genital ridge, there is short period of massive mitotic expansion, after which these oocyte precursors initiate meiosis, but then arrest at prophase 1 and remain dormant in the developing ovary until after puberty. Each month a small subset of this pool of oocytes will undergo a growth phase with only one fully maturing to ovulation. Over their development, oocytes acquire unique DNAm and histone modification profiles. In the late phase of oocyte growth, DNAm increases from 7–14% in PGCs up to 72% in mature oocytes, though remains less than the 86% in mature sperm (Table 5.1) [9, 10]. Unlike somatic cells or spermatocytes, de novo DNAm in oocytes is uncoupled from DNA replication and is acquired in a DNMT3A and DNMT3L dependent manner [22]. In contrast to sperm, where DNAm is more widespread and enriched at enhancers and repetitive short interspersed nuclear elements, DNAm in oocytes is uniquely distributed across actively transcribed gene bodies and CpG islands (CGI) [10], resulting in a oocyte-specific bimodal distribution of hypo- and hyper-methylated regions. Interestingly, DNMT1 and UHRF1, which are canonically responsible for the maintenance of DNAm, function in this context to complete *de-novo* methylation [15, 23]. Compared to other cell types, oocytes also show increased non-CpG methylation [15]. However, DNAm in oocytes is non-essential for the completion of oogenesis [24, 25]. Lower DNAm in oocytes may allow for subsets of transposable elements

Table 5.1 Major epigenetic differences between tissues and stages in development

	Gametes	Post-fertilization/ preimplantation	Somatic tissues (ICM-derived)	PLACENTAL TROPHOBLAST (TE-derived)
DNA methylation (DNAm)	Genome-wide erasure of DNAm, drops from 80% to 7–14% in PGCs	Loss of DNAm active (paternal) and passive (maternal)	Hypomethylation at CGI promoters of tissue-specific genes; Hypermethylation in gene bodies and intergenic regions	Lower relative genome-wide DNAm, owing to large PMDs in gene-poor regions
Partially methylated domains (PMDs)			Present in only a few tissues and with less genomic coverage relative to placenta; A key epigenomic feature when cells become malignant	Large regions that cover mostly intergenic regions; PMD-contained promoters are enriched for controlling placental genes; Placental-specific gene pathways co-opted in some cancers
Imprinting	Established in spermatogenesis and oogenesis	Imprints protected from DNA demethylation	Arises from a small number of gametic differences in DNAm that are resistant to reprogramming and act as imprinting control centers. Most imprinting is stable and pervasive across tissues; A small minority show tissue specificity in parent-of-origin expression	Most genes imprinted in somatic tissues are also imprinted in placenta. Some genes imprinted only in placenta show non-DNAm-dependent imprinting via maternal H3K27me3—Most of these are maternal and imprinting can be polymorphic
X-chromosome inactivation (XCI)	Erasure of inactive X-specific marks in female germ line	XIST and XACT expressed from both Xs in human female embryos initially. No initial imprinted XCI as there is in mouse	In females, the majority of CGI promoters are methylated on the inactive X and unmethylated on the active X. But chromosome-wide, the inactive X is less methylated	Less DNAm at CpG island promoters in female placentas, suggesting possible incomplete inactivation. Preferential paternal X inactivation in rodents, but not human

			than the active X. The inactive X also shows loss of promoter/enhancer histone acetylation; loss of promoter-associated H3K4me3 and H3K36me3; accumulation of H3K27me3, H4K20me1, H3K9me2, and macroH2A	
Histones	Low H3K9me2 and high H3K27me3 in PGCs; Sperm DNA packaged tightly around protamines; Oocytes have non-canonical distribution of H3K4me3	Paternal protamines are replaced by histones; Bivalent marks, marked by H3K27me3/H3K4me3, are established at promoters of developmental genes	Bivalent domains, marked by H3K4me3/H3K27me3, are prevalent in differentiation and cell type-specific	Histone deacetylation, increased H3K4me3, H3K9Ac, H3K27Ac, and H3K27me3 are associated with syncytialization. Less H3K27me3 in TE compared to ICM

DNAm, DNA methylation; PGC, primordial germ cell; XCI, X-chromosome inactivation; CGI, CpG Island; PMD, partially methylated domain; TE, trophectoderm; ICM, inner cell mass

to become active in late oocyte maturation, and serve as promoters or first exons for other genes, which can account for ~10% of oocyte-specific transcription [26].

Oocytes not only have a unique DNAm profile but also display non-canonical distribution of histone modifications. H3K4me3, which is associated with active promoters, is deposited across unusually broad intergenic domains, from the activity of the H3K4 methyltransferase, MLL2 [27–29]. H3K4me3 is required for genome-wide transcriptional silencing associated with oocyte maturation and for resumption of meiosis [27, 28, 30]. Although H3K4me3 is anti-correlated with DNAm in mouse oocytes, this inverse relation does not exist in humans [31]. Repressive H3K27me3 is found in large unmethylated regions with low transcriptional activity, but its functional role in oogenesis is unclear. However, a proportion of H3K27me3 in oocytes has been shown to regulate non-canonical DNAm-independent imprinting in the early embryo [32].

During maturation of the oocyte germinal vessicle, chromatin undergoes major conformational changes associated with transcriptional silencing. Upon resumption of meiosis, oocytes lose all higher-order chromatin structures, and interactions become uniform in strength across the chromosome [33, 34].

5.3 Post-fertilization

After fertilization, the paternal (sperm) and maternal (oocyte) genomes undergo extensive epigenetic reprogramming in order to equalize asymmetric epigenetic differences and prepare the totipotent zygote for cellular differentiation [35]. However, some genomic regions are resistant to this initial reprogramming, leading to the allele-specific expression of genes depending on parental origin, or genomic imprinting.

5.3.1 Post-fertilization Reprogramming of Gametic Genomes

Initially, the zygote is transcriptionally silent, relying on mostly maternally-inherited proteins and factors to carry out cellular processes during the first initial cell divisions. As maternally-inherited factors are degraded, the transcriptionally silent zygotic genome must become transcriptionally active, and this process, referred to as zygotic genome activation (ZGA), is associated with a variety of epigenetic changes in the paternal and maternal genomes [36]. In the paternal pronucleus, protamines are replaced with histones, and widespread erasure of DNAm is initiated before the first cell division through a rapid active demethylation process controlled by TET1. However, further demethylation occurs through replication-coupled passive dilution, which is aided by TET3 [8, 37]. TET3 converts 5'mC to 5'hmC which then impedes DNAm maintenance [38, 39], and TET3 activity itself can protect against DNMT3a-mediated de novo DNAm [40, 41]. Thus, TET proteins contribute to both active and passive modes of demethylation of the paternal genome after fertilization.

In contrast, the maternal pronucleus appears somewhat resistant to the initial TET demethylation steps, as evidenced by lower TET3 expression and lower TET3-

dependent 5'hmC [8]. The resistance of the maternal genome to this initial wave of DNA demethylation is due to H3K9me2-mediated recruitment of PGC7 (aka STELLA or DPPA3), which promotes maintenance of CpG DNAm and inhibits DNMT1-mediated de novo DNAm [42–45]. The maternal epigenome instead is demethylated by passive dilution through successive rounds of replication. By the blastocyst stage, about 20% of CpGs remain methylated, mostly at imprinted domains and repetitive elements [10, 22, 46].

Histone modifications are also dynamically reconfigured during preimplantation development. H3K4me3 is rapidly depleted after fertilization, but is then replaced by canonical H3K4me3 through H3K4 demethylases KDM5B and KDM1A [27, 28, 47]. In the zygote, there is also erasure of paternally-inherited H3K27me3, but maternally-inherited H3K27me3 at distal intergenic regions is maintained [48]. Despite promoters being devoid of H3K27me3, many still remain transcriptionally inactive, suggesting other silencing mechanisms are at play. H3K27me3 patterning is established by the blastocyst stage, where H3K27me3/H3K4me3 characterizes "bivalent" promoters of developmental genes, which are silent until rapid activation is needed for cell differentiation. Additionally, H3K27me3 maintains expression of some imprinted genes in early development until it is erased in the epiblast lineage at the blastocyst stage, but a subset of H3K27me3 imprinted genes are maintained in the extraembryonic lineage [32]. H3K9 di- and tri-methylation, which are associated to contribute to the silencing and protection of paternal DNAm at imprinted domains, are acquired by the 8-cell stage [43, 49].

