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Review Article

Inheritance of environment-induced phenotypic changes through epigenetic mechanisms

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Abstract

Growing evidence suggests that epigenetic changes through various parental environmental factors alter the phenotypes of descendants in various organisms. Environmental factors, including exposure to chemicals, stress and abnormal nutrition, affect the epigenome in parental germ cells by different epigenetic mechanisms, such as DNA methylation, histone modification as well as small RNAs via metabolites. Some current remaining questions are the causal relationship between environment-induced epigenetic changes in germ cells and altered phenotypes of descendants, and the molecular basis of how the abnormal epigenetic changes escape reprogramming in germ cells. In this review, we introduce representative examples of intergenerational and transgenerational inheritance of phenotypic changes through parental environmental factors and the accompanied epigenetic and metabolic changes, with a focus on animal species. We also discuss the molecular mechanisms of epigenomic inheritance and their possible biological significance.

Keywords: epigenome; inheritance; parental environmental factors; germ cells; DNA methylation

Introduction

It has been generally thought that phenotypic inheritance occurs only through genomic DNA; however, experimental evidence suggests that epigenetic changes via various parental environmental factors also contribute to phenotypic alterations in descendants of various organisms including humans. Although the detailed mechanisms of epigenomic inheritance are difficult to examine using human studies, studies using model organisms, including bacteria, fungi, plants and invertebrates as well as other mammals, have suggested molecular mechanisms [1, 2]. For instance, certain chemicals cause abnormal spermatogenesis and concomitant changes of DNA methylation in sperm over multiple generations [3–6], and a paternal high-fat diet (HFD) and stress result in metabolic diseases and behavioral changes in generations up to the grandchildren [7, 8]. However, most of these studies only showed the correlation of epigenetic and phenotypic changes, and their causal relationship is still unclear.

It is well known that germ cells undergo extensive epigenetic reprogramming [9], and it is therefore important to show how induced abnormal epigenetic changes in germ cells overcome reprogramming and are transmitted to successive generations. Another crucial issue is how environmental stimuli are converted into epigenetic alterations. In this regard, metabolism in germ cells may play a role, as environmental conditions affect metabolism, which may influence the epigenomic state in general and may do so in developing germ cells as well [10].

As a result of the increase in seemingly nontoxic artificial chemicals in our daily commodities and foods, as well as dietary and lifestyle changes, the unknown risks of abnormal epigenetic changes in our bodies and even in germ cells are likely to be heightened in current society. In this review, we summarize representative examples of phenotypic and associated epigenetic changes across generations that are induced by various parental environmental factors, and also touch on the possible involvement of metabolites and metabolism in epigenetic changes in germ cells. Finally, we discuss the mechanisms of epigenomic memory and provide a perspective of the possible biological significance of the inheritance of epigenomic changes.

Definition of Intergenerational and Transgenerational Inheritance

It is important to distinguish between 'intergenerational inheritance' and 'transgenerational inheritance' when discussing the inheritance of environmental influences [2]. Intergenerational inheritance is defined as the transmission of influences in an individual by an environmental stimulus to its offspring, derived from direct exposure of the fetus or germ cells to the stimulus. Male and female parental environmental influences in F1 progeny are intergenerational. In the case of mammals, environmental exposure of a pregnant female could directly affect her fetus as well as fetal germ cells, and therefore the influence in her F2 progeny is

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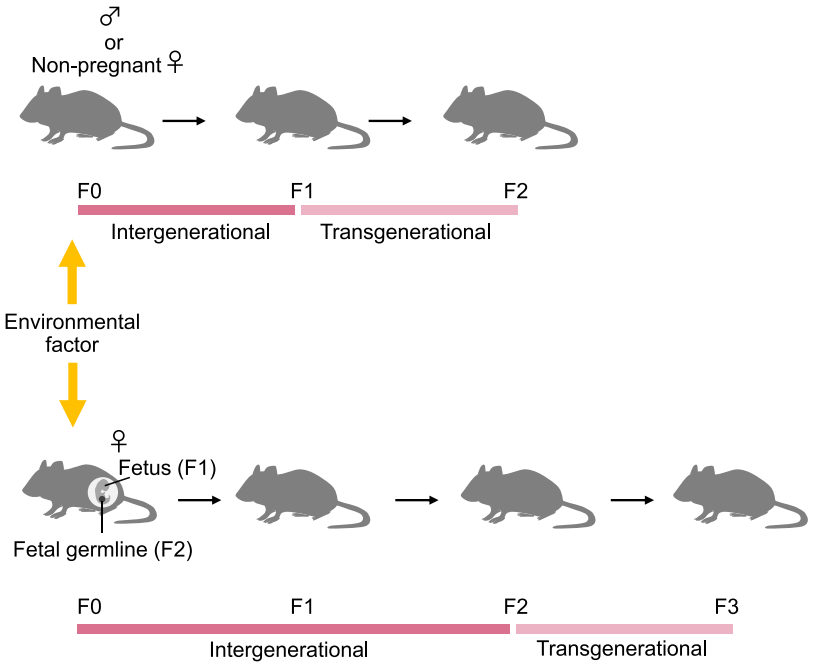

Figure 1: Intergenerational and transgenerational inheritance. Inheritance by the immediate offspring of an individual in which the change arose by an environmental stimulus is termed intergenerational inheritance. In the case of transgenerational inheritance, environmental influences are maintained even in the absence of the initial stimulus or epigenetic trigger

also considered to be intergenerational inheritance. Meanwhile, F2 progeny from a male and a non-pregnant female and F3 progeny from a pregnant female exposed to an environmental stimulus are derived from germ cells without direct exposure to the stimulus, which is defined as transgenerational inheritance (Fig. 1). The existence and mechanisms of transgenerational epigenetic inheritance (TEI) are emerging from studies using animal models, in particular flies and nematodes. On the other hand, in mammals, such studies have been mainly correlational, and there are few examples that have elucidated mechanisms, but observations suggesting TEI in mammals have been accumulating. The following sections describe examples of both intergenerational epigenetic inheritance (IEI) and TEI in animals through various environmental factors, as summarized in Table 1, and discuss their possible mechanisms.

How Does a Specific Environment Cause Epigenetic Changes in Germ Cells?

A variety of environmental factors affect offspring phenotypes. For example, it is well known that smoking and alcohol consumption during pregnancy adversely affect fetal development. Growing evidence has also suggested the effects of paternal environmental factors on offspring. In this section, we review representative environmental factors affecting offspring with associated epigenetic changes.

Influences of Chemical Exposure

A number of studies have reported that maternal exposure to several chemicals during gestation alters the phenotypes of offspring including germ cell development. Bisphenol-A (BPA) is one example of a chemical widely used as a material in plastic products. Epidemiological studies showed correlations of *in utero* exposure to BPA and anogenital distance in male offspring, which is a marker of androgenic and antiandrogenic effects of *in utero* chemical exposure, and is used as an endpoint for evaluating the reproductive toxicity of chemicals [11]. *In utero* exposure to BPA is also correlated to smaller fetus size during gestation, lower birth weight and high leptin and low adiponectin secretion in the F1 generation [12]. In mice, maternal exposure to BPA during embryonic day (E)12.5 to E18.5 inhibits the development of oocytes [13, 14] and alters DNA methylation of genomic imprinted genes in both male and female germ cells in the F1 generation [13].

Di(2-ethylhexyl) phthalate (DEHP) is another chemical used in a variety of plastic products. Several epidemiological studies have highlighted an increased risk of preterm birth after phthalate exposure *in utero*. Spontaneous preterm labor and preterm birth after obstetrical complications are correlated with the concentration of urinary phthalate metabolites [15]. Maternal exposure to DEHP during E7 to E14 in mice impairs spermatogenesis from F1 to F4 generations [3], and that during E8 to E18 in mice alters DNA methylation and gene expression in several genes associated with sperm physiology such as seminal vesicle secretory proteins gene (*Svs*) in sperm in maternally exposed F1 generations [4, 16]. In addition, we previously showed that promoter methylation of genes involved in spermatogenesis is increased in F1 fetal male germ cells immediately after maternal DEHP exposure from E8 to E18, and some of those genes persist in a hypermethylated state and are downregulated in adult sperm stem cells in F1 progeny [17].

