Lamarck rises from his grave: parental environment-induced epigenetic inheritance in model organisms and humans

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ABSTRACT

Organisms can change their physiological/behavioural traits to adapt and survive in changed environments. However, whether these acquired traits can be inherited across generations through non-genetic alterations has been a topic of debate for over a century. Emerging evidence indicates that both ancestral and parental experiences, including nutrition, environmental toxins, nurturing behaviour, and social stress, can have powerful effects on the physiological, metabolic and cellular functions in an organism. In certain circumstances, these effects can be transmitted across several generations through epigenetic (i.e. non-DNA sequence-based rather than mutational) modifications. In this review, we summarize recent evidence on epigenetic inheritance from parental environment-induced developmental and physiological alterations in nematodes, fruit flies, zebrafish, rodents, and humans. The epigenetic modifications demonstrated to be both susceptible to modulation by environmental cues and heritable, including DNA methylation, histone modification, and small non-coding RNAs, are also summarized. We particularly focus on evidence that parental environment-induced epigenetic alterations are transmitted through both the maternal and paternal germlines and exert sex-specific effects. The thought-provoking data presented here raise fundamental questions about the mechanisms responsible for these phenomena. In particular, the means that define the specificity of the response to parental experience in the gamete epigenome and that direct the establishment of the specific epigenetic change in the developing embryos, as well as in specific tissues in the descendants, remain obscure and require elucidation. More precise epigenetic assessment at both the genome-wide level and single-cell resolution as well as strategies for breeding at relatively sensitive periods of development and manipulation aimed at specific epigenetic modification are imperative for identifying parental environment-induced epigenetic marks across generations. Considering their diverse epigenetic architectures, the conservation and prevalence of the mechanisms underlying epigenetic inheritance in non-mammals require further investigation in mammals. Interpretation of the consequences arising from epigenetic inheritance on organisms and a better understanding of the underlying mechanisms will provide insight into how gene–environment interactions shape developmental processes and physiological functions, which in turn may have wide-ranging implications for human health, and understanding biological adaptation and evolution.

Key words: parental environment, acquired phenotype, epigenetic inheritance, DNA methylation, histone modification, small non-coding RNA.

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I. INTRODUCTION

Since the naturalist Jean Baptiste Lamarck famously proposed the inheritance of acquired traits, the ability of the environment to influence inherited traits independently of genetic sequence changes has been subject to debate (Droscher, 2015). Parental environmental factors, including nutrition, environmental toxins, nurturing behaviour, and social stress or fear, have been reported to play roles in shaping the phenotypes/traits of progeny across several generations (Lim & Brunet, 2013). To date, multiple environment-induced occurrences of epigenetic inheritance have been documented in antiviral and immune systems (Rechavi, Minevich & Hobert, 2011), metabolism (Carone et al., 2010; Ng et al., 2010; Ost et al., 2014; Rando & Simmons, 2015; Chen et al., 2016; Sharma et al., 2016), fertility (Anway et al., 2005; Katz et al., 2009), longevity (Greer et al., 2011, 2016), and emotionality (Weaver et al., 2004; Franklin et al., 2010; Gapp et al., 2014; Bohacek & Mansuy, 2015; Rodgers et al., 2015). It is widely accepted that organisms display physiological and behavioural alterations in response to changing environments and that they can also pass such changes on to their progeny. How these acquired traits are inherited from parents through non-DNA sequence-based modifications remains poorly understood (Heard & Martienssen, 2014).

Since the coining of this term over half a century ago (Waddington, 1959), ‘epigenetics’ has been employed in a broad sense to refer to the long-term or stable regulation of gene expression and function induced by environmental
factors without a change in DNA sequence. Epigenetic modulations encompass covalent chemical modifications of DNA (Razin & Riggs, 1980) and histone (Strahl & Allis, 2000) as well as the production of non-coding RNAs such as small interfering RNA (siRNA), microRNA (miRNA), Piwi-interacting RNA (piRNA), and transfer RNA (tRNA) fragments (Bartel, 2004; Flanagan & Wild, 2007; Mohammad et al., 2012; Chen et al., 2016; Sharma et al., 2016). Increasing evidence suggests that certain epigenetic changes, such as DNA methylation (Morgan et al., 1999; Rakyan et al., 2000; Carone et al., 2010; Franklin et al., 2010; Padmanabhan et al., 2013; Radford et al., 2014; Wei et al., 2014), histone modification (Katz et al., 2009; Greer et al., 2011; Seong et al., 2011; Ost et al., 2014; Siklenka et al., 2015), siRNA (Rechavi et al., 2011, 2014; Conine et al., 2013; Houriet-Ze’evi et al., 2016), miRNAs (Gapp et al., 2014; Rodgers et al., 2013; He et al., 2016), piRNA (Ashe et al., 2012), and tRNA fragments (Chen et al., 2016; Sharma et al., 2016) (Table 1) can be transmitted from parents to their offspring through the germline. Thus, the inheritance of epigenetic states may allow organisms to deliver either adaptive or non-adaptive/pathological information related to the ancestral environment to their offspring.

Depending on the number of subsequent generations that express acquired traits similar to their ancestors but were not exposed to the environment that triggered the epigenetic change, three main models of transmission of parental experience-induced alterations have been proposed (Daxinger & Whitelaw, 2012; Siklenka et al., 2015; Szfyf, 2015): (i) intergenerational (or cross-generational) effects, such as the impact of in utero or paternal exposure to particular nutritional or stress environments on the developing embryo and its germline (only the F1 generation) (Carone et al., 2010; Ost et al., 2014; Radford et al., 2014); (ii) multigenerational effects (from the F1 to the F2 generation) (Dias & Ressler, 2014); and (iii) transgenerational effects that are found in more than three generations (Anway et al., 2005; Greer et al., 2011; Rechavi et al., 2011). Herein, we describe the epigenetic evidence for the inheritance of parental environment-induced phenotypes in nematodes, fruit flies, zebrafish, rodents, and humans acquired under diverse environmental conditions. To provide a broad view of epigenetic inheritance, we surveyed extensive examples of environmentally induced-epigenetic inheritance including transgenerational, multigenerational, and intergenerational effects. To understand better the underlying mechanisms and molecular marks associated with these acquired phenotypes, the epigenetic modifications recently demonstrated to be both susceptible to modulation by environmental cues and heritable, specifically, DNA methylation, histone modification, and small non-coding RNAs, have been summarized. We are particularly interested in environment-induced transgenerational effects that have been suggested to be transmitted through maternal and paternal germlines and in a gender-specific manner. Overall, a better understanding of epigenetic inheritance and the consequences of such acquired changes on individuals will likely provide insight into how gene–environment interactions shape development and physiological function.

II. WHAT ARE THE MECHANISMS UNDERLYING PARENTAL ENVIRONMENT-INDUCED EPIGENETIC INHERITANCE?

Epigenetic regulations including DNA methylation, histone modifications, and non-coding RNA, have been linked to the effects of environmental factors on physiology and behaviour (Tsankova et al., 2007). Epigenetic information undergoes extensive reprogramming in both gametes and the early embryo during normal germline transmission (Reik, 2007; Seisenberger et al., 2012; Seisenberger, Peat & Reik, 2013; Messerschmidt, Knowles & Solter, 2014). This is believed to enable the resetting of a largely naive state free of potentially deleterious marks caused by environmental impacts and the return to pluripotency in the zygote. In mammals, the epigenetic information that escapes the two major waves of epigenetic reprogramming, which occur after fertilization and during gametogenesis in the germline, represents a plausible transgenerational carrier of environmental information (Fig. 1) (Seisenberger et al., 2013). Thus, the inheritance of epigenetic information and germline reprogramming represent two sides of the same coin. How the patterns of epigenetic modifications are established and maintained in the specific tissues or cells of progeny to escape two waves of reprogramming has been the central question for epigenetic inheritance study.

(1) DNA methylation

Over the course of evolution, different epigenetic regulatory processes have become specialized in vertebrates and invertebrates. In mammals, DNA methylation occurs mainly at the palindromic dinucleotide sequence 5′-CpG-3′, specifically at the 5 position of cytosine (5mC), and comprises one of the major epigenetic regulations in mammalian cells (Bird, 2002). By contrast, 5mC is relatively sparse in invertebrates such as Caenorhabditis elegans (Greer et al., 2015; Hu et al., 2015) and Drosophila melanogaster (Lyko & Malezka, 2011; Raddatz et al., 2013; Zhang et al., 2015). DNA methylation machinery is relatively conserved among mammals (Hernando-Herraez et al., 2015). However, DNA methylation status may vary between different tissues and developmental stages (Bocklandt et al., 2011; Fritsche et al., 2013; Hannum et al., 2013) and can be directly modulated by extrinsic environmental cues. Notably, the heritability of DNA methylation has marked it as an attractive feature in the study of epigenetic inheritance. Specifically, during DNA replication, DNA methylation of newly generated CpG sequences occurs across from a methylated CpG in the parental strand, thus representing a biochemical mechanism for replicating an epigenetic mark (Law & Jacobsen, 2010). In mammals, DNA methylation is enzymatically catalysed by three active DNA methyltransferases DNMT3A, DNMT3B.
| Species | Models | Acquired phenotypes | Transmission | Epigenetic marks | References |
|---------|--------|---------------------|--------------|------------------|------------|
| Human   | Imprinting defect | Angelman and Prader-Willi syndrome | F1, F2 | DNA methylation of IGF2 | El-Maarri et al. (2001) and Buiting et al. (2003) |
| Mouse   | Taiwanese phenotype | Coat color and metabolic disorders | F1, F2, F3, F4 | DNA methylation of IGF2 | Anway et al. (2005) and Cropley et al. (2006) |
| Avy     | Coat color and metabolic disorders | F1, F2 | DNA methylation of IGF2 | Cropley et al. (2006) |
| AxinFu  | Coat color and metabolic disorders | F1, F2 | DNA methylation of IGF2 | Rakyan et al. (2003) and Waterland et al. (2006) |
| CUMS    | Depression-like behavior | F1 male, F2 female, F3 male and female | DNA methylation of Mecp2, Cb1 and Crfr2 | Franklin et al. (2010) |
| CUMS    | Developmental delay, congenital malformations | F1, F2, F3, F4, F5 | Reduction in DNA methylation | Padmanabhan et al. (2013) |
| Odour exposure | Heightened startle response | F1, F2 | CpG hypomethylation of the Olfr151 gene | Dias & Ressler (2014) |
| In utero undernourishment | Low birth mass and multiple metabolic defects | F1 | Altered DNA methylation in sperm | Radford et al. (2014) |
| Rat     | Increased maternal care | Decreased fearfulness and responses to stress | F1 female | Alterations of DNA methylation and histone modification of the Nr3c1 promoter | Weaver et al. (2004) |
| C. elegans | Paramutation | White tail tip and white feet | F1, F2, F3 | Redesigned H3K4me2 in sperm | Rassoulzadegan et al. (2006) |