From the zygote to blastocyst stages, higher-order chromatin structure such as long-range and local chromosome interactions are gradually established, including more enhancer–promoter interactions, increased number of DNase hypersensitivity sites, and increased chromatin accessibility [33, 34, 50].

5.3.2 Genomic Imprinting

Genomic imprinting is associated with human developmental disorders such as Prader–Willi Syndrome [51], Angelman Syndrome [52], and Beckwith–Wiedemann Syndrome [53, 54]. Many of the known imprinted genes are regulated by germline differentially methylated regions (gDMRs), where only one parental allele is methylated. As a consequence of differences in epigenetic reprogramming between oogenesis and spermatogenesis, over 1600 CGIs in mouse are gDMRs. Most of these gDMRs are subsequently lost either through removal of DNAm during preimplantation development [22, 55], or through acquisition of de novo DNAm on the alternate allele after implantation [56]. However, some gDMRs are resistant to early epigenetic reprogramming and stable throughout the lifespan, often acting as imprinting control regions (ICRs) that regulate the expression of nearby gene clusters. Mutations within ICRs can lead to loss of the ability to reprogram the appropriate parent-of-origin imprints and can result in unusual inheritance patterns in families. For example, mutations in the ICR regulating the *SNRPN* gene, implicated in Prader–Willi syndrome, can be passed from mother to child with no direct effect in the first generation. However, when a male inherits an ICR mutation from his mother, the maternal imprint

cannot be changed to a paternal imprint in his sperm, thus giving him a 50% risk of having a child affected by Prader–Willi syndrome [57].

In addition to canonical (DNAm-dependent) imprinting, there are a subset of non-canonical imprinted genes for which DNAm is absent, but instead imprinted gene expression is dependent on maternal H3K27me3 [32]. These sites of non-canonical imprinting may also lead to acquired DNAm differences (secondary imprinted DMRs) that are not derived from gDMRs. It is possible that even short-lived imprinting due to H3K27me3 on the maternal genome, leading to paternal-biased gene expression in human preimplantation embryos, could have lasting effects on embryonic development [58, 59]. Further, while the majority of maternal H3K27me3 is lost in embryonic lineages, it appears to be lost more gradually in the placenta and thus allelic differences in gene expression may play a greater role in placental function [12].

In mouse, the greatest numbers of imprinted loci were observed in early embryos, placenta, and brain [60]. A higher rate of imprinted genes in human placenta as compared to somatic tissues is also supported by the large number of placental-specific imprinted DMRs [61]. Placental-specific imprinted DMRs can arise from gDMRs (e.g., imprinted genes *Kcnq1* and *Igf2r*) or through acquisition of secondary DMRs via non-canonical imprinting (e.g., Gab1 and Sfmbt2), with loss of imprinting in the epiblast due to acquisition of DNAm, leading to bi-allelic silencing of the corresponding gene [62]. Interestingly, non-canonical imprinting was localized to endogenous retrovirus-K (ERVK) long terminal repeats, which can act as promoters in the placenta [63, 64]. It is possible that active ERVs in the placenta may interfere with epigenetic silencing in the placental lineages [62]. Placental-specific imprinting appears to be largely species specific and may have no clear function, but some knock-outs of placental-specific imprinted genes in mouse can lead to placental dysfunction [62].

5.4 Post-implantation

The first lineage-specification event occurs with the differentiation of the trophectoderm (TE) and inner cell mass (ICM) at the blastocyst stage of development. Trophoblast cells, which form both the outer layer of the placental chorionic villi, and the placental cells that invade into the maternal endometrium, are derived from the trophectoderm (TE). The remaining extraembryonic tissues (amnion, placental mesenchyme, and the inner layer of the chorion) derive from the ICM, as do all fetal tissues. At this critical developmental timepoint, there are already extensive genome-wide differences in epigenetic marks between ICM and TE that are essential for regulating the myriad of developmental events that follows.

5.4.1 Reprogramming of the Blastocyst

The blastocyst has the lowest levels of genome-wide DNAm compared to any other developmental stage, as a result of the genome-wide demethylation that occurs in the first few cell divisions after fertilization. At the blastocyst stage, DNAm begins to be

rapidly deposited across all ICM- and TE-derived tissues by DNMT3A and DNMT3B, which preferentially target broadly distributed H3K36me2/3-marked regions [9, 65, 66]. Although DNMT3A and -3B generally do not discriminate between genomic regions, the majority of CGI promoters remain unmethylated to avoid ectopic silencing of important genes. H3K4m3, which is associated with active promoters, protects promoter sequences from de novo DNAm by repelling the ADD domains of DNMT3A and -3B [67]. Developmental gene promoters, which are normally silent but must activate under specific developmental cues, are bivalently marked by PRC2-deposited H3K27me3 and H3K4me2 [68], and are devoid of CGI DNAm owing to TET1 activity [69]. Transcription factors, such as DPPA2, DPPA4, and others, are responsible for the targeted deposition of H3K4me3 and H3K27me3 at CGI promoters of bivalent genes in development [70–72].

5.4.2 Placental Trophoblasts

The embryo develops from the ICM but its growth also depends on the normal development of the placenta, which consists mostly of trophoblasts derived from TE. Due to early divergence of ICM- and TE-derived cell types, the resulting differentiated cell types are each epigenetically unique (Table 5.1). TE is resistant to the dramatic gain of methylation that ICM experiences starting at the blastocyst stage, resulting in a characteristic genome-wide hypomethylated profile of placental trophoblasts that make up the bulk of placenta [73, 74]. Fully developed somatic tissues have a bimodal distribution of genome-wide DNAm, where CGIs are mainly hypomethylated, and the rest of the genome is densely methylated. While unmethylated promoters are conserved in placenta, intergenic regions have lower DNAm compared to somatic tissues, and this lower methylation is organized into large partially methylated domains (PMDs) that are maintained throughout gestation [74, 75]. However, most CpG methylation increases in trophoblast over gestation, even in PMD regions [75, 76]. It is unclear if PMDs regulate transcription directly or are left from earlier transcription regulatory processes. However, even though these regions are relatively gene-poor overall, there is an enrichment for CGI promotors for genes involved in pathways related to the epithelial–mesenchymal transition, immune response, and inflammation [77]. Placental-specific genes and related pathways share epigenetic regulation that is similar to the epigenetic program in some cancers, where trophoblast-specific invasive and immune response pathways are co-opted [77, 78]. A proportion of placental hypomethylation is localized to various repetitive elements, such as LINE-1 [79] and human ERVs [80], which may regulate placental-specific functions [81]. Some repetitive elements serve as alternative promoters for trophoblast-related genes, such as for *KCNH5* and *IL2RB* [80, 82, 83]. Despite observations of PMD- and retrotransposon-driven gene expression in placenta, their contribution to placental function is relatively uncharacterized.

Histone modifications and chromatin dynamics are also under-characterized in placenta. Syncytiotrophoblasts, which line the outer surface of the placental chorionic villi are directly exposed to the maternal environment and govern molecular exchange, express hormones, and produce extracellular vesicles that are critical to

regulating interactions between mother and fetus. They are multi-nucleated, created by the fusion of underlying layer of cytotrophoblasts, and their DNA is heterogeneous; older nuclei are condensed into transcriptionally repressed heterochromatin-rich syncytial knots, whereas younger nuclei are euchromatic and transcriptionally active. Recent high-resolution studies indicate histone modifications are important to trophoblast differentiation. Differentiation of syncytiotrophoblast is associated with deacetylation of histone lysine residues, such as depletion of H3K27Ac and H3K9Ac at promoters by histone deacetylases HDAC1 and HDAC2 [84, 85]. Impairment of histone acetylation may be a feature of disease processes in placenta, as aberrant H3K27ac in placenta is associated with maternal preeclampsia and may be a more general feature of placental inflammation [76]. Other histone modifications, such as H3K27me3, H3K4me3, and H3K9me3, are dynamic in cytotrophoblast populations across gestation, but their functional impact on placental function is unclear [76]. H3K9me3, however, is associated with silencing of cytotrophoblast genes, and is correlated with placental DNA hypomethylation [76]. H3K27me3 might be important in establishing a trophoblast-specific transcription program in early placental development, as H3K27me3 has been shown to be uniquely distributed in trophoblast stem cells compared to other embryonic stem cells [86].

The dynamics of epigenetic regulation in the placenta throughout pregnancy is highly specific, especially in comparison to other cell types and tissues, and research in this exciting area is still in its infancy. However, growing interest in the early epigenetic regulation in the placenta promises that future research will lead to exciting new insights into human development.