Dichlorodiphenyltrichloroethane (DDT) is one of the well-studied chemicals used as a pesticide. Human cohort studies demonstrated the correlation of maternal serum DDT levels with preterm birth and small-for-gestational-age babies [18], and of the concentration of p,p′-dichlorodiphenyldichloroethylene (DDE), one of the primary metabolites of DDT in cord blood, with being overweight at 6.5 years of age [19]. In a rat model, maternal exposure to DDT in the gestation period from E8 to E14

Table 1: A summary of phenotypic change in offspring by parental environmental factors described in this review

Environmental factor	Organism	Exposed in	Phenotype	Phenotype inherited generations	Epigenome change	Epigenome inherited generations	References
Chemicals							
BPA	*H. sapiens*	Maternal	Androgenic and antiandrogenic effects, smaller size during gestation, lower birth weight, high leptin and low adiponectin secretion	F1			[11, 12]
BPA	*M. musculus*	Maternal	Defects of oocyte development	F1	DNA methylation	F1	[13, 14]
BPA	*M. musculus*	Maternal	Obesity	F6	DNA methylation	F6	[105]
Phthalate	*H. sapiens*	Maternal	Preterm birth	F1			[15]
DEHP	*M. musculus*	Maternal	Defects of spermatogenesis	F4	DNA methylation	F1	[3, 4, 16, 17]
DDT	*H. sapiens*	Maternal	Preterm births, small-for-gestational-age	F1			[18]
DDT	*R. norvegicus*	Maternal	Obesity, testis disease, ovary disease, kidney disease	F3	DNA methylation	F3	[6, 20–22]
DDE	*H. sapiens*	Maternal	Overweight at childhood	F1			[19]
DDE	*R. norvegicus*	Maternal	Alteration of male fertility	F3	DNA methylation	F3	[23]
Carbon tetrachloride	*R. norvegicus*	Paternal	Suppression of hepatic fibrosis	F2	DNA methylation, H2A.Z, H3K27me3	F2	[24]
Exercise							
Exercise	*M. musculus*	Maternal	Improvement of the metabolic health	F1	DNA methylation	F1	[58]
Stress							
Heat shock	*D. melanogaster*	Embryonic stage	De-repression of *white* gene in the PEV reporter	F2	H3K9me2	F2	[59]
Heat shock	*C. elegans*	Embryonic stage	Alteration of *daf-21p* expression	F2	H3K9me3	F2	[60]
Heat shock	*C. elegans*	Embryonic stage	Changes in expression of small RNA target mRNAs	F3	small RNAs	F3	[61]
Pathogen	*C. elegans*	Embryonic stage	Avoidance of a pathogen	F4	small RNAs	F4	[62, 63]
Low dose oxidant/Gut microbiota	*D. melanogaster*	Embryonic stage	Longer lifespan	F1			[90]
Separation from mother	*M. musculus*	Paternal	Alteration of behavior, insulin hypersensitivity	F3	small RNAs	F2	[8, 64]
Nutrition							
Protein restricted diet	*M. musculus*	Maternal	Impairment of lipid homeostasis	F1	DNA methylation	F1	[71. 72, 73]
Undernutritional diet	*M. musculus*	Maternal	Glucose intolerance	F2	DNA methylation	F1	[74, 75]
Undernutritional diet	*M. musculus*	Maternal	Downregulation of lipogenic genes	F2	DNA methylation H3K27me3	F2	[76]
High-fat diet	*M. musculus*	Maternal	Alteration of *Dlk1* expression	F2	DNA methylation microRNA	F1 F2 (F1 oocyte)	[77]
High-fat diet/Gut microbiota	*M. musculus*	Maternal	Obesity, glucose intolerance, insulin resistance	F1			[87]
High-fat diet	*M. musculus*	Paternal	Obesity, metabolic dysfunctions	F1	H3K4me3	F0	[7]
High-fat diet	*M. musculus*	Paternal	Obesity, insulin resistance	F2	DNA methylation microRNA	F0	[80]
Starvation	*C. elegans*	Embryonic stage	Longer lifespan	F3	small RNAs	F3	[82]

results in obesity, diseases of the kidneys, prostate and ovaries, as well as tumor development in F1 males and females, which are also observed in F3 through both paternal and maternal lineages [20], and differentially methylated regions (DMRs) in F3 sperm are associated with the pathological phenotypes [21]. A study using the same experimental design of maternal DDT exposure revealed that approximately 40–50% of the sperm DMRs in the F1 generation are retained in the sperm of F2 and F3 generations, suggesting transgenerational inheritance of altered DNA methylation [6]. In addition, the increase of spermatogenic cell apoptosis and decrease of sperm number in the F3 generation but not in the F1 generation from DDT-exposed mothers were observed [20, 22]. Alterations in male fertility and expression of genomic imprinted genes *H19* and *Igf2* with hypomethylation of *Igf2* DMR2 in sperm from F1 to F3 generations through paternal line were also observed via maternal exposure to DDE from E8 to E15 [23]. Although their causal relationship is unclear, the results together imply that maternal exposure to several chemicals alters the epigenomic state of fetal germ cells, which may be transgenerationally inherited and affect fertility and other phenotypes in the offspring.

Besides the negative influences of parental chemical exposure on their descendants, paternal carbon tetrachloride (CCl_4) exposure causes heritable adaptation of hepatic wound healing in rats [24]. In this study, CCl_4 exposure in male rats caused fibrosis in the F0 male liver, but suppressed the hepatic fibrosis in the F2 male liver through the paternal linage with hypomethylation of DNA, hyperacetylation of histone H3 and upregulation of an antifibrogenic gene, peroxisome proliferator activated receptor *(Ppar)-γ*, and the opposite epigenetic modifications and downregulation of a profibrogenic gene, *Tgf-β1*. In addition, CCl_4 treatment induces enrichment of one of histone variants, H2A.Z, which is exclusive to DNA methylation, and of trimethylation of lysine 27 in histone H3 (H3K27me3), a histone modification which represses transcription, in a *Ppar-γ* promoter region in the CCl_4-treated F0 sperm. Remarkably, serum transfer from CCl_4-treated rats also induces enrichment of the same histone modifications in the *Ppar-γ* promoter in CCl_4-untreated rat sperm, suggesting that the liver damage by treatment of CCl_4 results in the accumulation of an unknown soluble factor in the serum that might modify the chromatin signatures in sperm, and influence the heritable suppression of CCl_4-induced hepatic fibrosis.

During spermatogenesis, histones are progressively exchanged with more basic and smaller protamine proteins. However, a recent study has shown that sperm retains a significant amount of histones, and around 7.5% and 4% of that in somatic cells in mouse and human sperm, respectively [25, 26]. These histones bear modifications including methylation and acetylation, which may influence paternal chromatin landscapes and prefigure the expression patterns of genes in the early embryo [25, 27, 28]. This supports the idea that abnormal histone modifications at certain loci in sperm detected after environmental exposure can be inherited by the offspring.

Possible Mechanisms of DNA Methylation by Chemicals

It is currently unclear how chemicals cause epigenetic changes in germ cells, but reactive oxygen species (ROS) are one of the candidate molecules involved in chemical-mediated epigenetic changes. For instance, DDT and BPA induce ROS in various cell types [29, 30], and mono (2-ethylhexyl) phthalate (MEHP), a hydrolyzed metabolite of DEHP, also induces ROS production in adult testicular germ cells [31] and other cell types [32–34]. Possible mechanisms of ROS-mediated DNA methylation have been proposed [35], one of which is DNA methylation by ROS as a catalyst [36]. A superoxide molecule could react with the C-5 atom in cytosine and deprotonate it, which then acquires a nucleophilic property because of its negative charge. The nucleophilic C-5 atom then reacts with a positively charged S-atom in a methyl group in S-adenosyl methionine (SAM), which generates a methylated cytosine (Fig. 2A).

The formation of DNA methyltransferase (DNMT)-containing silencing complexes may also play a role in DNA methylation by oxidative damage of DNA by ROS. ROS is known to induce double strand breaks (DSBs), and an *in vitro* study using inducible DSBs in an exogenous E-cadherin gene promoter with a CpG Island, which is frequently DNA hypermethylated in epithelial cancer cells, demonstrated a model for DSB-induced gene silencing [37]. In this model, the key epigenetic regulators involved in establishing and maintaining transcriptional repression, namely SIRT1, EZH2, DNMT1 and DNMT3B, are recruited to the damaged sites in the exogenous E-cadherin CpG Island in human breast cancer cells during the DSB-induced DNA repair (DSBR) process. Although the promoter activity is preserved in most cells after DSBR, a small percentage of the cells demonstrate heritable DNA methylation and gene silencing, probably by the recruited DNMTs (Fig. 2B).