CpG, cytosine–phosphate–guanine; CUMS, chronic and unpredictable maternal separation; dsRNA, double-stranded RNA; H3K4me2, histone H3 lysine 4 dimethylation; IGMR, intergenerational metabolic reprogramming; KDM1A, lysine (K) demethylase 1A; let-7, lethal-7; miRNAs, microRNA; piRNA, Piwi-interacting RNA; sRNA, small RNA; tRFs, transfer RNA fragments; tRNA, transfer RNA; HPA-axis, hypothalamic–pituitary–adrenal-axis; IGAR, intergenerational metabolite reprogramming; KDM, lysine (K) demethylase; 22G-RNA, small RNAs (22G-RNA).
Fig. 1. Epigenetic information undergoes extensive reprogramming both in the gametes and the early embryo during normal germline transmission. In mammals, the epigenetic information that escapes the two major waves of epigenetic reprogramming occurring after fertilization and during sperm development in the germline is inherited and represents a plausible transgenerational carrier of environmental information. In particular, in different stages of male and female germ cell development, such as during embryogenesis and early postnatal life, epigenetic regulations, including DNA methylation (Reik, 2007; Seisenberger et al., 2012, 2013; Messerschmidt et al., 2014), 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) (Hackett et al., 2013; Wang et al., 2014) and histone modifications such as H3K9me2, H3K9ac, and H3K27me3 at certain genes (Hammoud et al., 2009; Bryczynska et al., 2010; Liu et al., 2016), as well as RNA granules, such as processing bodies (P-bodies) (Brengues, Teixeira & Parker, 2005), present dynamic changes that are displayed as gradient colours.

and DNMT1 (Klose & Bird, 2006; Li & Zhang, 2014). Among them, DNMT1 is highly specific for the hemi-methylated CpGs generated during DNA replication and faithfully copies CpG methylation from the parental to the daughter strand (Fatemi et al., 2001; Goll & Bestor, 2005; Sharif et al., 2007), whereas, DNMT3A and DNMT3B play critical roles in establishment of de novo DNA methylation patterns during development (Smith & Meissner, 2013).

DNA methylation in both the female and male germlines is largely eliminated from the genome during gametogenesis and post fertilization, seemingly diminishing the possibility of replicative inheritance. However, it is now clear that whereas the erasure of DNA methylation during two waves of genome-wide reprogramming is fairly extensive, it is not complete (Guibert, Forne & Weber, 2012; Seisenberger et al., 2012; Hackett et al., 2013). Epigenetic cytosine methylation states can escape erasure following fertilization and be largely maintained at differentially methylated regions of imprinted genes (parent-of-origin-specific allelic expression). For example, individuals who were prenatally exposed to famine during the Dutch ‘Hunger Winter’ exhibited reduced levels of DNA methylation at the imprinted IGF2 locus (paternally expressed) compared with unexposed siblings (Heijmans et al., 2008). Moreover, repetitive sequences, such as the retrovirus element intracisternal A-particle (IAP) in Avy (Cropley et al., 2006) and Axin<sup>E</sup> mice (Rakyan et al., 2003), have been shown to exhibit incomplete demethylation from post-fertilization to preimplantation development (Lane et al., 2003). Thus, it is likely that imprinted genes and repetitive sequences may function as molecular carriers to transmit epigenetic memory to the progeny. Therefore, DNA methylation has the potential to act as a heritable epigenetic mark, although it remains unclear whether the same mechanism of heritability during DNA replication can be extended to meiosis to enable non-genetic inheritance across generations.

(2) Histone modification

Chromatin protein modification occurs through the chemical modification of the N-terminal tails of histones via methylation (Jenuwein, 2001), acetylation (Wade, Pruss & Wolffe, 1997), phosphorylation (Rosetto, Avvakumov & Cote, 2012), sumoylation [the covalent attachment of the ubiquitin-related modifier (SUMO) to proteins] (Shiio & Eisenman, 2003), and ubiquitination (the covalent attachment of ubiquitin to proteins) (Shilatifard, 2006). Histone methylations have been observed to play a role in the inheritance of acquired phenotypes in species, such as C. elegans, D. melanogaster, and Schizosaccharomyces pombe (Katz et al., 2009; Ost et al., 2014; Audergon et al., 2015) from which DNA methylation is largely absent. In addition, species
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Agrawal, 2012; Norouzitallab adapted features

Artemia the shrimp

methylation and histone modification, such as plants and exhibiting epigenetic regulations mediated by both DNA transcription in germ cells, are specific to gamete-mediated epigenetic inheritance (Ashe et al., 2012). Furthermore, it was recently demonstrated that rRNA-derived small RNA fragments in sperm represent a paternal epigenetic factor and contribute to intergenerational inheritance of paternal high-fat or low-protein diet-induced metabolic disorders, suggesting roles for these molecules in transmitting metabolic disorders from father to offspring (Chen et al., 2016; Sharma et al., 2016).

III. WHICH EPIGENETIC MODIFICATIONS AND TRAITS CAN BE INHERITED FROM PARENTAL ENVIRONMENTS?

The first example of epigenetic inheritance was reported in plants following the discovery of transposable elements by McClintock (1953). In particular, because the plant germ line develops from somatic cells exposed to developmental and environmental cues, the inheritance of acquired traits through epigenetic regulation is extensive throughout this system (Heard & Martiensen, 2014). Subsequently, the epigenetic inheritance of gene silencing, antiviral and immune responses, fertility, metabolism, emotional traits, olfactory imprinting and odour-fear conditioning, and longevity has been described in the progeny of nematodes, fruit flies, zebrafish, rodents, and humans, as detailed below. The growing body of evidence suggests that epigenetic inheritance could occur in most species.

(1) Evidence for epigenetic inheritance in Caenorhabditis elegans

Nematodes have acquired specialized epigenetic machineries during evolution. In particular, the model organism C. elegans has a highly efficient RNAi system, including an extended family of Argonaute proteins (Yigit et al., 2006) and the small RNA amplification process mediated by a short RNA-dependent RNA polymerase (RdRP) (Smardon et al., 2000; Sijen et al., 2001). DNA methylation mainly occurs at adenine N6 (6mA) in C. elegans rather than at 5mC as in most other organisms (Greer et al., 2015). Accordingly, C. elegans has been used to study transgenerational epigenetic effects resulting from siRNA (Fire et al., 1998; Rechavi et al., 2011, 2014) and histone modifications (Katz et al., 2009; Greer et al., 2011), rather than from DNA methylation (Fig. 2).

(a) Exogenous double-stranded RNA-induced epigenetic inheritance

Double-stranded RNA (dsRNA) can trigger the sequence-specific degradation of messenger RNA (mRNA) via the process known as RNAi (Fire et al., 1998; Kennerdell & Carthew, 1998; Ngo et al., 1998; Waterhouse, Graham & Wang, 1998). In C. elegans, dsRNA is able to spread across cellular boundaries and can be transmitted from the soma to the germ line (Fire et al., 1998), thereby allowing the transgenerational inheritance of silencing responses to foreign transcribed DNA introduced into the species. For

(3) Small non-coding RNAs

Changes in epigenetic marks in germ cells have been reported after exposure to a range of environmental triggers. However, conclusive proof for their direct involvement in information transfer across generations only exists for RNA. By means of deep sequencing, a large number of RNAs of all classes have been identified in growing oocytes (Tam et al., 2008; Watanabe et al., 2008). Although spermatozoa have a highly condensed nucleus and contain little cytoplasm, a large portion of the RNA population has been detected in sperm (Peng et al., 2012; Chen et al., 2016; Sharma et al., 2016). This observation suggests that RNAs may be involved in the transmission of acquired phenotypes. Initially, miRNAs and siRNAs, two types of small non-coding RNAs (sncRNAs), that function within the RNA interference (RNAi) pathway through base-pairing with complementary sequences within target RNA molecules, were shown to be involved in parental environment-induced epigenetic inheritance (Fire et al., 1998; Lau et al., 2006; Rassoulzadegan et al., 2006; Rechavi et al., 2011, 2014; Shirayama et al., 2012; Grandjean et al., 2015). piRNAs, which specialize in targeting transposon transcription in germ cells, are specific to gamete-mediated
Fig. 2. Inheritance paradigm and molecular mechanisms underlying transgenerational inheritance in *Caenorhabditis elegans*. (A) Epigenetic inheritance in *C. elegans* is induced by various environmental factors including double-stranded RNA (dsRNA) or foreign virus, starvation, and Piwi-interacting RNA (piRNA), through exogenous or endogenous RNA interference (RNAi) pathways. Upon entering the cytoplasm, exogenous dsRNAs are cleaved by the double-stranded RNA-specific endoribonuclease (DICER)-1 to produce small interfering RNAs (siRNAs) that are targeted to sequence-specific messenger RNA (mRNA) by the Argonaute protein RNAi defective-1 (RDE-1) (Rechavi et al., 2011; Buckley et al., 2012; Gu et al., 2012). In the endo-RNAi pathway, starvation triggers RDE-4-dependent siRNAs in the soma and heritable RNAi defective-1 (HRDE-1)-dependent siRNAs in germ cells to induce changes in the longevity of progeny worms (Rechavi et al., 2014). In addition, the silencing triggered by both piRNAs and environmental RNAi is transmitted across worm generations through chromatin factors, such as the histone modification H3K9me3, and the worm-specific Argonaute-9 (WAGO-9)–22G-RNA pathway (WAGO–22G-RNA pathway). (B) Genetic manipulation or a history of genetic manipulation in worm ancestors induces epigenetic inheritance in *C. elegans*. spr-5 mutation induces gradual accumulation of H3K4me2 and infertility in their descendants (Katz et al., 2009). The lifespan of genetically manipulated wild-type descendants from *wdr-5, ash-2*, or *set-2* mutants was extended for up to four generations via retinoblastoma-related binding protein 2 (RBR-2) and H3K4me3 (Greer et al., 2011). (C) General scheme of transgenerational inheritance in *C. elegans*. endo-siRNA, endogenous siRNA; exo-siRNA, exogenous siRNA; HPL-2, heterochromatin protein 1 (HP1)-like protein 2.