5.4.3 X-Chromosome Inactivation

As female 46,XX embryos have two X chromosomes compared to one in 46,XY males, there is initially a sex difference in the expression of X-chromosome genes. As a mechanism for dosage compensation in female embryos, most genes on one of the two X chromosomes are transcriptionally silenced. X-chromosome inactivation (XCI) has been well studied and is a useful paradigm for understanding how monoallelic epigenetic silencing can occur during development. A critical trigger for XCI is the expression of XIST RNA, a 15–17 kb long non-coding RNA that accumulates along one X chromosome in *cis* and is required for the initiation and stable maintenance of XCI throughout development [87]. While XCI has been most well studied in mouse, there are some key differences with humans. For example, in mouse Xist expression is initiated at the 4–8 cell stage when only the paternal X is inactivated, followed by later reactivation of the paternal X and random XCI in the epiblast. In human embryos, XCI is not initiated until implantation or after, and there is no parental bias in XCI [88]. As human XCI is only completed after implantation and early tissue differentiation, there is a dosage imbalance of most X-linked genes up to this point [89]. Furthermore, as cells can differ by which parental X chromosome is inactivated in females, different cells can express different genetic variants. This not only has consequences for the presentation of genetic disorders but presents challenges to epigenome-wide association studies. Unfortunately, the

X-chromosome data is often discarded from epigenome-wide association studies (EWASs) as a consequence.

In human preimplantation embryos, XIST is expressed from both X's in females, as well as from the single X in males [89]. XACT, a primate-specific X-linked lncRNA, which eventually coats the active X chromosome, is also expressed biallelically at this time [90]. The mechanism by which XIST expression is subsequently dampened to keep only one X active is unclear, although it is hypothesized that XACT may antagonize XIST activity, and XIST may be able to trigger XCI on one randomly chosen X once XACT expression is lost [91]. It is also hypothesized that there is a dosage-sensitive repressor of XIST, encoded by an autosomal gene, that acts prior to the initiation of XCI [88]. In mouse, the RNA binding molecule SPEN has been shown to bind to Xist RNA, and once recruited to the X chromosome, targets active enhancers and promoters and induces repressive chromatin changes that shut down transcription [92]. Regardless of the mechanism, a series of sequential chromatin alterations are established as Xist RNA spreads along the X [93]. First, most euchromatic histone marks are lost, such as promoter/enhancer histone acetylation, and promoter-associated H3K4me3 and H3K36me3. Heterochromatic marks including H3K27me3, H2AK119Ub, and H4K20me1 accumulate later, and there is also accumulation of H3K9me2, and macroH2A. Lastly, CGI promoter methylation secures the stable nature of chromatin compaction characteristic of the inactive. Interestingly, even though H4K20me1 and H3K27me3 are early marks associated with the inactive X chromosome, they are not necessary for silencing and may accumulate as secondary effects of the inactive heterochromatin [94]. These epigenetic changes on the inactive X chromosome result in condensation of the inactive X into a transcriptionally silent structure called a Barr body that localizes to the nuclear periphery.

In addition to higher expression of X-linked genes in females prior to XCI, some genes escape from XCI altogether, and may show persistent higher expression in females compared to males. Roughly 15% of genes in human somatic tissues escape XCI, defined as having expression from the inactive X that is at least 10% of the active X [95]. Genes that escape XCI across different species tend to be enriched for CTCF-binding sites, ATAC-sequencing signal (indicative of open chromatin) and LTR repeats, compared to genes that are subject to silencing by XCI [96]. Thus, some sex differences may be due to persistent gene expression differences on the X, as well as to secondary effects on autosomal gene expression [97]. In addition, some genes show variable XCI, being variably inactivated in some cells, tissues, and individuals. Comparisons across tissues within individuals and in twin pairs show high concordance, indicating that a large portion of variability in XCI escape may be due to *cis*-acting genetic variation [95, 98].

The process of XCI in the placenta is less well studied and associated with distinct properties. In mouse, there is preferential inactivation of the paternal X, while in human placenta, the process is random but occurs in a patchy fashion across the placenta due to its clonal development [99, 100]. In comparison to males, placental DNAm on the X chromosome in females is greater at gene promoters and lower in gene bodies than expected for X-chromosome inactivation [101]. However, in

comparison to blood there is substantial hypomethylation of X-linked promoters in the female placenta, suggesting that there might be more escape from XCI in the placenta [79]. If this is true, then sex differences in fetal development may be driven in part by sex differences in placental function.

5.5 Factors That Contribute to Variability in Development

Epigenetic mechanisms ensure cellular identities are maintained throughout not only development but the entirety of human life, resulting in wide-ranging types of cellular function that fulfill highly tissue-specific niches. At the same time, extrinsic factors such as environment and aging can perturb the epigenetic program, increasing risk of aberrations that can result in disease. Intrinsic genetic variation is another factor that is increasingly becoming appreciated as a major factor contributing to epigenetic variation, highlighting the importance of considering human diversity.

5.5.1 Cell-Specific Epigenetics

Fundamentally, epigenetic marks are a cellular process. Despite this, most epigenetic studies rely on whole tissue samples, which are mixtures of distinct cell populations. Because every distinct cell population also has a distinct epigenetic profile, measurements of whole tissues are an average epigenetic profile of the cellular soup that consists of each sample. This results in challenges in interpreting epigenetic changes associated with disease, environment, or development; as observed epigenetic variation can be attributed to either changes that are present in a subset of cells, in all or most cells, or are simply reflecting cell composition variability without any changes in the epigenetic footprint [102]. For example, placental epigenetics, including canonical features like PMDs and placental-specific imprinting, are often features of trophoblast cells, rather than all component cell populations of the placenta [75, 76]. Fortunately, these challenges are surmountable. At the experimental design stage, cell populations most relevant to the research question should be as homogeneous as possible by using cell-specific isolation protocols. When this is not possible, cell composition can be estimated from whole tissue samples, which is commonly done in DNAm studies [103]. Single-cell technologies can address cell-specificity challenges, and have been useful in characterizing early developmental timepoints [104], but current costs and sample preparation requirements remain as major hurdles to employing these technologies at scale on a population level resolution to study disease and environment-related processes.

5.5.2 Genetic Influences on Epigenetic Variation

Genetic variation at the single nucleotide level is also a major factor contributing to epigenetic variation, especially in contexts where multiple human populations are

included. Methylation quantitative trait loci (mQTLs) are where a SNP affects the DNAm of a nearby CpG, which can account for up to 75% of the variance in DNAm [105, 106]. However, it is important to note that joint SNP plus environmental variation are larger contributors to DNAm variation than SNP variation alone [107]. The contribution of genetic and environmental factors can vary depending on tissue and cell type [108], and different human populations [109, 110]. The contribution of genotype-specific DNAm in human development is relatively uncharacterized. Even though the majority of mQTLs in adult brain are also found in fetal brain, a subset are fetal-specific mQTLs and enriched for variants associated with schizophrenia [111] and autism spectrum disorder [112]. Placental-specific mQTLs have also been identified, many of which are associated with expression changes, and a subset overlap known loci linked to fetal growth, diabetes, and inflammation [113]. In a study including blood samples from 4 timepoints, ranging from birth (cord blood) and adulthood (mean age = 47.5), a majority (>84%) of mQTLs were replicated across at all timepoints, suggesting that genetic effects are largely stable across life [114]. These studies highlight the importance of considering the contribution of human diversity and genetic influences on epigenetic processes in human development.

5.6 Transgenerational Epigenetics

It has been demonstrated in many organisms that a subset of environmentally induced epigenetic changes may be transmitted through the germline over multiple generations [115]. For example, exposures to endocrine disrupting chemicals, altered nutrition (e.g., high fat or low calorie), and stress have all been associated with transgenerational epigenetic alterations in rodent models [116]. In mammals, there is more evidence for male transgenerational epigenetics, as it is difficult to separate the in utero effects of maternal or grandmaternal exposures from true transgenerational inheritance in females [115, 116]. Epigenetic inheritance may potentially involve DNAm, histone modifications, short or long non-coding RNAs, and structural chromatin proteins [117, 118]. Although germline remodeling in spermatogenesis involves DNAm erasure and replacement of most histones with protamines, a small number of histones still remain, particularly in active CpG-rich gene regulatory regions [119, 120]. In a transgenic mouse model with overexpression of the histone demethylase KDM1A leading to depletion of H3K4me2, there was enrichment for H3K4me3 that escaped reprogramming in the embryo, supporting that some histone marks can serve as a potential mechanism for transgenerational inheritance [121]. Other studies have supported a role for small non-coding RNAs in transgenerational epigenetic inheritance. For example, male mice subjected to early life stress showed altered stress response pathways in their offspring, and by isolating microRNAs from the sperm of the stress-exposed males and injecting them into zygotes, similar behavioral effects were reproduced in offspring from control (non-stressed) parents [122, 123]. In another model whereby rats were transiently exposed to agricultural fungicide vinclozolin or pesticide DDT,

it was observed that changes in DNAm, histone localization, and non-coding RNAs colocalized to the same chromosomal regions, implying integrated effects that are mediated by non-coding RNA directed DNAm and DNAm-directed histone retention [124].