Another example of ROS-induced DNA methylation by formation of a DNMT-containing silencing complex is the enhancement of DNMT1 binding to ROS-induced damaged regions in guanine and cytosine-rich promoters by 8-oxoguanine DNA glycosylase (OGG1), a DNA glycosylase responsible for excising ROS-induced 8-oxo-2′deoxyguanosine during base excision repair (BER) [38]. Since guanine is prone to oxidation compared to other deoxyribonucleosides [39], GC-enriched genomic regions may readily undergo BER, in which DNMTs may be recruited to methylate the regions.

An additional possible mechanism of DNA methylation by ROS is the induction of *Dnmt* gene expression by ROS [40]. The activator protein-1 (AP-1) transcription factor (TF) is activated by ROS via mitogen-activated protein kinase (MAPK) signaling [41, 42], and *Dnmt1* expression is upregulated by activation and binding of AP-1 to the *Dnmt1* promoter via Ras signaling [43], together suggesting transcriptional regulation of *Dnmt1* by ROS (Fig. 2C). In addition, upregulation of DNMT1 by H_2O_2 treatment [44] and downregulation of *Dnmt3b* by treatment with the antioxidant N-acetyl-L-cysteine (NAC) [40] support the induction of DNMTs' expression by ROS. The mechanism of selective DNA methylation in specific gene promoters by chemical exposure is not yet well understood, but as described above, methylation of CpG Islands (CGIs) by DNMT recruitment after ROS-induced DNA damage may contribute to preferential DNA methylation of specific genes. Another possibility is that the highly accessible chromatin structure around the spermatogenesis-related genes in fetal germ cells [45] may facilitate preferential DNA methylation of those genes in fetal germ cells by DEHP exposure [17].

Effects of Maternal Exercise

Recent cohort studies have shown that exercise during pregnancy has a positive impact on the health of offspring including prevention of metabolic disease, cardiovascular disease and cancer [46, 47]. Several rodent strains with different duration and modalities of maternal exercise also showed the effects of maternal exercise on the metabolic health of offspring [48–54], and the

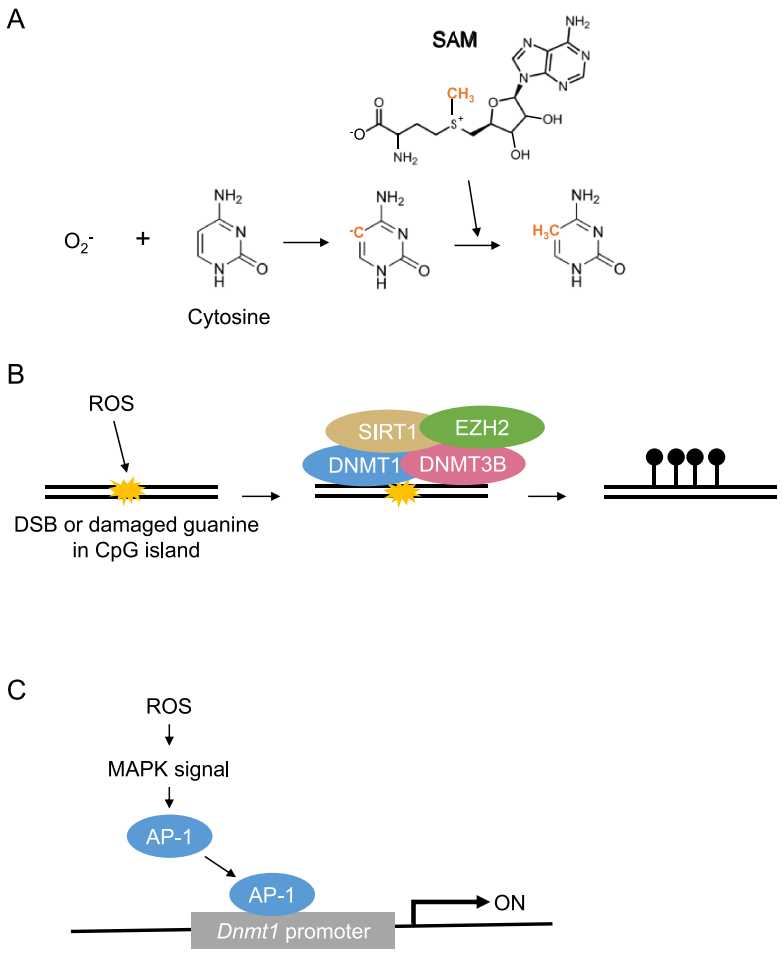

Figure 2: Possible mechanisms of DNA methylation via reactive oxygen species (ROS). (A) In DNA methylation by ROS as a catalyst, a superoxide molecule deprotonates the C-5 atom of cytosine. (B) Recruitment of a DNA methyltransferase (DNMT)-containing silencing complex by double strand break (DSB) or damaged guanine induced by ROS in the CpG Island. (C) Induction of *Dnmt* gene expression by ROS via mitogen-activated protein kinase (MAPK) signaling and activator protein-1 (AP-1)

majority of those studies showed that maternal exercise before and during pregnancy improves glucose tolerance and insulin sensitivity in F1 offspring.

Exercise during pregnancy has multiple effects on the placenta including vascularization [55], distribution of nutrient transporters [56] and autocrine signaling [57]. These findings suggest that the placenta functions as a secretory organ in response to exercise during pregnancy. Additionally, a study showed that maternal exercise throughout pregnancy improves the metabolic health of F1 offspring through a vitamin D receptor (VDR)-mediated increase in placental superoxide dismutase 3 (SOD3) expression and secretion in mice [58]. In more detail, maternal exercise induces the expression of VDR, which in turn activates SOD3 expression in trophoblasts in the placenta. SOD3 secreted from trophoblasts then induces glucose metabolic genes in the fetal liver by ten-eleven translocation (TET)-mediated DNA demethylation of their promotors, resulting in improved glucose homeostasis in F1 offspring. Although the results only demonstrated the effect of maternal exercise in the F1 generation, the influence of maternal exercise in subsequent generations is an attractive possibility, which requires investigation in further studies.

Influences of Parental Stress

Changes in phenotypes associated with epigenomic alterations in response to certain stresses have been reported in a diverse range of animal species, from invertebrates to mammals. In addition, these phenotypes and epigenetic changes are transgenerationally inherited. For instance, stress-induced loss of H3K9 methylation and subsequent de-repression of certain genes are heritable over multiple generations in both *Drosophila melanogaster* [59] and *Caenorhabditis elegans* [60]. In *D. melanogaster*, 1 h heat shock (HS) at 37°C induces de-repression of *white* gene in the position effect variegation (PEV) reporter via phosphorylation of *Drosophila* homologue of activation transcription factor 2 (dATF-2). The activated dATF-2 is released from H3K9me2, which causes loss of H3K9me2 and subsequent heterochromatic disruption in early embryonic somatic cells as well as in primordial germ cells (PGCs) by HS [59], and the heterochromatic disruption represented by white eye color by *white* gene activation is transmitted to the next generation (G1). Although the influence of HS is also inherited by the second generation (G2), the *white* gene activation is decreased, and is not observed in the following generations (G3–G5), indicating that HS-induced heterochromatic disruption is de-stabilized over generations.

In *C. elegans*, upregulation of the HS gene (*daf-21*) reporter by HS stress in the embryonic stage is inherited by at least two generations after single exposure of HS through both maternal and paternal lines [60]. In addition, decreased H3K9me3 is observed in the F2 progeny from grandparents who developed under a high temperature condition, at 25°C for 48 h from embryos to young adult stage, and upregulation of the reporter in F1 progeny from the heat-shocked parents is dependent on inactivation of the putative histone methyltransferase SET-25. De-repression of multiple classes of repetitive elements in the descendants was also dependent on SET-25 inactivation. Although the physiological significance of the HS-induced changes in histone modifications and gene expression in *Drosophila* and *C. elegans* is currently unclear, these results suggest that transgenerational inheritance of histone modifications could occur under environmental stress in these animals. In addition, heat stress also alters gene expression transgenerationally through small RNAs in *C. elegans*, lasting two to three generations after a return to normal temperature conditions [61].