example, it has been found that the interference effects induced by exogenous dsRNA that targeted genes such as *pos-1*, *mom-2*, or *sgg-1* could be transmitted through the germline of *C. elegans* for two or three generations (Grishok, Tabara & Mello, 2000). Owing to the short generation time of a worm (approximately 3 days), however, this 2–3 generational effect could simply be explained by the dilution of the silencing factors. However, a convincing study of dsRNA-induced epigenetic inheritance in worms demonstrated that the transcriptional silencing mediated by direct injection of dsRNA or by feeding the worms bacteria that expressed dsRNA could persist for more than 80 generations in the absence of trigger RNA (Vastenhouw et al., 2006).
Further studies aimed to uncover the biological function underlying the transmission of RNAi-mediated gene-silencing effects in *C. elegans*. In these, the Flock House virus, a plus-strand RNA nodavirus, was introduced into the worms to generate siRNAs and initiate a defence against the virus (Rechavi *et al.*, 2011). The antiviral effect was reported to persist for 50 generations in a manner independent of the virus that had generated it (Rechavi *et al.*, 2011). In such conditions, an acquired ability to respond to specific viruses through the production of targeted siRNAs might serve as an epigenetic defence mechanism against viruses in *C. elegans*. Thus, the ability to transmit information such as siRNAs derived from ancestral exogenous virus exposure to descendants might provide adaptive benefits for future generations. Furthermore, once the environments of the progeny and the ancestors differ, the long-term transmission of epigenetic responses may be adaptive as well (Houri-Ze’evi *et al.*, 2016). For example, the duration of epigenetic inheritance can be extended by exposure to ‘secondary dsRNA triggers’ that target a gene unrelated to the primary dsRNA trigger (Houri-Ze’evi *et al.*, 2016). Such an extension of an ancestral heritable RNAi-based response is determined by feedback between exogenous siRNA (exo-siRNA) and endogenous RNAi genes, in that the exo-siRNA inheritance system is initiated by an RNAi response that produces exo-siRNAs at the expense of endogenous siRNA (endo-siRNA) populations (Houri-Ze’evi *et al.*, 2016). Therefore, ‘secondary triggers’ can initiate new RNAi responses to extend the inheritance of ancestral silencing and reset the ‘transgenerational timer’ (Houri-Ze’evi *et al.*, 2016). By contrast, altering the regulation of endo-siRNAs that target genes required for the inheritance of siRNAs has the potential to turn off the siRNA inheritance mechanism. Considering that ancestral responses would likely be detrimental if the parental environment differed from that of the progeny, a mechanism capable of fixing these epigenetically driven phenotypic changes and of adaptive control of the duration of environmental responses might be of particular relevance and could thus affect the course of evolution. However, considering that worms are self-fertilizing and crossed under experimentally controlled conditions, whether the long-term transmission of epigenetic responses might be adaptive in more complex organisms such as vertebrates remains an open question.

With respect to the mechanism underlying multigenerational RNAi inheritance, multiple studies have consistently revealed that dsRNA-induced epigenetic inheritance in *C. elegans* involves nuclear RNAi and chromatin factors such as Argonaute proteins and small RNA pathways mediating H3K9 methylation (H3K9me) (Burton, Burkhart & Kennedy, 2011; Buckley *et al.*, 2012; Gu *et al.*, 2012). For example, the exposure of worms to dsRNA can trigger the locus-specific enrichment of H3K9me3, preferentially surrounding the targeting sequences of RNAi, in diverse genes (Gu *et al.*, 2012). In particular, the H3K9me3 marks have been shown to be maintained in the absence of dsRNA for at least two generations before being lost (Gu *et al.*, 2012). These results provided direct evidence that dsRNA-induced chromatin modifications persist through generations. In a related study, it was found that an Argonaute protein nuclear RNAi defective 3 (NRDE-3), together with other components of the nuclear RNAi pathway, maintained heritable RNAi silencing, siRNA expression, and H3K9me at the specific genomic sites targeted by RNAi in the progeny of worms exposed to dsRNA (Burton *et al.*, 2011). The above studies represent examples that nuclear RNAi promotes RNAi inheritance in somatic tissues, suggesting that the chromatin and RNAi machinery may form a self-reinforcing loop that coordinates and maintains dsRNA-induced transgenerational effects.

The nuclear gene-silencing events are key determinants of RNAi memory in diverse cell types of *C. elegans*, including not only somatic tissues but also germ cells (Buckley *et al.*, 2012). In a genetic screen for nematodes defective in transmitting RNAi silencing signals to future generations, Buckley *et al.* (2012) identified the heritable RNAi defective 1 (hrde-1) gene, encoding an Argonaute protein that associates with siRNAs, in the germ cells of progeny of worms exposed to dsRNA. In the nuclei of these germ cells, HRDE-1 engages the nuclear RNAi defective pathway to direct the trimethylation of H3K9 at RNAI-targeted genomic loci and promote RNAi inheritance (Buckley *et al.*, 2012). These results suggest a model in which endogenous heritable RNAs that engage HRDE-1 act as specificity factors to direct epigenetic maintenance in the germ-cell lineage.

(b) Endo-siRNA-induced epigenetic inheritance

In *C. elegans*, endo-siRNAs are mainly formed from RdRP transcripts, which are produced as part of the response to exo-siRNA and as part of the endogenous gene regulatory mechanisms mentioned previously (Han *et al.*, 2009). In particular, 22G-RNAs, which have a length of ∼22 nucleotides (nt) and possess a triphosphorylated 5′ guanosine (G), comprise the most abundant type of endo-siRNA in *C. elegans* (Pak & Fire, 2007). Recently, it was found that the absence of food in the first larval stage could induce changes in the levels of 22G-RNAs and in the worm lifespan (Rechavi *et al.*, 2014). These starvation-induced changes in 22G-RNA levels and longevity were passed on for at least three generations in the presence of sufficient food (Rechavi *et al.*, 2014). Multiple genes involved in fat regulation, protein turnover (a balance between protein synthesis and protein degradation), stress resistance, longevity, and reproduction were found to be regulated by 22G-RNAs in response to starvation (Rechavi *et al.*, 2014). Furthermore, this transgenerational transmission of starvation-induced 22G-RNAs was shown to be dependent on the germline expression of nuclear Argonaute heritable RNAi defective 1 (HRDE-1 or WAGO-9) and of the dsRNA binding protein RDE-4, indicating that a specific RNAi pathway was involved (Rechavi *et al.*, 2014). This was the first study to demonstrate that an environmental nutrition-induced endogenous response can trigger small RNA inheritance. In addition, this study suggests that epigenetic inheritance is
not limited to the defence against exogenous viruses but also represents a general adaptive phenomenon.

(c) piRNA-induced epigenetic inheritance

piRNAs, identified by large-scale sequencing of small RNAs in *C. elegans*, are a class of sncRNAs characterized as being 21 nucleotides in length with a 5′ uracil, therefore alternatively termed 21U-RNAs (Ruby et al., 2006; Batista et al., 2008; Wang & Reinke, 2008). To date, over 15000 unique 21U-RNAs have been identified, most of which are expressed in the germline to regulate endogenous targets (Baggin et al., 2012). Piwi-related gene 1 (PRG-1), a member of the Piwi family, is an Argonaute protein especially expressed in the germline and is known to promote, process, or stabilize 21U-RNAs expression. The expressed 21U-RNAs can in turn interact with PRG-1 to promote proper expression of spermatogenesis transcripts (Batista et al., 2008; Wang & Reinke, 2008). piRNAs have been found to trigger a multigenerational epigenetic memory of endogenous gene expression profile in *C. elegans* (Ashe et al., 2012).

Using a piRNA sensor in which a short sequence complementary to an endogenous piRNA was fused with a green fluorescent protein (GFP) reporter gene, Baggin et al. (2012) found that piRNAs and PRG-1 could trigger transcriptional silencing in *trans* by generating endogenous 22G-RNAs at imperfectly complementary sites. Notably, when single-copy transgenes consisting of an endogenous germline gene (piRNA) fused to a foreign sequence (environmental RNAi) were inserted at a given chromosomal site, the silencing triggered by both the piRNA and environmental dsRNA was permanent and transmitted through generations (Ashe et al., 2012; Shirayama et al., 2012). However, when single-copy transgenes were inserted into a prg-1 mutant, they could not be silenced and thus were expressed (Ashe et al., 2012; Shirayama et al., 2012). The maintenance of the silencing in the descendants, as found by both these studies, was dependent on chromatin factors such as H3K9me3, as well as on the worm-specific Argonaute (AGO)-9/WAGO-9/HRDE-1–22G-RNA pathway, rather than on PRG-1 (Ashe et al., 2012; Shirayama et al., 2012). They also ruled out the possibility that chromosomal pairing was required for transfer of the silent state. These results indicate that PRG-1 and piRNAs are required for the initial recognition of the foreign sequence but are not necessary to maintain the silencing.