The possibility of epigenetic inheritance in humans is suggested by the Dutch famine, whereby the offspring of males, but not of females, prenatally exposed to caloric restriction showed increased adiposity [125]. Furthermore, small non-coding RNA expression and DNAm differed in the sperm from obese as compared to lean men [126]. Altered microRNA profiles were also reported in the sperm of men who smoked [127], while altered DNAm was reported in the sperm of infertile men taking high dose folate supplements [128]. However, direct evidence for epigenetic inheritance in humans is scarce, and in many cases where abnormal DNAm appeared to be transmitted in families, were actually due to genetic mutations that influence DNAm (e.g., imprinting mutations) [117, 129]. Likely, the same mechanisms shown in other mammals occur also in humans, but their significance in the context of the extensive genetic, environmental, and cultural variation in humans remains to be determined.

5.7 Conclusion

In conclusion, an important area of human epigenetics is understanding the epigenetic processes that occur during early development and gametogenesis. Histone modifications, chromosome structure, DNAm, and non-coding RNAs are highly dynamic in development, and are essential in setting up the epigenetic profiles that regulate gene expression in differentiated cells in later life. Recent and ongoing advancements in areas such as single-cell and low-input sequencing technologies are rapidly enabling characterization of the multifaceted epigenome at critical developmental timepoints. An important area for future research will be to not only continue measuring these epigenetic marks with advancing technologies, but also to understand the functional relevance of various epigenetic processes, how they contribute to development itself, and what factors disturb these processes that results in increased risk of disease in normal development. Recently developed ex vivo organoid technologies are promising, as more mechanistic studies and particularly human ones are needed. Although major epigenetic processes are often conserved across mammals, there is often evolutionary divergence in the timing and function of the proteins involved. Population studies measuring epigenetic marks in early life tissues, will also be important to understand how variability in normal development occurs under the context of genetic and environmental-related variation. Technology advancements and growing interest promise that the dynamics of the epigenome in development will be characterized at an unprecedented level of detail than ever before. This will lead to many exciting new insights that will aid our understanding of how genetic and environmental factors interact with the epigenome to create the vast diversity of human phenotypes.

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References

1. Surani MA, Hayashi K, Hajkova P (2007) Genetic and epigenetic regulators of pluripotency. Cell 128:747–762. https://doi.org/10.1016/j.cell.2007.02.010
2. Hajkova P, Ancelin K, Waldmann T, Lacoste N, Lange UC, Cesari F, Lee C, Almouzni G, Schneider R, Surani MA (2008) Chromatin dynamics during epigenetic reprogramming in the mouse germ line. Nature 452:877–881. https://doi.org/10.1038/nature06714
3. Seki Y, Yamaji M, Yabuta Y, Sano M, Shigeta M, Matsui Y, Saga Y, Tachibana M, Shinkai Y, Saitou M (2007) Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development 134:2627–2638. https://doi.org/10.1242/dev.005611
4. Seki Y, Hayashi K, Itoh K, Mizugaki M, Saitou M, Matsui Y (2005) Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev Biol 278:440–458. https://doi.org/10.1016/j.ydbio.2004.11.025
5. Ancelin K, Lange UC, Hajkova P, Schneider R, Bannister AJ, Kouzarides T, Surani MA (2006) Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. Nat Cell Biol 8:623–630. https://doi.org/10.1038/ncb1413
6. Hackett JA, Sengupta R, Zylicz JJ, Murakami K, Lee C, Down TA, Surani MA (2013) Germline DNA demethylation dynamics and imprint erasure through 5-Hydroxymethylcytosine. Science 339:448–452. https://doi.org/10.1126/science.1229277
7. Hargan-Calvopina J, Taylor S, Cook H, Hu Z, Lee SA, Yen M-R, Chiang Y-S, Chen P-Y, Clark AT (2016) Stage-specific demethylation in primordial germ cells safeguards against precocious differentiation. Dev Cell 39:75–86. https://doi.org/10.1016/j.devcel.2016.07.019
8. Gu T-P, Guo F, Yang H, Wu H-P, Xu G-F, Liu W, Xie Z-G, Shi L, He X, Jin S, Iqbal K, Shi YG, Deng Z, Szabó PE, Pfeifer GP, Li J, Xu G-L (2011) The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature 477:606–610. https://doi.org/10.1038/nature10443
9. Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, Popp C, Thienpont B, Dean W, Reik W (2012) The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. Mol Cell 48:849–862. https://doi.org/10.1016/j.molcel.2012.11.001
10. Zhu P, Guo H, Ren Y, Hou Y, Dong J, Li R, Lian Y, Fan X, Hu B, Gao Y, Wang X, Wei Y, Liu P, Yan J, Ren X, Yuan P, Yuan Y, Yan Z, Wen L, Yan L, Qiao J, Tang F (2018) Single-cell DNA methylome sequencing of human preimplantation embryos. Nat Genet 50:12–19. https://doi.org/10.1038/s41588-017-0007-6
11. Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA (2002) Epigenetic reprogramming in mouse primordial germ cells. Mech Dev 117:15–23. https://doi.org/10.1016/S0925-4773(02)00181-8
12. Hanna CW, Demond H, Kelsey G (2018) Epigenetic regulation in development: is the mouse a good model for the human? Hum Reprod Update 24:556–576. https://doi.org/10.1093/humupd/dmy021
13. Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for De novo methylation and mammalian development. Cell 99:247–257. https://doi.org/10.1016/S0092-8674(00)81656-6
14. Kato Y, Kaneda M, Hata K, Kumaki K, Hisano M, Kohara Y, Okano M, Li E, Nozaki M, Sasaki H (2007) Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. Hum Mol Genet 16:2272–2280. https://doi.org/10.1093/hmg/ddm179