As a good example of transgenerational inheritance of a physiologically meaningful phenotypic change by pathogenic stress, exposure of a pathogenic strain of *Pseudomonas aeruginosa* (PA14) to *C. elegans* larvae induces avoidance of the same pathogen up to the F4 generation [62]. This transgenerational avoidance behavior depends at least to some extend on the expression of the TGF-β ligand DAF-7 in ASI sensory neurons. In more detail, ingested small non-coding RNAs (ncRNAs) in PA14 are processed by the RNAi pathway in the gut of a host organism, followed by further processing by the piRNA pathway in the germ line, which may transmit the pathogenic memory to descendants. The processed small RNA subsequently targets *maco-1* in ASI neurons and downregulates it, and decreased MACO-1 upregulates *daf-7* expression in ASI neurons, which results in the avoidance behavior [63].

In the case of traumatic stress in mice, daily 3 h proximal separation of male pups (F1) from the mother during postnatal days 1–14, namely unpredictable maternal separation combined with unpredictable maternal stress (MSUS), induces alteration of behavior including reduced avoidance, fear and an altered response to aversive conditions in F1 and F2 male offspring through the paternal line [8]. In addition, glucose metabolism is also affected, such as lower blood insulin and glucose levels as well as insulin hypersensitivity, especially in F2 males, after MSUS exposure [8]. MSUS also causes alteration of the expression of several micro RNAs (miRNAs) in F1 sperm and in F2 serum and hippocampus, but not in F2 sperm, although F3 mice also show similar abnormal behaviors as F1 and F2 mice [64], suggesting that the MSUS-induced changes of miRNAs in F1 may be converted to other epigenetic marks in F3 mice. Intriguingly, injection of total RNA of MSUS sperm to fertilized oocytes obtained from untreated females could recapitulate MSUS-induced behavioral and metabolic abnormalities in the resulting offspring [8], suggesting the involvement of the affected sperm RNA-dependent mechanisms in transmission of the MSUS-induced traits to the next generation.

Not only model animals, but also non-model animals have been studied for epigenetic inheritance. One example is influence of heat stress in wild Guinea pigs [65]. Exposure of F0 adult male Guinea pig to an increased ambient temperature at 30°C for 2 months resulted in hypermethylation and downregulation of Signal Transducer and Activator of Transcription 3 (Stat3) in F1 male liver. Studies of birds also demonstrated the influence of parental stress on offspring [66]. For instance, combination of stresses such as food deprivation, physical restraint or social isolation of laying hen influences the offspring's hypothalamus-pituitary-adrenal-axis response by elevation of corticosterone (CORT), a stress-related hormone [67]. These findings show the parental stresses affect offspring in non-model animals as well.

Roles of Nutrition

A growing number of studies focusing on the developmental origins of health and disease have identified links between early nutrition and diseases in humans [68–70]. Studies using model animals also demonstrated the epigenetic changes coupled with phenotypic alterations in the offspring through parental or early developmental nutritional conditions, which are transgenerationally inherited in the case of nutritional changes in paternal mice and nematodes in early development.

In mouse models of maternal nutritional changes, phenotypic and epigenetic changes occur up to the F2 generation, namely intergenerational inheritance. As an example of maternal nutritional influence only on the F1 generation, feeding a protein-restricted diet during pregnancy induces hypomethylation of the glucocorticoid receptor (*Gr*) and *Ppar-α* gene promoters, accompanied by their upregulation and that of downstream genes in juvenile and adult livers and impaired lipid homeostasis in F1 offspring [71–73].

Concerning the influence of maternal nutrition over generations, feeding of a poor nutritional diet in pregnant mice from E12.5 until birth results in inheritance of glucose intolerance in F1 as well as F2 generations through both male and female F1 parents [74]. Further study showed that maternal undernutrition during E12.5 to E18.5 causes locus-specific hypomethylation in intergenic regions and CGIs including regulatory elements as well as in nucleosome-retaining regions in adult F1 sperm [75]. Although the abnormal methylation status is lost in liver and brain in the F2 embryo, the glucose tolerance-related genes near the undernutrition-induced DMRs in F1 sperm persisted in abnormal expression in the F2 embryonic tissues, suggesting that the undernutrition had induced hypomethylation in F1 sperm, and influenced the expression of the genes nearby via uncharacterized mechanisms in F2 tissues [75]. A similar maternal undernutrition model from another research group demonstrated downregulation of lipogenic genes including the liver X receptor-alpha (*Lxra*) gene encoding an upstream TF regulating *de novo* lipogenesis, concomitant reduction of DNA methylation in its untranslated region (UTR) and enrichment of repressive histone modifications in the F2 liver [76]. In addition, the abnormal DNA methylation is also found in F1 sperm.

In the case of the influences of excess nutrition, a luciferase reporter knock-in mouse for the genomic imprinted *Dlk1* locus was applied to visualize epigenetic fidelity after maternal exposure to a HFD from E0.5 to E18.5 [77]. As a result, normally silent maternal *Dlk1* reporter is activated and expressed in tissues such as brain, liver, thyroid, testes and adipose tissues with increased DNA methylation in male and female F1 offspring. The mechanistic connection between the increased DNA methylation in *Dlk1* DMR and upregulation of the *Dlk1* reporter is currently unclear. However, the results showed that maternal HFD feeding induces the loss of imprinting in F1 offspring. In F2 progeny from maternally HFD-exposed F1 females but not males, the *Dlk1* reporter is variably expressed among tissues. DNA methylation of the *Dlk1* DMR in oocytes is not largely different between HFD-exposed and control F1 females, but the expression of some genes including those encoding miRNAs in the *Dlk1* cluster is upregulated in the HFD-exposed F1 oocytes, which may cause the misexpression of

Dlk1 in F2 offspring. The results suggest that two different epigenetic influences, i.e. altered DNA methylation in *Dlk1* DMR in F1 somatic tissues and aberrant upregulation of miRNAs in the *Dlk1* cluster in F1 oocytes by a maternal HFD, may result in loss of imprinting and its upregulation in F1, and misexpression of *Dlk1* in F2, respectively. These studies suggest that changes in maternal nutritional status during gestation, such as HFD or nutritionally poor diets, induce epigenetic changes in fetal germ cells, which influences F2 phenotypes.

In addition to maternal effects, a paternal HFD also affects offspring phenotypes associated with epigenomic alterations, which are transgenerationally inherited. Paternal obesity by HFD induces alteration of H3K4me3 in genes implicated in metabolic, inflammatory and developmental processes in F0 sperm, which is associated with the altered expression of metabolism-related genes in preimplantation embryos but not in the adult liver, and with metabolic dysfunctions in F1 adult male mice [7]. In this model, transgenerational susceptibility to metabolic abnormalities in F2 offspring is observed only when obese F0 have a pre-existing abnormally modified sperm epigenome by overexpression of a transgene for the histone demethylase KDM1A. This suggests that the risk of transgenerational transmission of abnormal nutrition-induced diseases may be greater if an ancestor has pre-existing damage to the sperm epigenome, possibly induced by prior exposure to adverse environments. Using the same *Kdm1a* overexpression male mice, it was demonstrated that developmental defects induced by KDM1A overexpression in one generation last for two subsequent generations without a *Kdm1a* transgene in germ cells [78, 79], implying a transgenerational influence of the abnormal histone modification in mammals. As in the case of paternal traumatic stress, as mentioned above, paternal HFD also alters miRNA expression in F0 sperm [80], although the relationship between alteration of miRNA and phenotypic changes is currently unclear.

As in the case of the influences of stresses, parental nutritional status also affects epigenomic changes in offspring in a non-model animal, wild Guinea pig, and low protein diet results in DNA methylation changes in liver and testis in F1 male [81]. The transgenerational influence of nutritional changes has also been studied in *C. elegans*. Starvation at the larval stage induces expression of small RNAs targeting several nutritional genes, which are inherited for at least three generations after returning the organism to nutritionally rich conditions, and these descendants have an increased lifespan compared to control organisms [82].

Cross-talk of Metabolites and Epigenetic Changes in the Fetus Induced by Maternal Environmental Factors

Epigenetic Regulation in Offspring via Metabolites from Maternal Gut Microbiota

The gut microbiota could modify the host epigenome and gene expression through microbiota-derived bioactive compounds [83, 84]. Epigenetic changes in somatic tissues mediated by gut microbial metabolites participate in metabolic disorders via alterations in intestinal permeability, immune responses, inflammatory reactions and insulin resistance [85]. In addition, gut microbiota in pregnant mothers could also influence fetal physiology and the epigenome via microbial metabolites.