Similar to the mechanisms underlying environmental RNAi-induced transgenerational effects, the transmission of piRNA-mediated silencing in *C. elegans* is dependent on common RNAi and chromatin pathways. Specifically, RdRP-dependent 22G-RNA biosynthesis, initiated by the piRNA/PRG-1 complex, is recognized by HRDE-1 that then triggers NRDE-mediated H3K9me3 at the targeted locus. The established H3K9me3 is transmitted to the progeny and results in transcriptional silencing even in the absence of PRG-1 (Ashe et al., 2012; Shirayama et al., 2012). These findings suggest the means by which an individual worm can utilize RNA-induced epigenetic silencing to detect foreign sequences and to protect bona fide germline transcripts from one generation to the next.

Related to piRNA-induced gene silencing of foreign sequences, an essential Argonaute protein chromosome segregation and RNAi deficient 1 (CSR-1), which is particularly abundant in mature sperm, represents a candidate factor for self-recognition of endogenous germline-expressed transcripts to evade piRNA-induced silencing (Seth et al., 2013; Wedeles, Wu & Claycomb, 2013). It has been found that CSR-1, together with two other argonaute like genes ALG-3 and ALG-4, can transmit and amplify the memory of spermatogenesis gene expression to offspring (Conine et al., 2013). Specifically, the heterozygous offspring (carrying only one mutant allele) of homozygous alg-3/4 (carrying two mutant alleles) exhibit reduced fertility in *C. elegans* (Conine et al., 2013). Repeated backcrossing (five times) heterozygous hermaphrodites (worms having both male and female reproductive organs) to homozygous mutant males resulted in a progressive loss of fertility in heterozygous descendants (Conine et al., 2013). Mechanistically, male sperm were found to harbour an extensive repertoire of CSR-1-associated small RNAs targeting oogenesis-specific mRNAs (Conine et al., 2013). These studies provide a clear insight regarding how *C. elegans* can recognize self from non-self and transmit a memory of gene expression in the form of Argonaute/small RNA complexes from preceding generations.

(d) Histone methylation and the epigenetic inheritance of fertility

In *C. elegans*, primordial germ cells undergo a massive and rapid loss of histone H3 lysine 4 dimethylation (H3K4me2) to erase the memory of zygotic transcription (Schaner et al., 2003). The histone demethylase suppressor of preselin (spr)-5 may participate in this resetting process in the worm, thereby preventing a decline of epigenetic stability and viability in the germ cells. Katz et al. (2009) showed that loss of spr-5 results in progressive sterility across generations. They further demonstrated that the gradual accumulation of H3K4me2 in the two primordial germ cells Z2 and Z5 occurred in a spr-3 mutant and became evident over many worm generations. Accordingly, genes associated with spermatogenesis were gradually derepressed in these worms (Katz et al., 2009). Conversely, the expression of spermatogenesis genes dramatically decreased in sperm after 20 generations despite the continual increase in H3K4me2 levels. This phenomenon correlated with a decline in worm fertility and brood size as well as with eventual death of the germ cells (Katz et al., 2009). Notably, they performed a single cross of the homozygous mutant worms with wild-type worms to generate heterozygous demethylase activity in the germline. Following this, the heterozygous worms were returned to a homozygous mutant state to score for sterility. Their results indicated that the outcrossing decreased the frequency of sterility to wild-type levels in the first generation after hemizygosity, suggesting that the most severely sterile phenotype was completely rescued after a single cross (Katz et al., 2009). This study provided
the first evidence that histone marks serve as important mechanisms for epigenetic inheritance. However, as gene expression and H3K4me2 levels were measured in a mixed population of worms at all developmental stages in this study, there are several remaining questions. For example, it is unclear what the specific transcriptional alteration is in primordial germ cells, what the mechanism underlying the progressive degeneration of the germline is, and what causes the eventual loss of expression of spermatogenesis genes after 20 generations.

(e) Histone methylation and the epigenetic inheritance of longevity

Researchers have found that longevity can be epigenetically inherited across generations in C. elegans, and that this is linked to histone methylation. The H3K4me3 regulatory complex [absent small or homeotic 2 (ASH-2), WD repeat-containing protein 5 (WDR-5), and SET family member 2 (SET-2)] functions as a chromatin modifier to trimethylate H3K4 and plays a pivotal role in the regulation of longevity in C. elegans (Greer et al., 2010). To investigate the influence of epigenetic regulation and exclude the original genetic manipulation, Greer et al. (2011) generated wild-type descendants by crossing wild-type males with wdr-5 mutant hermaphrodites to generate F1 heterozygous hermaphrodites, which were then self-crossed for up to five generations. They demonstrated that the lifespan of genetically manipulated wild-type descendants from wdr-5 mutant ancestors was extended for up to four generations despite the fact that the initial trigger of the genetic mutation was no longer present (Greer et al., 2011). The transgenerational inheritance of longevity was further shown to be dependent on retinoblastoma-related binding protein 2 (RBR-2), which functions as an H3K4me3 demethylase and is necessary for the lifespan extension caused by deficiencies in members of the H3K4me3 regulatory complex (Greer et al., 2011). Depletion of other chromatin modifiers (e.g. H3K9 methylase and H3K27 demethylases) failed to elicit a similar inheritance of longevity (Greer et al., 2011). These results indicated that the transgenerational inheritance of lifespan extension is relatively specific for the H3K4me3 chromatin modifiers, which may therefore be important for the epigenetic memory of lifespan across generations (Greer et al., 2010). However, a direct epigenetic basis for transgenerational inheritance, e.g. whether there is incomplete reprogramming of H3K4me3 at certain loci, remains missing in this study. Nevertheless, this finding suggests that ageing can be regulated epigenetically, which raises the intriguing possibility that limited manipulation of specific chromatin modifiers in parents might induce an epigenetic memory of longevity in their descendants.

(f) Olfactory imprinting

In C. elegans, the presence of food is required for olfactory imprinting, which is a learned olfactory response and can only be acquired during a defined developmental time window or during a specific physiological state. In particular, an association between odour and food can be encoded during the L1 stage of C. elegans (Remy & Hobert, 2005). Notably, the imprinting is memorized over several consecutive generations and leads to the stable inheritance of a behavioural response for over 40 generations (Remy, 2010). However, the molecular mechanisms underlying the recurrence of this sensory experience over generations have yet to be uncovered.

(2) Evidence for epigenetic inheritance in Drosophila melanogaster

Similar to C. elegans, DNA methylation is largely absent from D. melanogaster (Lyko & Maliszka, 2011; Raddatz et al., 2013; Zhang et al., 2015); accordingly, researchers have focused on how chromatin modifications are involved in epigenetic inheritance in this species (Seong et al., 2011; Ost et al., 2014).

(a) Heat shock-induced epigenetic inheritance

In D. melanogaster, heat shock or osmotic stress can induce the disruption of heterochromatin, which is a tightly compact form of chromatin characterized by gene silencing. It has been found that heat shock-induced changes in heterochromatin are inherited by successive generations (Seong et al., 2011). Specifically, a transcription factor, drosophila activation transcription factor 2 (dATF-2), the homolog of which functions in the nucleation and spread of heterochromatin in fission yeast (Jia, Noma & Grewal, 2004), was shown to be involved in heat shock-induced epigenetic inheritance (Seong et al., 2011). Using position-effect variegation-mediated alterations in white gene silencing as a read-out of heterochromatin formation, Seong et al. (2011) revealed that, upon heat shock or osmotic stress, dATF-2 was phosphorylated by stress-activated protein kinases such as p38, which in turn led to the release of phosphorylated dATF-2 from the heterochromatin region. The stress-induced heterochromatin disruption was found to be transmitted through the germline. The phenotypes examined (eye colour and wing notches) eventually faded and disappeared in successive generations unless the stress was applied again (Seong et al., 2011). Furthermore, stress-induced heterochromatin formation occurs when unstressed insects harbouring the white gene are mated with stressed insects, suggesting that these acquired traits are inherited in a non-Mendelian manner. This result resembles the extensively studied phenomenon of paramutation in plants, in which a paramutagenic allele causes another allele in the same nucleus to become silenced (Chandler, 2010). Although the stress-induced, dATF-2-dependent epigenetic change described in this study has high penetrance, the phenomenon has not received much attention to date, in part owing to a lack of morphological, physiological, or behavioural phenotypes in successive generations in response to the new epigenetic states. Nevertheless, this work presents a striking example that environmental cues such as stress induce changes in chromatin state and thereby alter gene expression across generations.
(b) Paternal diet-induced epigenetic inheritance

Dietary effects may also produce epigenetic inheritance in D. melanogaster. For example, in a Drosophila model of paternal-diet-induced intergenerational metabolic reprogramming, as little as 24 h of acute sugar dietary intervention in fathers had the ability to elicit obesity in F1 offspring via the male germline (Ost et al., 2014). Using identical or comparable position-effect variegation lines, Ost et al. (2014) further revealed that this intergenerational reprogramming in response to dietary manipulation modified the chromatin state and transcription in offspring in a manner sensitive to the functions of Polycomb, enhancer of zeste [E(z)], a histone H3K27 methyltransferase, SetDB1 (a H3K9 histone methyltransferase), Su(var)3-9 (a H3K9 histone methyltransferase), and heterochromatin protein 1 (HP1) (Ost et al., 2014). Notably, numerous genes important to both cytosolic and mitochondrial metabolism appeared to be embedded into H3K9me3- and polycomb-controlled regions (Ost et al., 2014). Chromatin-dependent transcriptional derepression in the sperm of high-sugar-fed males was also observed, suggesting that chromatin-dependent signatures of metabolic reprogramming are forecast in the paternal germline (Ost et al., 2014). The same epigenetic signatures predictive of obesity susceptibility were found in murine and human obesity samples (Ost et al., 2014), suggesting that a similar mechanism controls this metabolic reprogramming in insects and mammals. Together, these data indicate that acute environmental exposure can induce phenotypic variation in the offspring through epigenetic changes such as H3K9me3-dependent chromatin regulation. Considering that acute feeding is a rather common phenomenon for wild animals over evolutionary timescales, such a mechanism thus carries profound implications for our understanding of phenotypic diversity and evolution.