15. Shirane K, Toh H, Kobayashi H, Miura F, Chiba H, Ito T, Kono T, Sasaki H (2013) Mouse oocyte Methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. PLoS Genet 9:e1003439. https://doi.org/10.1371/journal.pgen.1003439
16. Barau J, Teissandier A, Zamudio N, Roy S, Nalesso V, Hérault Y, Guillou F, Bourc'his D (2016) The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. Science 354:909–912. https://doi.org/10.1126/science.aah5143
17. Pezic D, Manakov SA, Sachidanandam R, Aravin AA (2014) piRNA pathway targets active LINE1 elements to establish the repressive H3K9me3 mark in germ cells. Genes Dev 28: 1410–1428. https://doi.org/10.1101/gad.240895.114
18. Zamudio N, Barau J, Teissandier A, Walter M, Borsos M, Servant N, Bourc'his D (2015) DNA methylation restrains transposons from adopting a chromatin signature permissive for meiotic recombination. Genes Dev 29:1256–1270. https://doi.org/10.1101/gad.257840.114
19. Balhorn R, Brewer L, Corzett M (2000) DNA condensation by protamine and arginine-rich peptides: analysis of toroid stability using single DNA molecules. Mol Reprod Dev 56:230–234. https://doi.org/10.1002/(SICI)1098-2795(200006)56:2+<230::AID-MRD3>3.0.CO;2-V
20. Bao J, Bedford MT (2016) Epigenetic regulation of the histone-to-protamine transition during spermiogenesis. Reprod Camb Engl 151:R55–R70. https://doi.org/10.1530/REP-15-0562
21. Yoshida K, Muratani M, Araki H, Miura F, Suzuki T, Dohmae N, Katou Y, Shirahige K, Ito T, Ishii S (2018) Mapping of histone-binding sites in histone replacement-completed spermatozoa. Nat Commun 9:3885. https://doi.org/10.1038/s41467-018-06243-9
22. Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G (2011) Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet 43:811–814. https://doi.org/10.1038/ng.864
23. Maenohara S, Unoki M, Toh H, Ohishi H, Sharif J, Koseki H, Sasaki H (2017) Role of UHRF1 in de novo DNA methylation in oocytes and maintenance methylation in preimplantation embryos. PLoS Genet 13:e1007042. https://doi.org/10.1371/journal.pgen.1007042
24. Bourc'his D, Xu G-L, Lin C-S, Bollman B, Bestor TH (2001) Dnmt3L and the establishment of maternal genomic imprints. Science 294:2536–2539. https://doi.org/10.1126/science.1065848
25. Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H (2004) Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. Nature 429:900–903. https://doi.org/10.1038/nature02633
26. Franke V, Ganesh S, Karlic R, Malik R, Pasulka J, Horvat F, Kuzman M, Fulka H, Cernohorska M, Urbanova J, Svobodova E, Ma J, Suzuki Y, Aoki F, Schultz RM, Vlahovicek K, Svoboda P (2017) Long terminal repeats power evolution of genes and gene expression programs in mammalian oocytes and zygotes. Genome Res 27:1384–1394. https://doi.org/10.1101/gr.216150.116
27. Dahl JA, Jung I, Aanes H, Greggains GD, Manaf A, Lerdrup M, Li G, Kuan S, Li B, Lee AY, Preissl S, Jermstad I, Haugen MH, Suganthan R, Bjørås M, Hansen K, Dalen KT, Fedorcsak P, Ren B, Klungland A (2016) Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. Nature 537:548–552. https://doi.org/10.1038/nature19360
28. Zhang B, Zheng H, Huang B, Li W, Xiang Y, Peng X, Ming J, Wu X, Zhang Y, Xu Q, Liu W, Kou X, Zhao Y, He W, Li C, Chen B, Li Y, Wang Q, Ma J, Yin Q, Kee K, Meng A, Gao S, Xu F, Na J, Xie W (2016) Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. Nature 537:553–557. https://doi.org/10.1038/nature19361
29. Hanna CW, Taudt A, Huang J, Gahurova L, Kranz A, Andrews S, Dean W, Stewart AF, Colomé-Tatché M, Kelsey G (2018) MLL2 conveys transcription-independent H3K4 trimethylation in oocytes. Nat Struct Mol Biol 25:73–82. https://doi.org/10.1038/s41594-017-0013-5

30. Andreu-Vieyra CV, Chen R, Agno JE, Glaser S, Anastassiadis K, Stewart AF, Matzuk MM (2010) MLL2 is required in oocytes for bulk histone 3 lysine 4 Trimethylation and transcriptional silencing. PLoS Biol 8:e1000453. https://doi.org/10.1371/journal.pbio.1000453
31. Xia W, Xu J, Yu G, Yao G, Xu K, Ma X, Zhang N, Liu B, Li T, Lin Z, Chen X, Li L, Wang Q, Shi D, Shi S, Zhang Y, Song W, Jin H, Hu L, Bu Z, Wang Y, Na J, Xie W, Sun Y-P (2019) Resetting histone modifications during human parental-to-zygotic transition. Science 365: 353–360. https://doi.org/10.1126/science.aaw5118
32. Inoue A, Jiang L, Lu F, Suzuki T, Zhang Y (2017) Maternal H3K27me3 controls DNA methylation-independent imprinting. Nature 547:419–424. https://doi.org/10.1038/nature23262
33. Ke Y, Xu Y, Chen X, Feng S, Liu Z, Sun Y, Yao X, Li F, Zhu W, Gao L, Chen H, Du Z, Xie W, Xu X, Huang X, Liu J (2017) 3D chromatin structures of mature gametes and structural reprogramming during mammalian embryogenesis. Cell 170:367–381.e20. https://doi.org/10.1016/j.cell.2017.06.029
34. Du Z, Zheng H, Huang B, Ma R, Wu J, Zhang X, He J, Xiang Y, Wang Q, Li Y, Ma J, Zhang X, Zhang K, Wang Y, Zhang MQ, Gao J, Dixon JR, Wang X, Zeng J, Xie W (2017) Allelic reprogramming of 3D chromatin architecture during early mammalian development. Nature 547:232–235. https://doi.org/10.1038/nature23263
35. Xu R, Li C, Liu X, Gao S (2021) Insights into epigenetic patterns in mammalian early embryos. Protein Cell 12:7–28. https://doi.org/10.1007/s13238-020-00757-z
36. Schulz KN, Harrison MM (2019) Mechanisms regulating zygotic genome activation. Nat Rev Genet 20:221–234. https://doi.org/10.1038/s41576-018-0087-x
37. Inoue A, Zhang Y (2011) Replication-dependent loss of 5-Hydroxymethylcytosine in mouse preimplantation embryos. Science 334:194–194. https://doi.org/10.1126/science.1212483
38. Hashimoto H, Liu Y, Upadhyay AK, Chang Y, Howerton SB, Vertino PM, Zhang X, Cheng X (2012) Recognition and potential mechanisms for replication and erasure of cytosine hydroxymethylation. Nucleic Acids Res 40:4841–4849. https://doi.org/10.1093/nar/gks155
39. Otani J, Kimura H, Sharif J, Endo TA, Mishima Y, Kawakami T, Koseki H, Shirakawa M, Suetake I, Tajima S (2013) Cell cycle-dependent turnover of 5-Hydroxymethyl cytosine in mouse embryonic stem cells. PLoS One 8:e82961. https://doi.org/10.1371/journal.pone.0082961
40. Amouroux R, Nashun B, Shirane K, Nakagawa S, Hill PWS, D'Souza Z, Nakayama M, Matsuda M, Turp A, Ndjetehe E, Encheva V, Kudo NR, Koseki H, Sasaki H, Hajkova P (2016) De novo DNA methylation drives 5hmC accumulation in mouse zygotes. Nat Cell Biol 18:225–233. https://doi.org/10.1038/ncb3296
41. Richard Albert J, Au Yeung WK, Toriyama K, Kobayashi H, Hirasawa R, Brind'Amour J, Bogutz A, Sasaki H, Lorincz M (2020) Maternal DNMT3A-dependent de novo methylation of the paternal genome inhibits gene expression in the early embryo. Nat Commun 11:5417. https://doi.org/10.1038/s41467-020-19279-7
42. Li Y, Zhang Z, Chen J, Liu W, Lai W, Liu B, Li X, Liu L, Xu S, Dong Q, Wang M, Duan X, Tan J, Zheng Y, Zhang P, Fan G, Wong J, Xu G-L, Wang Z, Wang H, Gao S, Zhu B (2018) Stella safeguards the oocyte methylome by preventing de novo methylation mediated by DNMT1. Nature 564:136–140. https://doi.org/10.1038/s41586-018-0751-5
43. Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, Sekimoto T, Ikawa M, Yoneda Y, Okabe M, Tanaka S, Shiota K, Nakano T (2007) PGC7/Stella protects against DNA demethylation in early embryogenesis. Nat Cell Biol 9:64–71. https://doi.org/10.1038/ncb1519
44. Nakamura T, Liu Y-J, Nakashima H, Umehara H, Inoue K, Matoba S, Tachibana M, Ogura A, Shinkai Y, Nakano T (2012) PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. Nature 486:415–419. https://doi.org/10.1038/nature11093
45. Zeng T-B, Han L, Pierce N, Pfeifer GP, Szabó PE (2019) EHMT2 and SETDB1 protect the maternal pronucleus from 5mC oxidation. Proc Natl Acad Sci U S A 116:10834–10841. https://doi.org/10.1073/pnas.1819946116

46. Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, Yang L, Zhang J, Li G, Ci W, Li W, Zhou Q, Aluru N, Tang F, He C, Huang X, Liu J (2014) Programming and inheritance of parental DNA Methylomes in mammals. Cell 157:979–991. https://doi.org/10.1016/j.cell.2014.04.017
47. Ancelin K, Syx L, Borensztein M, Ranisavljevic N, Vassilev I, Briseño-Roa L, Liu T, Metzger E, Servant N, Barillot E, Chen C-J, Schüle R, Heard E (2016) Maternal LSD1/KDM1A is an essential regulator of chromatin and transcription landscapes during zygotic genome activation. eLife 5:e08851. https://doi.org/10.7554/eLife.08851
48. Zheng H, Huang B, Zhang B, Xiang Y, Du Z, Xu Q, Li Y, Wang Q, Ma J, Peng X, Xu F, Xie W (2016) Resetting epigenetic memory by reprogramming of histone modifications in mammals. Mol Cell 63:1066–1079. https://doi.org/10.1016/j.molcel.2016.08.032
49. Quenneville S, Verde G, Corsinotti A, Kapopoulou A, Jakobsson J, Offner S, Baglivo I, Pedone PV, Grimaldi G, Riccio A, Trono D (2011) In embryonic stem cells, ZFP57/KAP1 recognize a methylated Hexanucleotide to affect chromatin and DNA methylation of imprinting control regions. Mol Cell 44:361–372. https://doi.org/10.1016/j.molcel.2011.08.032
50. Lu F, Liu Y, Inoue A, Suzuki T, Zhao K, Zhang Y (2016) Establishing chromatin regulatory landscape during mouse preimplantation development. Cell 165:1375–1388. https://doi.org/10.1016/j.cell.2016.05.050
51. Nicholls RD, Knoll JHM, Butler MG, Karam S, Lalande M (1989) Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. Nature 342:281–285. https://doi.org/10.1038/342281a0
52. Knoll JH, Nicholls RD, Magenis RE, Graham JM, Lalande M, Latt SA (1989) Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 32:285–290. https://doi.org/10.1002/ajmg.1320320235
53. Junien C (1992) Beckwith-Wiedemann syndrome, tumourigenesis and imprinting. Curr Opin Genet Dev 2:431–438. https://doi.org/10.1016/S0959-437X(05)80154-6
54. Ohlsson R, Nyström A, Pfeifer-Ohlsson S, Töhönen V, Hedborg F, Schofield P, Flam F, Ekström TJ (1993) IGF2 is parentally imprinted during human embryogenesis and in the Beckwith–Wiedemann syndrome. Nat Genet 4:94–97. https://doi.org/10.1038/ng0593-94
55. Kobayashi H, Sakurai T, Imai M, Takahashi N, Fukuda A, Yayoi O, Sato S, Nakabayashi K, Hata K, Sotomaru Y, Suzuki Y, Kono T (2012) Contribution of intragenic DNA methylation in mouse Gametic DNA Methylomes to establish oocyte-specific heritable Marks. PLoS Genet 8:e1002440. https://doi.org/10.1371/journal.pgen.1002440
56. Proudhon C, Duffié R, Ajjan S, Cowley M, Iranzo J, Carbajosa G, Saadeh H, Holland ML, Oakey RJ, Rakyan VK, Schulz R, Bourc'his D (2012) Protection against De novo methylation is instrumental in maintaining parent-of-origin methylation inherited from the gametes. Mol Cell 47:909–920. https://doi.org/10.1016/j.molcel.2012.07.010
57. Ohta T, Gray TA, Rogan PK, Buiting K, Gabriel JM, Saitoh S, Muralidhar B, Bilienska B, Krajewska-Walasek M, Driscoll DJ, Horsthemke B, Butler MG, Nicholls RD (1999) Imprinting-mutation mechanisms in Prader-Willi syndrome. Am J Hum Genet 64:397–413. https://doi.org/10.1086/302233
58. Duffié R, Ajjan S, Greenberg MV, Zamudio N, Escamilla del Arenal M, Iranzo J, Okamoto I, Barbaux S, Fauque P, Bourc'his D (2014) The Gpr1/Zdbf2 locus provides new paradigms for transient and dynamic genomic imprinting in mammals. Genes Dev 28:463–478. https://doi.org/10.1101/gad.232058.113
59. Zhang W, Chen Z, Yin Q, Zhang D, Racowsky C, Zhang Y (2019) Maternal-biased H3K27me3 correlates with paternal-specific gene expression in the human morula. Genes Dev 33:382–387. https://doi.org/10.1101/gad.323105.118
60. Babak T, DeVeale B, Tsang EK, Zhou Y, Li X, Smith KS, Kukurba KR, Zhang R, Li JB, van der Kooy D, Montgomery SB, Fraser HB (2015) Genetic conflict reflected in tissue-specific maps of genomic imprinting in human and mouse. Nat Genet 47:544–549. https://doi.org/10.1038/ng.3274

61. Hanna CW, Peñaherrera MS, Saadeh H, Andrews S, McFadden DE, Kelsey G, Robinson WP (2016) Pervasive polymorphic imprinted methylation in the human placenta. Genome Res 26: 756–767. https://doi.org/10.1101/gr.196139.115
62. Hanna CW (2020) Placental imprinting: emerging mechanisms and functions. PLoS Genet 16. https://doi.org/10.1371/journal.pgen.1008709
63. Bogutz AB, Brind'Amour J, Kobayashi H, Jensen KN, Nakabayashi K, Imai H, Lorincz MC, Lefebvre L (2019) Evolution of imprinting via lineage-specific insertion of retroviral promoters. Nat Commun 10:5674. https://doi.org/10.1038/s41467-019-13662-9
64. Hanna CW, Pérez-Palacios R, Gahurova L, Schubert M, Krueger F, Biggins L, Andrews S, Colomé-Tatché M, Bourc'his D, Dean W, Kelsey G (2019) Endogenous retroviral insertions drive non-canonical imprinting in extra-embryonic tissues. Genome Biol 20:225. https://doi.org/10.1186/s13059-019-1833-x
65. Borgel J, Guibert S, Li Y, Chiba H, Schübeler D, Sasaki H, Forné T, Weber M (2010) Targets and dynamics of promoter DNA methylation during early mouse development. Nat Genet 42: 1093–1100. https://doi.org/10.1038/ng.708
66. Dhayalan A, Rajavelu A, Rathert P, Tamas R, Jurkowska RZ, Ragozin S, Jeltsch A (2010) The Dnmt3a PWWP domain reads histone 3 lysine 36 Trimethylation and guides DNA methylation*. J Biol Chem 285:26114–26120. https://doi.org/10.1074/jbc.M109.089433
67. Zhang Y, Jurkowska R, Soeroes S, Rajavelu A, Dhayalan A, Bock I, Rathert P, Brandt O, Reinhardt R, Fischle W, Jeltsch A (2010) Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. Nucleic Acids Res 38:4246–4253. https://doi.org/10.1093/nar/gkq147
68. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES (2006) A bivalent chromatin structure Marks key developmental genes in embryonic stem cells. Cell 125:315–326. https://doi.org/10.1016/j.cell.2006.02.041
69. Verma N, Pan H, Doré LC, Shukla A, Li QV, Pelham-Webb B, Teijeiro V, González F, Krivtsov A, Chang C-J, Papapetrou EP, He C, Elemento O, Huangfu D (2018) TET proteins safeguard bivalent promoters from de novo methylation in human embryonic stem cells. Nat Genet 50:83–95. https://doi.org/10.1038/s41588-017-0002-y
70. Eckersley-Maslin MA, Parry A, Blotenburg M, Krueger C, Ito Y, Franklin VNR, Narita M, D'Santos CS, Reik W (2020) Epigenetic priming by Dppa2 and 4 in pluripotency facilitates multi-lineage commitment. Nat Struct Mol Biol 27:696–705. https://doi.org/10.1038/s41594-020-0443-3
71. Gretarsson KH, Hackett JA (2020) Dppa2 and Dppa4 counteract de novo methylation to establish a permissive epigenome for development. Nat Struct Mol Biol 27:706–716. https://doi.org/10.1038/s41594-020-0445-1
72. Kremsky I, Corces VG (2020) Protection from DNA re-methylation by transcription factors in primordial germ cells and pre-implantation embryos can explain trans-generational epigenetic inheritance. Genome Biol 21:118. https://doi.org/10.1186/s13059-020-02036-w
73. Ehrlich M, Gama-Sosa MA, Huang L-H, Midgett RM, Kuo KC, McCune RA, Gehrke C (1982) Amount and distribution of 5-methylcytosine in human DNA from different types of tissues or cells. Nucleic Acids Res 10:2709–2721. https://doi.org/10.1093/nar/10.8.2709
74. Schroeder DI, Blair JD, Lott P, Yu HOK, Hong D, Crary F, Ashwood P, Walker C, Korf I, Robinson WP, LaSalle JM (2013) The human placenta methylome. Proc Natl Acad Sci 110: 6037–6042. https://doi.org/10.1073/pnas.1215145110
75. Yuan V, Hui D, Yin Y, Peñaherrera MS, Beristain AG, Robinson WP (2021) Cell-specific characterization of the placental methylome. BMC Genomics 22:6. https://doi.org/10.1186/s12864-020-07186-6
76. Zhang B, Kim MY, Elliot G, Zhou Y, Zhao G, Li D, Lowdon RF, Gormley M, Kapidzic M, Robinson JF, McMaster MT, Hong C, Mazor T, Hamilton E, Sears RL, Pehrsson EC, Marra MA, Jones SJM, Bilenky M, Hirst M, Wang T, Costello JF, Fisher SJ (2021) Human placental