Short chain fatty acids (SCFAs) including butyrate, propionate and acetate are produced from carbohydrates and fibers by the gut microbiota as a product of anaerobic fermentation, but not in host cells. Rodent models showed that commensal microbiota-derived SCFAs emerge as central players mediating crosstalk between the microbiota and host [84, 86]. Since the maternal gut microbiota appears to constitutively supply SCFAs to embryos via the bloodstream, the plasma levels of SCFAs are significantly lower in germ-free (GF) mothers and their embryos than in the specific pathogen-free (SPF) controls, which are free from only mouse-specific pathogens. The offspring from GF mothers are susceptible to obesity, glucose intolerance and insulin resistance by HFD after weaning, because SCFAs supplied from the mother are sensed by SCFA receptors, G-protein coupled receptor 41 (GPR41) and GPR43 expressed in the embryonic tissues including pancreatic β cells, and promote their differentiation and subsequent insulin secretion [87]. In addition to GPR activation by SCFAs, the expression of *Gpr43* may be stimulated by SCFAs. Studies showed that intracellular butyrate and propionate [88] as well as acetate [89] inhibit the activity of histone deacetylases (HDACs), implying a possible epigenetic mechanism of *Gpr43* gene expression by HDACs [87]. Meanwhile, it is currently unknown whether SCFAs could influence the epigenome of germ cells, but as mentioned below, the gut microbiota likely affects germ cells in *Drosophila*.

In *Drosophila*, transient exposure to low concentrations of oxidants during development leads to an extension of adult lifespan via selective depletion of *Acetobacter aceti*, one of the dominant species of gut microbiota in flies, which causes gut dysfunction and shortens the lifespan [90]. In addition, this effect of low-dose antioxidants can be passed to the next generation, which lives longer with decreased *A. aceti* without additional exposure to oxidants. Although its mechanism is currently unclear, the results suggest that changes in gut microbes in the parents by low-dose oxidants transmit some signals that influence the epigenome of their germ cells. In addition to shortened life span, depletion of *A. aceti* in *Drosophila* also causes repression of oogenesis and enhancement of embryonic development of a subsequent generation [91]. Taken together, dysbiosis of the parental microbiota likely influences the epigenome of the offspring.

Another major gut-derived metabolite is folate [84]. Because folate is involved in SAM production, which serves as a methyl-donor in methylation of DNA and histones, maternal folate likely influences epigenetic changes in fetal germ cells after passing through the placenta. Supporting this idea, wild-type F2 mice from a mother carrying a mutation in methionine synthase reductase (*Mtrr*), a gene involved in folate metabolism, show congenital malformations, developmental delays and tissue-specific loss of DNA methylation [92]. In addition, maternal supplementation of methyl-donor increases DNA methylation at the CpG sites of A^{vy} alleles and suppresses the expression of the corresponding genes, resulting in changed coat color in the offspring [93]. However, it is currently unclear whether maternal gut bacteria-derived folate influences the epigenome of the offspring.

Metabolism of Fetal Germ Cells

Cellular metabolism is closely correlated to environmental conditions including nutrition, and in terms of metabolites that cause epigenetic changes, the metabolic state of germ cells is an important factor influencing their epigenome [10, 94, 95]. We previously showed that the major energy metabolic pathway is gradually converted from glycolysis to OXPHOS in fetal germ cells during differentiation, and that glycolysis plays a role in PGC specification and reprogramming to pluripotent stem cells [96]. In addition, the Tricarboxylic acid (TCA) cycle, Ser-Gly-one carbon metabolism (SGOC) including the folate and methionine cycles, is consistently upregulated in fetal male germ cells during differentiation [97],

and those pathways are closely related to epigenetic regulation via several metabolites such as α-ketoglutaric acid (α-KG), flavin adenine dinucleotide (FAD), succinate, fumarate in the TCA cycle and SAM in SGOC [98]. Meanwhile, in fetal female germ cells, mitochondrial metabolism as well as protein stability are increased during differentiation, suggesting their possible importance in oogenesis [97]. Further, pyruvate and fatty acid metabolism are involved in the early steps of oocyte maturation [99], and the DNA demethylase TET1, whose cofactor is α-KG, plays an important role in regulating meiosis through DNA demethylation of a subset of meiotic genes in oocytes [100].

The various above-mentioned environmental factors likely result in metabolic and subsequent epigenetic changes in the fetus as well as in fetal germ cells, which may consequently cause phenotypic alterations in the offspring. Among maternal environmental factors, certain chemicals, metabolites and proteins may pass through the placenta, while others may be metabolically converted in maternal tissues and by the gut microbiota, and resultant substances in the blood stream may be transferred to the fetus, which likely influences the epigenome of fetal germ cells. Yet, the connection among certain environmental factors, metabolites in parent and fetal germ cells and their epigenetic changes are currently largely unknown, which should be addressed in future studies.

Mechanisms Underlying TEI in Mammals

In the previous sections, we mainly discussed the correlation between phenotypic and related epigenetic changes through parental environmental factors in the offspring. We then focus on the mechanisms underlying TEI in more detail and discuss a possible causal relationship between environment-induced epigenetic changes and offspring phenotypes.

Causal Relationship between Changes in DNA Methylation of Germ Cells and Phenotypic Alterations in Offspring

Although a number of studies have shown that the transmission of DNA methylation changes by parental environmental factors to the next generation is correlated to changes in the expression of phenotype-related genes [24, 76], little information is available on whether changes in DNA methylation actually cause phenotypic consequences. Recently, a new method for DNA methylation editing enabled us to understand that artificially induced DNA methylation in particular gene loci is indeed transgenerationally inherited along with changes in their expression and related metabolic traits [101]. In this study, a previously established method to induce variable levels of de novo DNA methylation at promoter-associated CGIs by insertion of a CpG-free DNA cassette [102] was applied to two metabolic genes (*Ankrd26* and *Ldlr*) [101]. DNA hypermethylation and transcriptional downregulation of those genes are stably maintained across multiple cell divisions in the CpG-free DNA cassette-inserted mouse embryonic stem cells (ESCs), even after removal of the cassette. The manipulated ESCs were then injected into eight-cell embryos to generate DNA methylation-edited mice, whose descendants show hypermethylation and downregulation of *Ankrd26* and *Ldlr* along with related metabolic defects such as obesity and hypercholesterolemia across more than three generations. This study is therefore the first instance of transgenerational transmission of phenotypic changes induced by parental epigenetic alterations in mammals. Strikingly, the engineered CGI methylation was once erased in PGCs, and is faithfully re-established at early post-implantation stages of the subsequent generation. The nature of this DNA methylation memory is extremely interesting. It is likely that other epigenetic signatures or chromatin structures may temporally mediate altered DNA methylation, which may function as a heritable epigenomic memory to re-establish DNA methylation of the CGI in offspring. This possibility is further discussed in the following section. It is also an important open question whether CpG-free DNA insertion can be generally applied to a wide variety of genes harboring CGIs.

Transmission of Epigenomic Memories

The mechanisms of epigenomic memory in germ cells are crucial issues in understanding the transgenerational inheritance of environmentally induced phenotypic changes, because extensive epigenetic reprogramming in PGCs and fertilized eggs likely erases the altered epigenetic states [103]. For TEI, specific epigenomic information is likely protected from the reprogramming process and/or is converted into more robust chromatin information, which circumvents the programming in germ cells. A recent study suggested a TF-mediated control mechanism of epigenomic memory [104]. The study suggested that the DNA methylation status of genomic regions depends on binding of TF; TF-bound CpG sites remain hypomethylated before, during and after the reprogramming in germ cells and early embryonic cells, while TF-free CpG sites are hypermethylated before and after the reprogramming, which undergoes demethylation and re-methylation during the reprogramming (Fig. 3). This suggests that TFs impede methylation reprogramming of their bound loci, and act as carriers of epigenomic memory during germ cell and pre-implantation development.