(3) Evidence for epigenetic inheritance in zebrafish

The zebrafish Danio rerio is a model organism for vertebrate epigenetic studies and shares with mammals the basic vertebrate enzymes for DNA methylation such as Dnmt1 (Goll & Halpern, 2011; Wu et al., 2011), and for chromatin regulation (Vastenhouw & Schier, 2012). On the other hand, zebrafish lacks a Dnmt3L ortholog and thus is not required for parental imprinting (McGowan & Martin, 1997).

In zebrafish, oocytes are hypomethylated (75–80% CpG methylation) relative to sperm (91–95%), which is similar to the situation in mice (Jiang et al., 2012; Potok et al., 2013). However, a striking observation in zebrafish is that the sperm methylome is inherited without significant changes until zygotic gene activation (blastula stage, approximately 1000 cells, approximately 3 h post fertilization) as demonstrated using whole-genome bisulfite sequencing of gametes and early developmental embryos (Jiang et al., 2013; Potok et al., 2013; Jiang et al., 2013) and Potok et al. (2013) demonstrated that, upon fertilization, the paternally derived methylome is stably inherited throughout early zebrafish development. In parallel, the paternal methylome is initially stable but subsequently undergoes extensive remodelling through simultaneous demethylation of oocyte-specific hypermethylated regions and de novo methylation of oocyte-specific hypomethylated regions to reset its epigenetic state to that of the paternal genome (Jiang et al., 2013; Potok et al., 2013). The reprogramming strategy in zebrafish is essentially different from that in mice, in which both parental genomes undergo extensive DNA demethylation via active (paternal) and passive (maternal) mechanisms, leading to a shared hypomethylated state that is distinct from both gametic methylomes (Gu et al., 2011; Inoue & Zhang, 2011; Wossiello et al., 2011; Smith et al., 2012).

Considering the striking similarity between the blastula stage methylome and the sperm methylome, the fidelity of the sperm methylome until the blastula stage raises the additional possibility that the stably maintained paternal methylome might be inherited through subsequent germ cell development to mature sperm and throughout the entire zebrafish life cycle. If so, this would suggest a potential route for epigenetic inheritance through the paternal germline in zebrafish. As the resetting of the parental epigenome influences the balance between reprogramming and inheritance, how the paternal methylome is protected from reprogramming during development and what the functional role is of the inherited parental epigenome in zebrafish remain open questions. Unlike in mice, zebrafish sperm do not contain protamines (Wu et al., 2011). Thus, whether the maintenance of the chromatin state therein is related to the maintained paternal methylome needs to be conclusively determined.

(4) Evidence for epigenetic inheritance in rodents

Mammals have acquired DNA methylation and histone modifications as well as non-coding RNA as their epigenetic regulators. Therefore, mammals differ greatly from the above model systems in their epigenetic architecture (Hardeland et al., 2003; Morgan et al., 2005). In rodents, studies of genetic manipulation-induced epigenetic inheritance, such as retrotransposon-derived models (Morgan et al., 1999), and models of exposure to dietary challenge (Carone et al., 2010; Ng et al., 2010), toxicants or chemicals (Anway et al., 2005), and social stress (Franklin et al., 2010; Gapp et al., 2014) support the transmission of parental experiences to offspring epigenetically. Because of their long generation time and complex development, however, the observed effects in most of the rodents studied have been restricted to the immediate offspring (F1, intergenerational effects), leaving open the possibility of transgenerational inheritance in mammals but not providing much experimental basis of it (Fig. 3).

(a) Genetic manipulation-induced epigenetic inheritance

Similar to the situation in C. elegans, genetic manipulation (Morgan et al., 1999; Rakyan et al., 2003) or a history of genetic manipulation in ancestors (Rassoulzadegan et al., 2006; Inoue & Zhang, 2011; Siklenka et al., 2013) can induce
Fig. 3. Models of parental environment-induced epigenetic inheritance in rodents. Effects of parental environments, such as exposure to dietary challenge, toxicants or chemicals, social stress or fear exposure, and odour conditioning, in the progeny of rodents. There are three main models for the transmission of parental experience in rodents: intergenerational effects (only the F1 generation) (Carone et al., 2010; Radford et al., 2014); multigenerational effects (from the F1 to the F2 generation) (Dias & Ressler, 2014); and transgenerational effects that are found in more than three generations (Anway et al., 2005). DNA methylation, histone modification, and non-coding RNA have been reported to be involved in the transmission of parental environment-induced phenotypes.
the inheritance of acquired phenotypes through epigenetic regulation in rodents.

(i) Retrotransposon manipulation-induced epigenetic inheritance.

In mammals, the best-studied epivariant loci at which epigenetic inheritance occurs through the gametes are in retrotransposon-derived strains. The agouti viable yellow (A\(^{v}\)) locus (Morgan et al., 1999; Cropley et al., 2006) and the axin-fused (Axin\(^{Fu}\)) locus (Rakyan et al., 2003) represent seminal examples of maternal diet-induced epigenetic programming.

In A\(^{v}\) mice, an IAP serving as a cryptic promoter was inserted into pseudoexon 1A of the Agouti gene (Dickies, 1962; Waterland & Jirtle, 2003), rendering the epigenetic state of A\(^{v}\) responsive to maternal dietary supplementation with methyl donors or endocrine-disrupting chemicals (Dolinoy, Huang & Jirtle, 2007) (Fig. 4A, B). A\(^{v}\) mice are epigenetic mosaics for IAP retrotransposon activity and DNA methylation, thus their coat colours range from yellow to brown in genetically identical A\(^{v}\) mice (Morgan et al., 1999; Daxinger & Whitelaw, 2012). When the A\(^{v}\) allele is unmethylated and active, the mice exhibit a syndrome characterized by yellow fur and excessive hunger, which can cause early-onset obesity and diabetes (Morgan et al., 1999). By contrast, mice whose mothers are fed with a methyl donor-rich diet are born with methylated and hence silent A\(^{v}\) alleles, resulting in agouti coloration and a metabolically normal phenotype (Wolff et al., 1998; Cooney, Dave & Wolff, 2002). The altered cytosine methylation profile and metabolic states of A\(^{v}\) offspring were found to persist well across two generations, even when these animals are fed normal diets (Cropley et al., 2006). However, the range of coat colours in offspring is unaffected by that of the father, indicating that the epigenetic modifications are completely erased after passage through the male germ line (Morgan et al., 1999). Such parent-of-origin effects probably arise because the resistance of IAPs to epigenetic reprogramming differs between the male and female germlines (Morgan et al., 1999; Cropley et al., 2006).

Axin\(^{Fu}\), first identified in 1937, is another classic example of a mammalian allele that displays extremely variable expression. In Axin\(^{Fu}\) mice, an IAP retrotransposon was similarly inserted into intron 6 of the Axin gene (Vasicek et al., 1997; Zeng et al., 1997), activating a downstream cryptic promoter that drives the expression of a biologically active 3′-truncated Axin transcript (Rakyan et al., 2003). Hypermethylation induced by a maternal diet enriched in methyl donors in this strain silences expression from the axin promoter and prevents tail kinks, whereas hypomethylation at this site correlates with expression of the 3′ Axin transcript and the penetrance of tail-kink phenotypes (Fig. 4C, D) (Waterland et al., 2006). Notably, the epigenetic state of Axin\(^{Fu}\) can be inherited following both maternal and paternal transmission, which is in contrast with the maternal-specific epigenetic inheritance of the A\(^{v}\) allele (Rakyan et al., 2003).

It has been suggested that DNA methylation primarily silences retrotransposons as a genomic defence mechanism to prevent them from transposing and disrupting gene expression (Walsh, Chailllet & Bestor, 1998); thus A\(^{v}\) and Axin\(^{Fu}\) provide examples of epigenetic variability and epigenetic inheritance of pleiotropic traits through DNA methylation. Although IAPs are not present in humans, the discovery of highly repetitive sequences such as long interspersed element-1 (LINE-1) and Alu elements in the human genome (Beck et al., 2010; Huang et al., 2010) raises the possibility that other genomic sequences may escape erasure of epigenetic reprogramming as well and be involved in the transmission of transgenerational phenotypes.

(ii) RNA manipulation-induced epigenetic inheritance. Paramutation represents a heritable epigenetic modification induced in plants by cross-talk between allelic loci (Chandler, 2010). A similar modification was reported in the mouse tyrosine kinase receptor Kit\(^{pm1Alf}\) allele, in which a lacZ-neo reporter cassette was inserted downstream of the initiator ATG codon of the Kit gene (Rassoulzadegan et al., 2006). As Kit has a critical role in several developmental processes, including germ cell differentiation and melanogenesis, Kit\(^{pm1Alf}\) heterozygotes exhibited a white tail tip and white feet (Rassoulzadegan et al., 2006). Wild-type offspring derived from Kit\(^{pm1Alf}\) heterozygotes maintained altered fur colouration with white spots to a variable extent. This altered fur colouration was transmitted to the F3 generation (Rassoulzadegan et al., 2006). The microinjection of either total RNA from Kit\(^{pm1Alf}/+\) heterozygotes or Kit-specific microRNAs into fertilized eggs induced a heritable white tail phenotype, indicating that the inheritance of this acquired phenotype was linked to RNA transmission (Rassoulzadegan et al., 2006). RNA bisulfite sequencing of sperm implicated Dnmt2-dependent cytosine methylation of Kit RNA in this inheritance (Kiani et al., 2013). However, the molecular basis for such inheritance remains unclear in this study. Paramutation in the Kit gene may provide a useful experimental model for further analysis of the function of RNA molecules in epigenetic inheritance.

(iii) Histone modification-induced epigenetic inheritance. The epigenetic inheritance of developmental abnormalities can also be triggered by abnormal histone demethylase activity in mice (Siklenka et al., 2015). In particular, overexpressing the human histone lysine 4 demethylase KDM1A during mouse spermatogenesis generated a mouse model that produced spermatozoa with reduced H3K4me2 within the CpG islands of genes implicated in development (Siklenka et al., 2015). Male transgenic offspring were bred with wild-type females to generate experimental heterozygous transgenic and non-transgenic mice, which were further crossed with wild-type females for several generations (Siklenka et al., 2015). Notably, a history of KDM1A overexpression in one generation severely impaired the development and survivability of offspring across two subsequent generations independent of KDM1A expression in the germ line (Siklenka et al., 2015).