cytotrophoblast epigenome dynamics over gestation and alterations in placental disease. Dev Cell 56:1238–1252.e5. https://doi.org/10.1016/j.devcel.2021.04.001
77. Nordor AV, Nehar-Belaid D, Richon S, Klatzmann D, Bellet D, Dangles-Marie V, Fournier T, Aryee MJ (2017) The early pregnancy placenta foreshadows dna methylation alterations of solid tumors. Epigenetics 00–00. https://doi.org/10.1080/15592294.2017.1342912
78. Smith ZD, Shi J, Gu H, Donaghey J, Clement K, Cacchiarelli D, Gnirke A, Michor F, Meissner A (2017) Epigenetic restriction of extraembryonic lineages mirrors the somatic transition to cancer. Nat Publ Group 549:4–3. https://doi.org/10.1038/nature23891
79. Cotton AM, Avila L, Penaherrera MS, Affleck JG, Robinson WP, Brown CJ (2009) Inactive X chromosome-specific reduction in placental DNA methylation. Hum Mol Genet 18:3544–3552. https://doi.org/10.1093/hmg/ddp299
80. Reiss D, Zhang Y, Mager DL (2007) Widely variable endogenous retroviral methylation levels in human placenta. Nucleic Acids Res 35:4743–4754. https://doi.org/10.1093/nar/gkm455
81. Chatterjee A, Macaulay EC, Rodger EJ, Stockwell PA, Parry MF, Roberts HE, Slatter TL, Hung NA, Devenish CJ, Morison IM (2016) Placental Hypomethylation is more pronounced in genomic loci devoid of Retroelements. G3 GenesGenomesGenetics 6:1911–1921. https://doi.org/10.1534/g3.116.030379
82. Cohen CJ, Rebollo R, Babovic S, Dai EL, Robinson WP, Mager DL (2011) Placenta-specific expression of the Interleukin-2 (IL-2) receptor β subunit from an endogenous retroviral promoter. J Biol Chem 286:35543–35552. https://doi.org/10.1074/jbc.M111.227637
83. Macaulay EC, Weeks RJ, Andrews S, Morison IM (2011) Hypomethylation of functional retrotransposon-derived genes in the human placenta. Mamm Genome 22:722–735. https://doi.org/10.1007/s00335-011-9355-1
84. Jaju Bhattad G, Jeyarajah MJ, McGill MG, Dumeaux V, Okae H, Arima T, Lajoie P, Bérubé NG, Renaud SJ (2020) Histone deacetylase 1 and 2 drive differentiation and fusion of progenitor cells in human placental trophoblasts. Cell Death Dis 11:1–17. https://doi.org/10.1038/s41419-020-2500-6
85. Kwak Y-T, Muralimanoharan S, Gogate AA, Mendelson CR (2019) Human trophoblast differentiation is associated with profound gene regulatory and epigenetic changes. Endocrinology 160:2189–2203. https://doi.org/10.1210/en.2019-00144
86. Rugg-Gunn PJ, Cox BJ, Ralston A, Rossant J (2010) Distinct histone modifications in stem cell lines and tissue lineages from the early mouse embryo. Proc Natl Acad Sci 107:10783–10790. https://doi.org/10.1073/pnas.0914507107
87. Lee JT, Jaenisch R (1997) Long-range cis effects of ectopic X-inactivation centres on a mouse autosome. Nature 386:275–279. https://doi.org/10.1038/386275a0
88. Migeon BR (2017) Choosing the active X: the human version of X inactivation. Trends Genet 33:899–909. https://doi.org/10.1016/j.tig.2017.09.005
89. Petropoulos S, Edsgärd D, Reinius B, Deng Q, Panula SP, Codeluppi S, Plaza Reyes A, Linnarsson S, Sandberg R, Lanner F (2016) Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos. Cell 165:1012–1026. https://doi.org/10.1016/j.cell.2016.03.023
90. Vallot C, Huret C, Lesecque Y, Resch A, Oudrhiri N, Bennaceur-Griscelli A, Duret L, Rougeulle C (2013) XACT, a long noncoding transcript coating the active X chromosome in human pluripotent cells. Nat Genet 45:239–241. https://doi.org/10.1038/ng.2530
91. Vallot C, Patrat C, Collier AJ, Huret C, Casanova M, Liyakat Ali TM, Tosolini M, Frydman N, Heard E, Rugg-Gunn PJ, Rougeulle C (2017) XACT noncoding RNA competes with XIST in the control of X chromosome activity during human early development. Cell Stem Cell 20:102–111. https://doi.org/10.1016/j.stem.2016.10.014
92. Dossin F, Pinheiro I, Żylicz JJ, Roensch J, Collombet S, Le Saux A, Chelmicki T, Attia M, Kapoor V, Zhan Y, Dingli F, Loew D, Mercher T, Dekker J, Heard E (2020) SPEN integrates transcriptional and epigenetic control of X-inactivation. Nature 578:455–460. https://doi.org/10.1038/s41586-020-1974-9

93. Brockdorff N, Bowness JS, Wei G (2020) Progress toward understanding chromosome silencing by Xist RNA. Genes Dev 34:733–744. https://doi.org/10.1101/gad.337196.120
94. Tjalsma SJD, Hori M, Sato Y, Bousard A, Ohi A, Raposo AC, Roensch J, Le Saux A, Nogami J, Maehara K, Kujirai T, Handa T, Bagés-Arnal S, Ohkawa Y, Kurumizaka H, da Rocha ST, Żylicz JJ, Kimura H, Heard E (2021) H4K20me1 and H3K27me3 are concurrently loaded onto the inactive X chromosome but dispensable for inducing gene silencing. EMBO Rep 22. https://doi.org/10.15252/embr.202051989
95. Tukiainen T, Villani A-C, Yen A, Rivas MA, Marshall JL, Satija R, Aguirre M, Gauthier L, Fleharty M, Kirby A, Cummings BB, Castel SE, Karczewski KJ, Aguet F, Byrnes A, Lappalainen T, Regev A, Ardlie KG, Hacohen N, MacArthur DG (2017) Landscape of X chromosome inactivation across human tissues. Nature 550:244–248. https://doi.org/10.1038/nature24265
96. Balaton BP, Fornes O, Wasserman WW, Brown CJ (2021) Cross-species examination of X-chromosome inactivation highlights domains of escape from silencing. Epigenetics Chromatin 14. https://doi.org/10.1186/s13072-021-00386-8
97. Raznahan A, Parikshak NN, Chandran V, Blumenthal JD, Clasen LS, Alexander-Bloch AF, Zinn AR, Wangsa D, Wise J, Murphy DGM, Bolton PF, Ried T, Ross J, Giedd JN, Geschwind DH (2018) Sex-chromosome dosage effects on gene expression in humans. Proc Natl Acad Sci 115:7398–7403. https://doi.org/10.1073/pnas.1802889115
98. Cotton AM, Price EM, Jones MJ, Balaton BP, Kobor MS, Brown CJ (2015) Landscape of DNA methylation on the X chromosome reflects CpG density, functional chromatin state and X-chromosome inactivation. Hum Mol Genet 24:1528–1539. https://doi.org/10.1093/hmg/ddu564
99. Moreira de Mello JC, de Araújo ESS, Stabellini R, Fraga AM, de Souza JES, Sumita DR, Camargo AA, Pereira LV (2010) Random X inactivation and extensive mosaicism in human placenta revealed by analysis of allele-specific gene expression along the X chromosome. PLoS One 5:e10947. https://doi.org/10.1371/journal.pone.0010947
100. Peñaherrera MS, Jiang R, Avila L, Yuen RKC, Brown CJ, Robinson WP (2012) Patterns of placental development evaluated by X chromosome inactivation profiling provide a basis to evaluate the origin of epigenetic variation. Hum Reprod Oxf Engl 27:1745–1753. https://doi.org/10.1093/humrep/des072
101. Gong S, Johnson MD, Dopierala J, Gaccioli F, Sovio U, Constância M, Smith GC, Charnock-Jones DS (2018) Genome-wide oxidative bisulfite sequencing identifies sex-specific methylation differences in the human placenta. Epigenetics 13:228–239. https://doi.org/10.1080/15592294.2018.1429857
102. Teschendorff AE, Relton CL (2017) Statistical and integrative system-level analysis of DNA methylation data. Nat Rev Genet 19:129–147. https://doi.org/10.1038/nrg.2017.86
103. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT (2012) DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 13:86–86. https://doi.org/10.1186/1471-2105-13-86
104. Wen L, Tang F (2019) Human germline cell development: from the perspective of single-cell sequencing. Mol Cell 76:320–328. https://doi.org/10.1016/j.molcel.2019.08.025
105. Bell JT, Pai AA, Pickrell JK, Gaffney DJ, Pique-Regi R, Degner JF, Gilad Y, Pritchard JK (2011) DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. Genome Biol 12:R10–R10. https://doi.org/10.1186/gb-2011-12-1-r10
106. Teh AL, Pan H, Chen L, Ong M-L, Dogra S, Wong J, MacIsaac JL, Mah SM, McEwen LM, Saw S-M, Godfrey KM, Chong Y-S, Kwek K, Kwoh C-K, Soh S-E, Chong MFF, Barton S, Karnani N, Cheong CY, Buschdorf JP, Stünkel W, Kobor MS, Meaney MJ, Gluckman PD, Holbrook JD (2014) The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes. Genome Res 24:1064–1074. https://doi.org/10.1101/gr.171439.113
107. Czamara D, Eraslan G, Page CM, Lahti J, Lahti-Pulkkinen M, Hämäläinen E, Kajantie E, Laivuori H, Villa PM, Reynolds RM, Nystad W, Håberg SE, London SJ, O'Donnell KJ,