Consistent with this hypothesis, a mouse model of maternal BPA exposure-induced obesity suggested TEI by environmental factors via TFs [105]. In this study, BPA exposure to pregnant mice from E7.5 to E13.5, which corresponds to the period of global DNA demethylation in PGCs, caused obesity in F1 through F6 offspring via both paternal and maternal lineages. Maternal exposure to BPA results in increased chromatin accessibility in *cis*-regulatory elements (CREs) containing binding motifs of CCCTC-binding factor (CTCF) within the *Fto* gene in F1 to F5 sperm. In addition, the CREs increase interaction with neighboring *Irx3* and *Irx5* genes in F3 sperm by maternal BPA exposure. *Irx3* and *Irx5* are involved in the differentiation of appetite-controlling neurons, and this interaction possibly affects those gene expressions. Importantly, deletion of the CTCF site in the *Fto* CRE results in restoration of the obesity phenotype induced by maternal BPA in the F2 and F3 mice, supporting the importance of the CTCF site in the *Fto* CRE for transgenerational inheritance of BPA-induced obesity. Because this study also showed hypomethylation of the *Fto* CRE in F3 sperm by maternal BPA; it is likely that TF binding by increased chromatin accessibility in the hypomethylated site may protect from its DNA re-methylation to serve the epigenomic memory (Fig. 3).

As an additional mechanism for epigenomic memory, interaction of different epigenetic machinery may also be involved. Maternal exposure to vinclozolin or DDT in rats alters sperm ncRNA expression in the F1 generation, whose sequences have substantial overlap with the sequences in the DMRs suggesting their interaction in the F1–F3 generations. In addition, the DMRs also have significant overlap with the differential histone retention sites in sperm, suggesting a potential role for RNA-directed DNA methylation as well as DNA methylation-directed histone retention [6]. This suggests the possibility that changes in certain

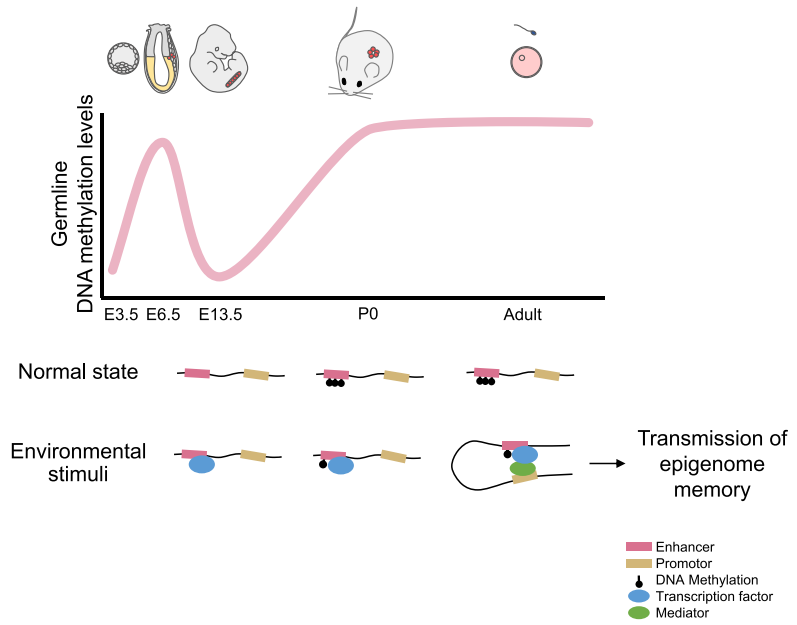

Figure 3: A model for the transmission of transcription factor (TF)-mediated epigenome memories by exposure to parental environmental factors during pregnancy. TF-binding sites escape epigenetic reprogramming in germ cells. TFs also mediate change in the chromatin structure, which is inherited by the next generation

types of epigenomic information are interpreted differently in sperm, which may subsequently result in altered chromatin structures and gene expression after fertilization.

Possible Biological Meaning of Epigenetic Inheritance and Future Perspectives

If environment-induced epigenetic changes are transgenetically inherited, what does this mean for organisms? As described above, many studies showed parental environmental factors negatively affect offspring; however, adaptation to chemically induced liver damage in rats, prolonged life span by low-dose oxidants in *Drosophila* and prolonged life span by starvation and avoidance behavior for pathogens by pathogenic stress in *C. elegans* are apparently positive influences for descendants. This supports the idea that environmentally induced heritable epigenetic alterations could, in some situations, play a role in the adaptation towards better fitness for living in a changing environment. Finding a positive influence of the parental environment on descendants is not as easy as finding a negative influence, which likely resulted in bias in the number of medical, stress and trauma studies. Meanwhile, there are positive outcomes in human studies on meditation [106, 107], exercise as mentioned above and also the EpiTrain project showing changes of DNA methylation by the training intervention [108], though their influences in germ cells are unknown. Therefore, it would be interesting to examine tolerance to parental-stress-related environments in offspring in an effort to identify positive consequences of the parental environment by using small organisms with a short lifespan, such as invertebrates and small fishes. Such an experimental approach may be helpful for further understanding of possible benefits of ancestral environmental influences on offspring. On the other hand, the negative effects of the parental environment on offspring could be useful for identifying pollutants and undesirable lifestyle habits in today's society, and even for predicting the susceptibility to some diseases in offspring based on parental epigenomic information.

Considering the possible involvement of epigenomic inheritance in adaptation to environmental changes over generations, and ultimately in evolution, the question remains of how long epigenomic inheritance could contribute to phenotypic adaptation in offspring in light of the short-term nature of epigenomic memory over generations. Finding examples of environmentally induced long-lasting epigenomic memory in future studies may shed light on this issue. Meanwhile, studies have suggested that methylated CpGs are prone to mutations, indicating the possibility that environmentally induced epigenetic changes are converted into gene mutations [109, 110]. Uncovering how non-mutagenic environmental factors could cause long-lasting heritable consequences in germ cells will help to understand the possible biological significance of TEI.

Data Availability

All data mentioned in this manuscript are piblished, and are properly cited.