Chromatin immunoprecipitation (ChIP) combined with sequencing (ChIP-Seq) analysis revealed a reduction in the levels of H3K4me2 enrichment in sperm from transgenic mice. Additionally, microarray analysis of the RNA profiles identified over 560 transcripts differentially regulated.
between F1 transgenic and non-transgenic sperm, 41 of which were associated with genes that exhibited reduced levels of H3K4me2 at their promoters in F1 transgenic sperm (Siklenka et al., 2015). Altered DNA methylation in CpG-rich regions was ruled out as a mechanism for transmission. Instead, the investigators suggested that the abnormal RNA profiles transferred to the wild-type embryo by transgenic sperm might be responsible for the observed transmission of developmental defects in offspring (Siklenka et al., 2015). In addition, microarray expression analysis of two-cell embryos revealed that over 100 of the genes that exhibited reduced histone methylation in transgenic sperm were differentially regulated in embryos sired by transgenic and non-transgenic mice (Siklenka et al., 2015). Given that abnormal RNAs can induce changes in chromatin structure and thus allow abnormal RNA expression in C. elegans (Ashe et al., 2012; Le Thomas et al., 2014), it is possible that a similar RNA-based feedback loop exists in the wild-type embryo from transgenic sperm, which may be part of the mechanisms underlying their reported epigenetic inheritance. This was the first study in mammals to demonstrate that altered histone methylation and sperm RNA could function as potential mediators of epigenetic inheritance in developing sperm cells to induce developmental abnormalities.

(b) Environmental chemical-induced epigenetic inheritance

Environmental toxicants appear to have broad effects on future generations even when the stressful environments no longer exist (Anway et al., 2005). A classic study illustrating this phenomenon involved the exposure of female rats to two different endocrine disruptors, vinclozolin and methoxychlor, at a critical time during gonadal sex determination (embryonic day (E) 8–15 in the
rat. Strikingly, the toxicant exposure resulted in adult testis phenotypes characterized by decreased spermatogenic capacity and male infertility (20% decrease in sperm count and a 25–35% reduction in sperm motility) over at least four generations (Anway et al., 2005). In particular, this phenotype was found to be associated with altered DNA methylation in a subset of genes in the male germline (Anway et al., 2005). Considering that chemical exposure can cause mutations, however, genetic factors were not fully excluded in this study. They also did not identify the specific gene(s) responsible for this environmental toxicant-induced inheritance nor did they exclude the involvement of other epigenetic regulations, such as histone modification or snRNAAs. Additionally, as the exposure levels used in these studies were higher than anticipated for environmental levels, the possible toxicological impact of this epigenetic inheritance on animal populations remains to be determined.

Furthermore, a variety of other environmental chemicals, including dioxin, pesticides and insect-repellent mixtures, and plastics-derived endocrine disruptors, have been shown to induce the epigenetic inheritance of abnormal phenotypes (Manikkam et al., 2012a,b; Skinner et al., 2013). Together, these findings indicate that environmental chemicals are able to affect epigenetic marks and create long-lasting changes over generations.

c Parental nutrition-induced epigenetic inheritance

The quantity and trophic structure of the diet, particularly during the early stages of development, constitute primary factors in the development and health of an individual. Therefore, numerous studies have demonstrated close associations between the risk of chronic diseases in the next generation and unbalanced nutritional intake early in life. For example, the study of intrauterine growth restriction (IUGR) and its potential adverse impacts on lifelong health, such as cardiovascular disease in adulthood, has attracted attention (Zohdi et al., 2015). To obtain a better understanding of the effects of undernutrition and/or malnutrition, multiple rodent models have been established under controlled conditions, such as maternal protein restriction, global dietary restriction, or a high-saturated-fat diet fed over specific periods, including preconception, pregnancy, lactation, or a combination thereof.

(i) Protein restriction-induced epigenetic inheritance. One of the most extensively studied and well-characterized IUGR models is that of maternal protein restriction in rats, in which the female breeder rats are fed a low-protein diet (6–8.7% casein) from 2 weeks prior to mating until 2 weeks after lactation (Kwong et al., 2000; Sathishkumar et al., 2009). According to the severity of protein restriction, this model has been shown to result in IUGR characterized by a reduction in the body mass of the offspring at birth, with subsequent higher systolic blood pressure, hyperinsulinemia (higher insulin in the bloodstream), hyperleptinemia (higher leptin levels in the bloodstream), abnormal appetite, reduced locomotion, and obesity in the adult offspring (Langley-Evans, Welham & Jackson, 1999; Cheema et al., 2005). Furthermore, the strains of the rodents studied, the levels of maternal protein restriction in the diet, and the timing of administration of the diet to the dams influence the cardiovascular and metabolic phenotypes in the offspring generated in this model (Zohdi et al., 2015). Notably, protein restriction-induced phenotypes can be passed to more than one generation through a non-genetic mechanism. In rats, for example, feeding a protein restriction diet to the F0 mother during pregnancy resulted in elevated blood pressure and endothelial dysfunction (Torrens, Poston & Hanson, 2008) as well as insulin resistance in the F1 and F2 generations despite their receiving adequate nutrition (Woodall et al., 1996; Martin et al., 2000). Moreover, the adverse effects of gestational protein restriction on glucose homeostasis have also been observed in the offspring up to the F3 generation (Benyshek, Johnston & Martin, 2006).

The epigenetic mechanisms involved in the transmission of parental metabolic phenotypes to offspring were further investigated in an elegant study by Carone et al. (2010). In this study, male mice were fed chow or low-protein diets (LPD, 11% protein, with the remaining mass made up with sucrose) and bred with normal chow-fed females. Both the male and female offspring derived from LPD males displayed increased hepatic expression of lipid and cholesterol synthesis genes (Carone et al., 2010). Subsequently, reduced representation bisulfite sequencing (RRBS) was used to profile the DNA methylome in the livers of the male offspring fed LPD. Carone et al. (2010) observed numerous modest alterations (approximately 20%) in cytosine methylation depending on the paternal diet. Among these changes, the methylation levels of a likely enhancer for the key lipid regulator peroxisome proliferator activated receptor-α (PPARα) gene were confirmed to be reproducible (Carone et al., 2010). However, an effect of paternal diet on the methylation of this locus was not identified in LPD sperm. Thus, whether this site-specific change in methylation is a functional resultant remains to be determined. There are other limitations in this study. For example, considering that RRBS is only able to characterize cytosine methylation across about 10% of the mouse genome, with a greater coverage of CpG islands especially in the promoter region, more sensitive assays of sperm methylation may uncover other loci with differential DNA methylation (Meissner et al., 2008; Bock et al., 2010; Harris et al., 2010). In addition, as Carone et al. (2010) observed some differences in histone modification and RNA expression in the sperm of LPD offspring, the possible contribution of these epigenetic modifications to non-genetic paternal inheritance cannot been ruled out.

The function of tRNA fragments (tRFs, approximately 28–34 nt) in LPD-induced epigenetic inheritance has also been studied (Chen et al., 2016; Sharma et al., 2016). tRFs, which are predominantly derived from the 5′ ends of tRNAs, represent the most abundant class of RNA in mature sperm [approximately 80% of small RNAs (sRNA)] (Peng et al., 2012; Sharma et al., 2016). Sharma et al. (2016) indicated that the offspring generated via in vitro fertilization (IVF) using sperm obtained from animals consuming LPD...
Parental experience-induced epigenetic inheritance

(10% protein) exhibited significant hepatic up-regulation of the gene encoding the cholesterol biosynthesis enzyme squalene epoxidase. Notably, the IVF-derived offspring of males fed LPD also displayed alterations in sRNA levels, with increased amounts of tRFs and decreased levels of a known microRNA let-7 in mature sperm (Sharma et al., 2016). In particular, the 5’ fragments of tRNA-Gly-CCC, -TCC, and -GCC increased approximately two- to threefold in low-protein sperm. Functionally, it was demonstrated that the tRNA-glycine-GCC fragments repressed genes associated with the endogenous retroelement mouse endogenous retrovirus (ERV) like IAPs (MERVL) in both embryonic stem cells and embryos (Sharma et al., 2016). Similar to the effects observed in the IVF offspring of LPD-fed fathers, either injection of the spermatocyte sRNA fraction of LPD fathers or synthetic tRF-Gly-GCC oligos into naïve zygotes altered the expression of MERVL targets in early embryos (Sharma et al., 2016). The limitation of this study is that they provided neither the metabolic consequences in the offspring with altered MERVL target expression nor evidence whether their observations were relevant to the LPD model. Nevertheless, this study provided evidence for the function of sperm RNA, in particular tRNA fragments, in transmitting paternal acquired phenotypes.

(ii) In utero undernourishment-induced epigenetic inheritance. Similar to the effect of parental protein restriction, maternal undernourishment can induce intergenerational effects on metabolism through epigenetic regulation (Radford et al., 2014). In their model, Radford et al. (2014) implemented nutritional restriction in the outbred ICR mouse strain from E12.5 to 18.5 of pregnancy, when primordial germ cells are epigenetically reprogrammed and reacquire DNA methylation specifically in the male germline. Both the F1 and F2 male offspring of undernourished dams exhibited low birth mass and multiple metabolic defects, suggesting the inheritance of a metabolic phenotype through the paternal line (Radford et al., 2014). By using the genome-wide approach of methylated DNA immunoprecipitation (MeDIP)-sequencing (Weber et al., 2005; Jacinto, Ballestar & Esteller, 2008), they identified a total of 111 hypomethylated regions in the nutritionally restricted F1 sperm, about 70% of which were validated as true differentially methylated regions by further bisulfite pyrosequencing (Radford et al., 2014). However, the altered DNA methylation did not appear to persist in F2 E16.5 brain and liver, although considerable tissue-specific differences in the expression of the metabolic genes neighbouring differentially methylated regions were present (Radford et al., 2014). Notably, a considerable portion (21%) of the hypomethylated regions were overlapped with regions previously shown to be nucleosome-enriched, suggesting that nutritional restriction in utero might alter the chromatin architecture in sperm (Radford et al., 2014). As it has been suggested that 43% of the hypomethylated regions are resistant to zygotic reprogramming (Kobayashi et al., 2012), the differences in gene expression of the F2 generation might be attributed to altered methylation during early development or may represent the cumulative result of multiple locus-specific defects in the germline chromatin state. Nevertheless, this study provided a model of how whole-genome approaches followed by independent validation should be conducted in analogous studies of epigenetic inheritance.