Garg E, Meaney MJ, Entringer S, Wadhwa PD, Buss C, Jones MJ, Lin DTS, MacIsaac JL, Kobor MS, Koen N, Zar HJ, Koenen KC, Dalvie S, Stein DJ, Kondofersky I, Müller NS, Theis FJ, Räikkönen K, Binder EB (2019) Integrated analysis of environmental and genetic influences on cord blood DNA methylation in new-borns. Nat Commun 10:2548. https://doi.org/10.1038/s41467-019-10461-0

108. Gutierrez-Arcelus M, Ongen H, Lappalainen T, Montgomery SB, Buil A, Yurovsky A, Bryois J, Padioleau I, Romano L, Planchon A, Falconnet E, Bielser D, Gagnebin M, Giger T, Borel C, Letourneau A, Makrythanasis P, Guipponi M, Gehrig C, Antonarakis SE, Dermitzakis ET (2015) Tissue-specific effects of genetic and epigenetic variation on gene regulation and splicing. PLoS Genet 11. https://doi.org/10.1371/journal.pgen.1004958
109. Fraser HB, Lam LL, Neumann SM, Kobor MS (2012) Population-specificity of human DNA methylation. Genome Biol 13:R8–R8. https://doi.org/10.1186/gb-2012-13-2-r8
110. Galanter JM, Gignoux CR, Oh SS, Torgerson D, Pino-Yanes M, Thakur N, Eng C, Hu D, Huntsman S, Farber HJ, Avila PC, Brigino-Buenaventura E, LeNoir MA, Meade K, Serebrisky D, Rodríguez-Cintrón W, Kumar R, Rodríguez-Santana JR, Seibold MA, Borrell LN, Burchard EG, Zaitlen N (2017) Differential methylation between ethnic sub-groups reflects the effect of genetic ancestry and environmental exposures. eLife 6: . https://doi.org/10.7554/eLife.20532
111. Hannon E, Spiers H, Viana J, Pidsley R, Burrage J, Murphy TM, Troakes C, Turecki G, O'Donovan MC, Schalkwyk LC, Bray NJ, Mill J (2016) Methylation quantitative trait loci in the developing brain and their enrichment in schizophrenia-associated genomic regions. Nat Neurosci 19:48–54. https://doi.org/10.1038/nn.4182
112. Andrews SV, Ellis SE, Bakulski KM, Sheppard B, Croen LA, Hertz-Picciotto I, Newschaffer CJ, Feinberg AP, Arking DE, Ladd-Acosta C, Fallin MD (2017) Cross-tissue integration of genetic and epigenetic data offers insight into autism spectrum disorder. Nat Commun 8:1011. https://doi.org/10.1038/s41467-017-00868-y
113. Delahaye F, Do C, Kong Y, Ashkar R, Salas M, Tycko B, Wapner R, Hughes F (2018) Genetic variants influence on the placenta regulatory landscape. PLoS Genet 14:e1007785–e1007785. https://doi.org/10.1371/journal.pgen.1007785
114. Gaunt TR, Shihab HA, Hemani G, Min JL, Woodward G, Lyttleton O, Zheng J, Duggirala A, McArdle WL, Ho K, Ring SM, Evans DM, Davey Smith G, Relton CL (2016) Systematic identification of genetic influences on methylation across the human life course. Genome Biol 17:61. https://doi.org/10.1186/s13059-016-0926-z
115. Rando OJ (2016) Intergenerational transfer of epigenetic information in sperm. Cold Spring Harb Perspect Med 6. https://doi.org/10.1101/cshperspect.a022988
116. Sharma U (2019) Paternal contributions to offspring health: role of sperm small RNAs in intergenerational transmission of epigenetic information. Front Cell Dev Biol 7. https://doi.org/10.3389/fcell.2019.00215
117. Heard E, Martienssen RA (2014) Transgenerational epigenetic inheritance: myths and mechanisms. Cell 157:95–109. https://doi.org/10.1016/j.cell.2014.02.045
118. Legoff L, D'Cruz SC, Tevosian S, Primig M, Smagulova F (2019) Transgenerational inheritance of environmentally induced epigenetic alterations during mammalian development. Cell 8. https://doi.org/10.3390/cells8121559
119. Erkek S, Hisano M, Liang C-Y, Gill M, Murr R, Dieker J, Schübeler D, van der Vlag J, Stadler MB, Peters AHFM (2013) Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. Nat Struct Mol Biol 20:868–875. https://doi.org/10.1038/nsmb.2599
120. Jung YH, Sauria MEG, Lyu X, Cheema MS, Ausio J, Taylor J, Corces VG (2017) Chromatin states in mouse sperm correlate with embryonic and adult regulatory landscapes. Cell Rep 18:1366–1382. https://doi.org/10.1016/j.celrep.2017.01.034
121. Lismer A, Siklenka K, Lafleur C, Dumeaux V, Kimmins S (2020) Sperm histone H3 lysine 4 trimethylation is altered in a genetic mouse model of transgenerational epigenetic inheritance. Nucleic Acids Res 48:11380–11393. https://doi.org/10.1093/nar/gkaa712

122. Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, Farinelli L, Miska E, Mansuy IM (2014) Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci 17:667–669. https://doi.org/10.1038/nn.3695
123. Rodgers AB, Morgan CP, Leu NA, Bale TL (2015) Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. Proc Natl Acad Sci U S A 112:13699–13704. https://doi.org/10.1073/pnas.1508347112
124. Beck D, Ben Maamar M, Skinner MK (2021) Integration of sperm ncRNA-directed DNA methylation and DNA methylation-directed histone retention in epigenetic transgenerational inheritance. Epigenetics Chromatin 14:6. https://doi.org/10.1186/s13072-020-00378-0
125. Veenendaal MVE, Painter RC, de Rooij SR, Bossuyt PMM, van der Post J a. M, Gluckman PD, Hanson MA, Roseboom TJ (2013) Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine. BJOG Int J Obstet Gynaecol 120:548–553. https://doi.org/10.1111/1471-0528.12136
126. Donkin I, Versteyhe S, Ingerslev LR, Qian K, Mechta M, Nordkap L, Mortensen B, Appel EVR, Jørgensen N, Kristiansen VB, Hansen T, Workman CT, Zierath JR, Barrès R (2016) Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. Cell Metab 23:369–378. https://doi.org/10.1016/j.cmet.2015.11.004
127. Marczylo EL, Amoako AA, Konje JC, Gant TW, Marczylo TH (2012) Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? Epigenetics 7:432–439. https://doi.org/10.4161/epi.19794
128. Aarabi M, San Gabriel MC, Chan D, Behan NA, Caron M, Pastinen T, Bourque G, MacFarlane AJ, Zini A, Trasler J (2015) High-dose folic acid supplementation alters the human sperm methylome and is influenced by the MTHFR C677T polymorphism. Hum Mol Genet 24:6301–6313. https://doi.org/10.1093/hmg/ddv338
129. Horsthemke B (2018) A critical view on transgenerational epigenetic inheritance in humans. Nat Commun 9:2973. https://doi.org/10.1038/s41467-018-05445-5