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References

1. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. *Nature* 2019;**571**:489–99.
2. Fitz-James MH, Cavalli G. Molecular mechanisms of transgenerational epigenetic inheritance. *Nat Rev Genet* 2022;**23**:325–41.
3. Doyle TJ, Bowman JL, Windell VL *et al.* Transgenerational effects of Di-(2-ethylhexyl) phthalate on testicular germ cell associations and spermatogonial stem cells in mice. *Biol Reprod* 2013;**88**:112.
4. Stenz L, Escoffier J, Rahban R *et al.* Testicular dysgenesis syndrome and long-lasting epigenetic silencing of mouse sperm genes involved in the reproductive system after prenatal exposure to DEHP. *PLoS One* 2017;**12**:e0170441.
5. Skinner MK, Manikkam M, Tracey R *et al.* Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *BMC Med* 2013;**11**:1–16.
6. Beck D, Ben Maamar M, Skinner MK. Integration of sperm ncRNA-directed DNA methylation and DNA methylation-directed histone retention in epigenetic transgenerational inheritance. *Epigenetics Chromatin* 2021;**14**:6.
7. Pepin AS, Lafleur C, Lambrot R *et al.* Sperm histone H3 lysine 4 tri-methylation serves as a metabolic sensor of paternal obesity and is associated with the inheritance of metabolic dysfunction. *Mol Metab* 2022;**59**:101463.
8. Gapp K, Jawaid A, Sarkies P *et al.* Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nat Neurosci* 2014;**17**:667–9.
9. Lee HJ, Hore TA, Reik W. Reprogramming the methylome: erasing memory and creating diversity. *Cell Stem Cell* 2014;**14**:710–9.
10. Matsui Y, Hayashi Y. Metabolic pathways regulating the development and non-genomic heritable traits of germ cells. *J Reprod Dev* 2022;**68**:96–103.
11. Miao M, Yuan W, He Y *et al.* In utero exposure to bisphenol-A and anogenital distance of male offspring. *Birth Defects Res A Clin Mol Teratol* 2011;**91**:867–72.
12. Chou WC, Chen JL, Lin CF *et al.* Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. *Environ Health* 2011;**10**:94.
13. Zhang XF, Zhang LJ, Feng YN *et al.* Bisphenol A exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. *Mol Biol Rep* 2012;**39**:8621–8.
14. Wei Y, Han C, Li S *et al.* Maternal exposure to bisphenol A during pregnancy interferes ovaries development of F1 female mice. *Theriogenology* 2020;**142**:138–48.
15. Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. *JAMA Pediatr* 2014;**168**:61–7.
16. Prados J, Stenz L, Somm E *et al.* Prenatal exposure to DEHP affects spermatogenesis and sperm DNA methylation in a strain-dependent manner. *PLoS One* 2015;**10**:e0132136.
17. Tando Y, Hiura H, Takehara A *et al.* Epi-mutations for spermatogenic defects by maternal exposure to di(2-ethylhexyl) phthalate. *Elife* 2021;**10**:e70322.
18. Longnecker MP, Klebanoff MA, Zhou H *et al.* Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. *Lancet* 2001;**358**:110–4.
19. Valvi D, Mendez MA, Martinez D *et al.* Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: a prospective birth cohort study. *Environ Health Perspect* 2012;**120**:451–7.
20. Skinner MK, Manikkam M, Tracey R *et al.* Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *BMC Med* 2013;**11**:228.
21. King SE, McBirney M, Beck D *et al.* Sperm epimutation biomarkers of obesity and pathologies following DDT induced epigenetic transgenerational inheritance of disease. *Environ Epigenet* 2019;**5**:dvz008.
22. Maamar MB, Nilsson E, Sadler-Riggleman I *et al.* Developmental origins of transgenerational sperm DNA methylation epimutations following ancestral DDT exposure. *Dev Biol* 2019;**445**:280–93.
23. Song Y, Wu N, Wang S *et al.* Transgenerational impaired male fertility with an Igf2 epigenetic defect in the rat are induced by the endocrine disruptor p,p'-DDE. *Hum Reprod* 2014;**29**:2512–21.
24. Zeybel M, Hardy T, Wong YK *et al.* Multigenerational epigenetic adaptation of the hepatic wound-healing response. *Nat Med* 2012;**18**:1369–77.
25. Gold HB, Jung YH, Corces VG. Not just heads and tails: the complexity of the sperm epigenome. *J Biol Chem* 2018;**293**:13815–20.
26. Hammoud SS, Nix DA, Zhang H *et al.* Distinctive chromatin in human sperm packages genes for embryo development. *Nature* 2009;**460**:473–8.
27. 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.
28. Jung YH, Sauria MEG, Lyu X *et al.* Chromatin states in mouse sperm correlate with embryonic and adult regulatory landscapes. *Cell Rep* 2017;**18**:1366–82.
29. Pérez-Maldonado IN, Herrera C, Batres LE *et al.* DDT-induced oxidative damage in human blood mononuclear cells. *Environ Res* 2005;**98**:177–84.
30. Gassman NR. Induction of oxidative stress by bisphenol A and its pleiotropic effects. *Environ Mol Mutagen* 2017;**58**:60–71.
31. Kasahara E, Sato EF, Miyoshi M *et al.* Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl)phthalate. *Biochem J* 2002;**365**:849–56.
32. Tripathi A, Pandey V, Sahu AN *et al.* Di-(2-ethylhexyl) phthalate (DEHP) inhibits steroidogenesis and induces mitochondria-ROS mediated apoptosis in rat ovarian granulosa cells. *Toxicol Res* 2019;**8**:381–94.
33. Cho YJ, Park SB, Han M. Di-(2-ethylhexyl)-phthalate induces oxidative stress in human endometrial stromal cells in vitro. *Mol Cell Endocrinol* 2015;**407**:9–17.
34. Zhao ZB, Ji K, Shen XY *et al.* Di(2-ethylhexyl) phthalate promotes hepatic fibrosis by regulation of oxidative stress and inflammation responses in rats. *Environ Toxicol Pharmacol* 2019;**68**:109–19.
35. Wu Q, Ni X. ROS-mediated DNA methylation pattern alterations in carcinogenesis. *Curr Drug Targets* 2015;**16**:13–9.
36. Afanas'ev I. New nucleophilic mechanisms of ROS-dependent epigenetic modifications: comparison of aging and cancer. *Aging Dis* 2014;**5**:52–62.
37. O'Hagan HM, Mohammad HP, Baylin SB. Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA

methylation in an exogenous promoter CpG Island. *PLoS Genet* 2008;**4**:e1000155.
38. O'Hagan HM, Wang W, Sen S *et al.* Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer Cell* 2011;**20**:606–19.
39. Steenken S. Electron transfer in DNA? Competition by ultra-fast proton transfer? *Biol Chem* 1997;**378**:1293–7.
40. Campos ACE, Molognoni F, Melo FHM *et al.* Oxidative stress modulates DNA methylation during melanocyte anchorage blockade associated with malignant transformation. *Neoplasia* 2007;**9**:1111–21.
41. Hsu TC, Young MR, Cmarik J *et al.* Activator protein 1 (AP-1)- and nuclear factor kappaB (NF-kappaB)-dependent transcriptional events in carcinogenesis. *Free Radic Biol Med* 2000;**28**:1338–48.
42. WS W. The signaling mechanism of ROS in tumor progression. *Cancer Metastasis Rev* 2006;**25**:695–705.
43. MacLeod AR, Rouleau J, Szyf M. Regulation of DNA methylation by the Ras signaling pathway. *J Biol Chem* 1995;**270**:11327–37.
44. Zhang R, Kang KA, Kim KC *et al.* Oxidative stress causes epigenetic alteration of CDX1 expression in colorectal cancer cells. *Gene* 2013;**524**:214–9.
45. Li J, Shen S, Chen J *et al.* Accurate annotation of accessible chromatin in mouse and human primordial germ cells. *Cell Res* 2018;**28**:1077–89.
46. Blaize AN, Pearson KJ, Newcomer SC. Impact of maternal exercise during pregnancy on offspring chronic disease susceptibility. *Exerc Sport Sci Rev* 2015;**43**:198–203.
47. Harris JE, Baer LA, Stanford KI. Maternal exercise improves the metabolic health of adult offspring. *Trends Endocrinol Metab* 2018;**29**:164–77.
48. Carter LG, Lewis KN, Wilkerson DC *et al.* Perinatal exercise improves glucose homeostasis in adult offspring. *Am J Physiol Endocrinol Metab* 2012;**303**:1061–8.
49. Carter LG, Qi NR, De Cabo R *et al.* Maternal exercise improves insulin sensitivity in mature rat offspring. *Med Sci Sports Exerc* 2013;**45**:832–40.
50. Laker RC, Lillard TS, Okutsu M *et al.* Exercise prevents maternal high-fat diet-induced hypermethylation of the Pgc-1α gene and age-dependent metabolic dysfunction in the offspring. *Diabetes* 2014;**63**:1605–11.
51. Stanford KI, Lee MY, Getchell KM *et al.* Exercise before and during pregnancy prevents the deleterious effects of maternal high-fat feeding on metabolic health of male offspring. *Diabetes* 2015;**64**:427–33.
52. Raipuria M, Bahari H, Morris MJ. Effects of maternal diet and exercise during pregnancy on glucose metabolism in skeletal muscle and fat of weanling rats. *PLoS One* 2015;**10**:e0120980.
53. Sheldon RD, Nicole Blaize A, Fletcher JA *et al.* Gestational exercise protects adult male offspring from high-fat diet-induced hepatic steatosis. *J Hepatol* 2016;**64**:171–8.
54. Stanford KI, Takahashi H, So K *et al.* Maternal exercise improves glucose tolerance in female offspring. *Diabetes* 2017;**66**:2124–36.
55. Son JS, Liu X, Tian Q *et al.* Exercise prevents the adverse effects of maternal obesity on placental vascularization and fetal growth. *J Physiol* 2019;**597**:3333–47.
56. Mangwiro YTM, Cuffe JSM, Mahizir D *et al.* Exercise initiated during pregnancy in rats born growth restricted alters placental mTOR and nutrient transporter expression. *J Physiol* 2019;**597**:1905–18.
57. Mangwiro YTM, Cuffe JSM, Briffa JF *et al.* Maternal exercise in rats upregulates the placental insulin-like growth factor system with diet- and sex-specific responses: minimal effects in mothers born growth restricted. *J Physiol* 2018;**596**:5947–64.
58. Kusuyama J, Alves-Wagner AB, Conlin RH *et al.* Placental superoxide dismutase 3 mediates benefits of maternal exercise on offspring health. *Cell Metab* 2021;**33**:939–956.e8.
59. Seong KH, Li D, Shimizu H *et al.* Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell* 2011;**145**:1049–61.
60. Klosin A, Casas E, Hidalgo-Carcedo C *et al.* Transgenerational transmission of environmental information in *C. elegans*. *Science* 2017;**356**:320–3.
61. Schott D, Yanai I, Hunter CP. Natural RNA interference directs a heritable response to the environment. *Sci Rep* 2014;**4**:7387.
62. Moore RS, Kaletsky R, Murphy CT. Piwi/PRG-1 Argonaute and TGF-β mediate transgenerational learned pathogenic avoidance. *Cell* 2019;**177**:1827–1841.e12.
63. Kaletsky R, Moore RS, Vrla GD *et al. C. elegans* interprets bacterial non-coding RNAs to learn pathogenic avoidance. *Nature* 2020;**586**:445–51.
64. Franklin TB, Russig H, Weiss IC *et al.* Epigenetic transmission of the impact of early stress across generations. *Biol Psychiatry* 2010;**68**:408–15.
65. Weyrich A, Benz S, Karl S *et al.* Paternal heat exposure causes DNA methylation and gene expression changes of Stat3 in Wild Guinea pig sons. *Ecol Evol* 2016;**6**:2657–66.
66. Guerrero-Bosagna C, Morisson M, Liaubet L *et al.* Transgenerational epigenetic inheritance in birds. *Environ Epigenet* 2018;**4**:dvy008.
67. Ericsson M, Henriksen R, Bélteky J *et al.* Long-term and transgenerational effects of stress experienced during different life phases in chickens (*Gallus gallus*). *PLoS One* 2016;**11**:e0153879.
68. Chango A, Pogribny IP. Considering maternal dietary modulators for epigenetic regulation and programming of the fetal epigenome. *Nutrients* 2015;**7**:2748–70.
69. Lillycrop KA, Burdge GC. Maternal diet as a modifier of offspring epigenetics. *J Dev Orig Health Dis* 2015;**6**:88–95.
70. Lee HS. Impact of maternal diet on the epigenome during in utero life and the developmental programming of diseases in childhood and adulthood. *Nutrients* 2015;**7**:9492–507.
71. Burdge GC, Phillips ES, Dunn RL *et al.* Effect of reduced maternal protein consumption during pregnancy in the rat on plasma lipid concentrations and expression of peroxisomal proliferator-activated receptors in the liver and adipose tissue of the offspring. *Nutr Res* 2004;**24**:639–46.
72. Lillycrop KA, Phillips ES, Jackson AA *et al.* Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 2005;**135**:1382–6.
73. Lillycrop KA, Slater-Jefferies JL, Hanson MA *et al.* Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *British J Nutr* 2007;**97**:1064–73.
74. Jimenez-Chillaron JC, Isganaitis E, Charalambous M *et al.* Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. *Diabetes* 2009;**58**:460–8.
75. Radford EJ, Ito M, Shi H *et al.* In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science* 2014;**345**:1255903.