(iii) High-fat diet-induced epigenetic inheritance. High-fat diet (HFD)-induced epigenetic inheritance was demonstrated through pioneering research (Ng et al., 2010). In this study, male Sprague-Dawley rats were fed a chronic HFD from 4 weeks of age to induce obesity and then bred with control females at 14 weeks of age. The female offspring of the obese males exhibited glucose intolerance and reduced insulin secretion in their pancreatic islets. Microarray studies revealed that the impaired metabolic phenotype in female offspring was associated with altered IL13a2 expression and reduced methylation in a region proximal to its transcription start site in pancreatic islets (Ng et al., 2010). However, the results of this study left several essential questions unanswered. In particular, it was not determined whether similar defects could be observed in male offspring, whether HFD affected the epigenome in the germline of offspring, or whether the observed phenotypes and epigenetic changes could be transmitted to the second (F2) generation.

Furthermore, the function of tRFs in HFD-induced epigenetic inheritance has also been examined (Chen et al., 2016). Similar to previous reports, male mice fed a diet with 60% fat for 6 months beginning at 5 weeks of age became obese, glucose intolerant, and insulin resistant. Consistent with the study by Sharma et al. (2016; Chen et al., 2016) observed sRNA alterations in the sperm of mice fed HFD. They further utilized a high-throughput quantitative approach based on liquid chromatography tandem mass spectroscopy to examine changes in tRNA fragments that they termed tsRNAs. Two RNA modifications, 5-methylcytidine (m5C) and N2-methylguanosine (m2G), were significantly up-regulated in HFD sperm (Chen et al., 2016). A widespread modification of tRNAs in mammals, m5C, has been reported to contribute to tRNA stability (Tuorto et al., 2012) and is associated with RNA-mediated epigenetic inheritance (Kiani et al., 2013). Therefore, it was speculated that increased methyl modifications in RNAs, like m5C and m2G, might affect the stability of tsRNAs. Additionally, in a series of experiments in which tsRNA fractions from HFD sperm were injected into normal zygotes, they generated F1 offspring with metabolic disorders. The disorders were further associated with the altered expression of metabolic pathways in early embryos and in the islets of F1 offspring, demonstrating that the tsRNA-containing fraction, but not the miRNA- or long RNA-containing fractions of sperm RNA, was capable of transmitting glucose intolerance to offspring. However, the potential function of tsRNAs in embryos to relay information to the next generation remains to be elucidated. This study supports a role for sperm tRNA fragments in epigenetic inheritance. Their conclusion might be of particular relevance to human pathophysiology, as a
recent study observed germline alterations in non-coding RNAs in obese men (Donkin et al., 2016).

(iv) Nutrient deficiency-induced epigenetic inheritance. In addition to the animal models mimicking nutrition deficiency during foetal development, attempts have been made to investigate epigenetic effects of essential nutrients involved in one-carbon metabolism. Among these, the B vitamin folic acid serves as a carrier of methyl groups and its deficiency comprises one of the most common vitamin deficiencies in humans. In particular, methionine synthase reductase (MTRR) activates methionine synthase, which simultaneously converts 5-methyltetrahydrofolate to tetrahydrofolic acid (the biologically active form of folate) and homocysteine to methionine (Hart et al., 2002; Yamada et al., 2006). In a gene-trap hypomorph mutation of the Mtrr<sup>+/gt</sup> mouse harbouring a mutant allele of the Mtrr gene, Padmanabhan et al. (2013) observed reduced Mtrr mRNA levels and elevated levels of plasma homocysteine, suggesting the impaired conversion of homocysteine to methionine in these mice. Notably, Mtrr<sup>+/gt</sup> mice exhibited IUGR, developmental delay, and congenital malformations.

They further demonstrated that abnormal folate metabolism in Mtrr mice had distinct transgenerational functions that were responsible for specific developmental processes (Padmanabhan et al., 2013). In particular, the appearance of congenital malformations in the wild-type progeny derived from Mtrr<sup>+/gt</sup> intercrosses could persist for five generations and were specifically dependent upon the genotypes of the maternal grandparents (Padmanabhan et al., 2013). These results were further supported by embryo transfer experiments in which wild-type E3.25 embryos from heterozygous maternal grandparents were transferred to wild-type pseudopregnant females, after which they developed similar congenital malformations (Padmanabhan et al., 2013). To address the inheritance pattern of DNA methylation, the authors demonstrated that the livers and uteri of Mtrr<sup>+/gt</sup> and Mtrr<sup>+/gt</sup> (full knock-down-equivalent) adult mice showed a marked reduction in DNA methylation (44.9–74.2% of wild-type levels). Notably, despite the presence of two functional Mtrr alleles, the wild-type grand progeny of Mtrr-deficient maternal grandparents exhibited widespread epigenetic instability associated with altered gene expression (Padmanabhan et al., 2013), which likely explained the wide spectrum of phenotypes observed in these animals. However, the specific mechanism by which this information is inherited remains unknown. Whether RNA levels, DNA methylation, and histone or non-histone protein methylation are altered in the descendants of Mtrr mutant mice remains to be investigated. As most previous studies investigated extreme conditions such as famine, this study suggests that transgenerational dietary effects might be more common-place and might also occur under less-extreme conditions.

In addition to folic acid, the influence of prenatal dietary supplementation of choline, which provides methyl groups as a substrate for DNA methyltransferases (Batra, Sridhar & Devasagayam, 2010), has also attracted interest in terms of the physiological and psychological health of offspring (Zeisel, 2006; Jiang et al., 2012). In particular, high maternal dietary choline intake in humans leads to a lower risk of neural tube defects in infants (Shaw et al., 2004). In rodents, gestational choline supplementation advances hippocampal development (Mellott et al., 2004) and permanently enhances long-term potentiation and memory in the next generation (Cheng et al., 2008). However, whether the gestational choline supplementation-induced phenotype in F1 offspring can be transmitted to the F2 generation and the identification of epigenetic mechanisms underlying these phenotypes remain unknown at this time and require further investigation.

(d) Chronic parental stress and traumatic experience-induced epigenetic inheritance

Parental adverse and traumatic experiences during early life, including reduced maternal care (Weaver et al., 2004), unpredictable maternal separation (Franklin et al., 2010; Gapp et al., 2014), chronic variable stress (Morgan & Bale, 2011; Rodgers et al., 2013, 2015), and chronic paternal social defeat stress (Dietz et al., 2011), have been shown in rodents to contribute to behavioural and emotional disorders in offspring over several generations.

In particular, maternal deprivation and poor maternal care have been widely reported to perturb neurodevelopmental processes and represent a risk factor for the development of mood disorders. In rats, it has been known for decades that F1 female offspring that experienced increased grooming and licking and arch-back nursing from mothers display decreased fearfulness and more modest hypothalamic–pituitary–adrenal (HPA)-axis responses to stress than those who did not experience this care (Weaver et al., 2004). Alterations in both DNA methylation and histone modification at the nuclear receptor subfamily 3, group C, member 1 (Nr3c1) locus in the hippocampus of the F1 female pups receiving higher quality maternal care were identified during the first week of postnatal life and were correlated with changes in Nr3c1-encoded glucocorticoid receptor expression (Weaver et al., 2004). These results suggest that a high quality of maternal care positively influences appropriate behavioural responses and causes adaptive behaviours in F1 female offspring. In this case, however, the generation-to-generation acquisition of the nurturing behaviour does not occur through the gametes but represents a learned trait, likely through epigenetic regulation in genes such as oestrogen receptor α1b in the medial preoptic area of the brain (Champagne et al., 2006).

In addition to the quality of maternal care, social stress exposures such as chronic and unpredictable maternal separation (CUMS) and chronic variable stress during early development have also been reported to induce emotional alterations in the progeny through epigenetic regulations (Franklin et al., 2010; Rodgers et al., 2013; Gapp et al., 2014). Using an experimental paradigm for CUMS in early life, Franklin et al. (2010) provided evidence that CUMS could model the transgenerational transmission of complex behavioural alterations in C57Bl/6 mice.
Specifically, they demonstrated that subjecting mice to CUMS during postnatal days 1–14 induced depressive behaviours and altered the animals’ response to novel and aversive environments. Furthermore, some of the observed behavioural alterations were transmitted to F1 male offspring and to the subsequent generation (F2 female and F3 male offspring) as well. In this case, postnatal trauma-induced transmission of depression-like behaviour and anxiety occurred through a complex and sex-dependent mode. The mechanisms underlying this complex expression mode remain unclear. However, it is independent of maternal factors, as these acquired traits persist after cross-fostering (where offspring are removed from their biological parents at birth and raised by surrogates) (Franklin et al., 2010). CUMS led to altered DNA methylation at CpG islands of the methylated CpG binding protein 2 (Mecp2), cannabinoid receptor 2 (Cnr2), and corticotropin release factor receptor 2 (Crf2) genes in adult sperm of CUMS-stressed males. Similar changes in the DNA methylation were also found in the brain and sperm of the offspring (Franklin et al., 2010). This is the first study in mice to demonstrate that early traumatic stress alters behaviour and modifies the epigenetic profiles across generations, providing behavioural and molecular correlates to complex traits induced by the early environment.