76. Martínez D, Pentinat T, Ribó S *et al.* In utero undernutrition in male mice programs liver lipid metabolism in the second-generation offspring involving altered *Lxra* DNA methylation. *Cell Metab* 2014;**19**:941–51.
77. Van de Pette M, Dimond A, Galvão AM *et al.* Epigenetic changes induced by in utero dietary challenge result in phenotypic variability in successive generations of mice. *Nat Commun* 2022;**13**:2464.
78. Siklenka K, Erkek S, Godmann M *et al.* Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science* 2015;**350**:aab2006.
79. Lismer A, Siklenka K, Lafleur C *et al.* Sperm histone H3 lysine 4 trimethylation is altered in a genetic mouse model of transgenerational epigenetic inheritance. *Nucleic Acids Res* 2020;**48**:11380–93.
80. Fullston T, Teague EMCO, Palmer NO *et al.* Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. *FASEB J* 2013;**27**:4226–43.
81. Weyrich A, Jeschek M, Schrapers KT *et al.* Diet changes alter paternally inherited epigenetic pattern in male Wild Guinea pigs. *Environ Epigenet* 2018;**4**:dvy011.
82. Rechavi O, Houri-Ze'Evi L, Anava S *et al.* Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. *Cell* 2014;**158**:277–87.
83. Qin Y, Wade PA. Crosstalk between the microbiome and epigenome: messages from bugs. *J Biochem* 2018;**163**:105–12.
84. Woo V, Alenghat T. Epigenetic regulation by gut microbiota. *Gut Microbes* 2022;**14**:2022407.
85. Li D, Li Y, Yang S *et al.* Diet-gut microbiota-epigenetics in metabolic diseases: from mechanisms to therapeutics. *Biomed Pharmacother* 2022;**153**:113290.
86. Alenghat T, Artis D. Epigenomic regulation of host-microbiota interactions. *Trends Immunol* 2014;**35**:518–25.
87. Kimura I, Miyamoto J, Ohue-Kitano R *et al.* Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science* 2020;**367**:eaaw8429.
88. Waldecker M, Kautenburger T, Daumann H *et al.* Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J Nutr Biochem* 2008;**19**:587–93.
89. Soliman ML, Rosenberger TA. Acetate supplementation increases brain histone acetylation and inhibits histone deacetylase activity and expression. *Mol Cell Biochem* 2011;**352**:173–80.
90. Obata F, Fons CO, Gould AP. Early-life exposure to low-dose oxidants can increase longevity via microbiome remodelling in *Drosophila*. *Nature Commun* 2018;**9**:975.
91. Elgart M, Stern S, Salton O *et al.* Impact of gut microbiota on the fly's germ line. *Nat Commun* 2016;**7**:11280.
92. Padmanabhan N, Jia D, Geary-Joo C *et al.* Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. *Cell* 2013;**155**:81–93.
93. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 2003;**23**:5293–300.
94. Verdikt R, Allard P. Metabolo-epigenetics: the interplay of metabolism and epigenetics during early germ cells development. *Biol Reprod* 2021;**105**:616–24.
95. Hayashi Y, Matsui Y. Metabolic control of germline formation and differentiation in mammals. *Sex Dev* 2022;**16**:382–97.
96. Hayashi Y, Otsuka K, Ebina M *et al.* Distinct requirements for energy metabolism in mouse primordial germ cells and their reprogramming to embryonic germ cells. *Proc Natl Acad Sci U S A* 2017;**114**:8289–94.
97. Hayashi Y, Mori M, Igarashi K *et al.* Proteomic and metabolomic analyses uncover sex-specific regulatory pathways in mouse fetal germline differentiation. *Biol Reprod* 2020;**103**:717–35.
98. Haws SA, Leech CM, Denu JM. Metabolism and the epigenome: a dynamic relationship. *Trends Biochem Sci* 2020;**45**:731–47.
99. Tanaka K, Hayashi Y, Takehara A *et al.* Abnormal early folliculogenesis due to impeded pyruvate metabolism in mouse oocytes. *Biol Reprod* 2021;**105**:64–75.
100. Yamaguchi S, Hong K, Liu R *et al.* Tet1 controls meiosis by regulating meiotic gene expression. *Nature* 2012;**492**:443–7.
101. Takahashi Y, Morales Valencia M, Yu Y *et al.* Transgenerational inheritance of acquired epigenetic signatures at CpG Islands in mice. *Cell* 2023;**186**:715–731.e19.
102. Takahashi Y, Wu J, Suzuki K *et al.* Integration of CpG-free DNA induces de novo methylation of CpG Islands in pluripotent stem cells. *Science* 2017;**356**:503–8.
103. Sasaki H, Matsui Y. Epigenetic events in mammalian germ-cell development: reprogramming and beyond. *Nat Rev Genet* 2008;**9**:129–40.
104. Kremsky I, Corces VG. Protection from DNA re-methylation by transcription factors in primordial germ cells and pre-implantation embryos can explain trans-generational epigenetic inheritance. *Genome Biol* 2020;**21**:118.
105. Jung YH, Wang HLV, Ruiz D *et al.* Recruitment of CTCF to an *Fto* enhancer is responsible for transgenerational inheritance of BPA-induced obesity. *Proc Natl Acad Sci U S A* 2022;**119**:e2214988119.
106. Chaix R, Alvarez-López MJ, Fagny M *et al.* Epigenetic clock analysis in long-term meditators. *Psychoneuroendocrinology* 2017;**85**:210–4.
107. Chaix R, Fagny M, Cosin-Tomás M *et al.* Differential DNA methylation in experienced meditators after an intensive day of mindfulness-based practice: implications for immune-related pathways. *Brain Behav Immun* 2020;**84**:36–44.
108. Lindholm ME, Marabita F, Gomez-Cabrero D *et al.* An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. *Epigenetics* 2014;**9**:1557–69.
109. Skinner MK, Guerrero-Bosagna C, Haque MM. Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations. *Epigenetics* 2015;**10**:762–71.
110. Anastasiadi D, Piferrer F, Wittkopp P *et al.* Epimutations in developmental genes underlie the onset of domestication in farmed European Sea Bass. *Mol Biol Evol* 2019;**36**:2252–64.