Furthermore, the offspring of male mice exposed to 6 weeks of chronic variable stress either throughout puberty or in adulthood displayed significantly reduced HPA-axis stress responsivity (Rodgers et al., 2013). Through microarray and gene set enrichment analysis in the paraventricular nucleus (PVN) and bed nucleus of stria terminals (BNST), two brain regions that regulate stress, Rodgers et al. (2013) revealed global changes in transcription patterns including the increased expression of glucocorticoid-responsive genes in the PVN. Upon examination of the potential epigenetic mechanisms of germ cell transmission, nine specific sperm miRNAs were found to be significantly increased in paternal stress groups (Gannon et al., 2014). In a later study, they performed microinjection of the same nine miRNAs into a single-cell zygote, which was then implanted into surrogate dams, reared normally, and examined for subsequent cellular differentiation during brain development to guide the formation of specific anatomical structures of olfactory receptors. Nevertheless, this study represents a non-genetic mechanism for the quintessential evolutionary

alter metabolic responses in the progeny of mice (Gapp et al., 2014). Through deep sequencing of purified sperm RNA, several up-regulated miRNAs and downregulated piRNAs were identified in F1 CUMS sperm, indicating an effect of CUMS on several sncRNA populations. Furthermore, the injection of sperm RNAs from traumatized males into fertilized wild-type oocytes reproduced the behavioural and metabolic alterations in the resulting offspring, indicating that the effects were transmitted through the injected sperm RNAs. However, similar miRNAs were not affected in F2 sperm or F3 animals. It is possible that the changes in miRNAs that initially occurred in the sperm cells as a result of CUMS were transferred to other epigenetic marks, such as DNA methylation or histone post-translational modifications, for maintenance and further transmission of the altered emotional traits. These findings provide evidence for the concept that RNA-dependent processes contribute to the transmission of acquired traits in mammals. In addition, the identification of several miRNAs in the serum and their putative targets as mediators of these effects will provide molecular markers of traumatic stress for potential use in the diagnosis of stress predisposition and stress-induced disorders in humans.

(c) Odour-fear conditioning-induced epigenetic inheritance

Odour sensitivity has been a critical driving force in evolution. While modelling an ecologically relevant exposure, Dias & Ressler (2014) conditioned F0 male mice to fear an odour by pairing the odour with mild foot shocks prior to breeding. They found that the F1 and F2 generations sired by conditioned F0 mice also showed a heightened startle response in the presence of this odour. Furthermore, behavioural sensitivity and neuroanatomical changes such as in glomerular areas persisted after IVF, cross-fostering, and across two generations, indicating that the behaviour and structural alterations were multigenerationally inherited and were not socially transmitted from the F0 generation (Dias & Ressler, 2014). To identify the epigenetic marks responsible for this phenomenon, bisulfite sequencing of sperm DNA from conditioned F0 mice and their F1 offspring revealed the presence of CpG hypomethylation in the Olfr151 gene, encoding a known odorant receptor. However, the authors found no evidence of DNA methylation at the Olfr151 locus in the olfactory response neurons of the main olfactory epithelium in either the F1 or F2 generation (Dias & Ressler, 2014). Thus, more complex neuronal circuitry may be involved in the behaviour and structural alterations of progeny in addition to the identified olfactory receptor. There are other elusive issues in this study. For example, the mechanism by which the germ cells respond to both the olfactory signal and a fearful experience remains unclear. In addition, it remains to be determined how sperm maintain DNA methylation through the reprogramming and subsequent cellular differentiation during brain development to guide the formation of specific anatomical structures of olfactory receptors. Nevertheless, this study represents a non-genetic mechanism for the quintessential evolutionary
process. It suggests that evolution has equipped organisms with mechanisms to respond specifically and efficiently to certain critical novel experiences, such as odour and predator threat, and to transmit this information effectively to their offspring without the need for the typically slow process of natural selection (Szhyf, 2014).

(5) Evidence for epigenetic inheritance in humans

Human epidemiological data, including early-life parental and ancestral exposure data, support the notion that environmental and nutritional disturbance might result in the non-genetic transmission of altered phenotypes across generations. For example, a history of exposure to famine in either the paternal or maternal line was associated with reduced renal function, and mood disorders in the offspring (reviewed in Pembrey et al., 2014). Considering the difficulties in obtaining data in humans owing to long generation times, difficulty with accurate record keeping and relevant sample collection, epigenetic inheritance in the human population is difficult to measure and thus the precise mechanisms underlying the stable and heritable changes resulting from this environmental factor remain largely unknown.

(a) Parental nutrition-induced epigenetic inheritance

Gestational nutrition has been reported to result in long-term effects on the development and health of descendants (Li et al., 2010; Pembrey et al., 2014). For example, poor maternal nutrition, such as following maternal exposure to famine of the Dutch ‘Hunger Winter’ in World War II, is associated with lower birth mass and increased risk of type 2 diabetes over several generations (Gluckman, Hanson & Beedle, 2007; Lumey et al., 2007; Painter et al., 2008). Similarly, epidemiological data collected through following three Overkalix cohorts born in 1890, 1905, and 1920 in northern Sweden demonstrated that the nutrition experience in paternal grandparents was linked to obesity and cardiovascular disease two generations later. Specifically, insufficient food supply during the slow growth period of the paternal grandfather was associated with a low risk of cardiovascular disease mortality in their grandchildren; by contrast, diabetes mortality of the grandchildren increased when their paternal grandfathers were exposed to a surfeit of food during that period (Kaati, Bygren & Edvinsson, 2002; Pembrey et al., 2006). Further study of the Dutch Famine Birth Cohort indicated that offspring whose mothers were exposed to the famine exhibited altered DNA methylation levels in the proximal promoters of genes involved in metabolic processes as measured in whole blood (Heijmans et al., 2008; Tobi et al., 2009, 2014). However, it is unknown whether these observed differences in methylation are present in their germline. Thus, a truly documented epigenetic effect capable of transmitting the somatic response to famine to subsequent generations cannot be inferred.

Recently, it has been reported that the epigenomes of sperm from lean and obese men showed marked differences in snRNA expression and DNA methylation patterns (Donkin et al., 2016). These data differ considerably from the results in rodent studies. In particular, protamine-bound DNA, rather than histone-retained regions, showed a bimodal methylation distribution between obese- and lean-man sperm (Donkin et al., 2016). However, in the models of in utero nutritional environment and paternal low-protein diet, differentially methylated regions in the sperm are largely enriched in histone-retaining regions (Carone et al., 2010; Radford et al., 2014). Furthermore, they found that surgery-induced weight loss was associated with a dramatic remodelling of DNA methylation in sperm, especially at genetic locations controlling appetite (Donkin et al., 2016). This study indicates that the epigenetic landscape of human sperm is dynamic and influenced by environmental changes. Future investigations regarding whether and how obesity in men affects the metabolic function of their offspring may provide important information for understanding the influence of parental environment on human health.

(b) Epimutation-induced inheritance

The neurogenetic disorders Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are caused by the loss of function of distinct imprinted genes within the 15q11-q13 chromosomal locus. In a small group of patients (1% in PWS and 2–4% in AS) (El-Maarri et al., 2001), these diseases occur owing to aberrant imprinting, which disrupts the normal allele-specific methylation dependent on parental origin and the concomitant pattern of expression. In this subset of patients with AS, the imprinting defect occurred at a locus proximal to exon 1 of the SNURF-SNRPN gene, which encodes a polypeptide of a small nuclear ribonucleoprotein complex, and was inherited from the maternal grandfather or grandmother; however, in all informative patients with PWS, the imprinting defect at a region around exon 1 of SNURF-SNRPN was inherited only from the paternal grandmother, suggesting that the imprinting defect in PWS results from a failure to erase the maternal imprint during spermatogenesis (Buiting et al., 2003).

In addition, it has been reported that two patients with multiple primary non-polyposis colorectal cancer displayed soma-wide, allele-specific, and mosaic hypermethylation of the DNA mismatch repair gene MLH1, with no evidence of genetic mutation being further detected in this gene. This altered epigenetic status was also present in the spermatozoa of one of the individuals, indicating a germline defect and the potential for transmission to offspring (Suter, Martin & Ward, 2004).

(6) Evidence for epigenetic inheritance in other mammals

In addition to the species mentioned above, transgenerational effects have been examined in sheep (Louve et al., 2000), pigs (Kind et al., 1999), and baboons (Brans et al., 1986) through both maternal dietary manipulations and surgical intervention techniques (Oyama et al., 1992), suggesting
that the inheritance of acquired traits is a widespread phenomenon. Although the molecular nature and precise location of the epigenetic marks that are inherited is unknown in most of the above-mentioned cases, emerging evidence indicates that environmental cues have the potential to influence the establishment of epigenetic marks in the preimplantation embryo that can be transmitted from parent to offspring (Table 1).

IV. GERMLINE TRANSMISSION AND GENDER-SPECIFIC FEATURES OF PARENTAL ENVIRONMENT-INDUCED EPIGENETIC INHERITANCE

Although the epigenetic mechanisms underlying parental environment-induced transgenerational effects have been better illuminated in invertebrates, differences arising from paternal and maternal germline transmission and differences in the epigenetic inheritance outcomes between genders have been most frequently reported in mammals.

(1) Transmission through both paternal and maternal germines

Considering that the F1 offspring are directly exposed to the in utero environment, the observation that they possess some gestational environment-induced phenotypes is not particularly surprising. In particular, a number of gestational diet-induced epigenetic effects described above have been reported to be transmitted through maternal lines (Morgan et al., 1999; Padmanabhan et al., 2013). Female germ cells can mediate transgenerational effects through epigenetic mechanisms (Smallwood et al., 2011; Kobayashi et al., 2012). However, maternal transmission can also arise from the inheritance of mitochondrial DNA (Stewart & Larsson, 2014) or the effects of maternal metabolism (e.g., diabetes or obesity) (Gaillard, 2015). It is therefore difficult to separate nutrition-mediated effects from the direct effects of in utero environmental exposure on the offspring.

The burden of offspring phenotype can also be attributed to the physiological status of the father. In humans, food supply of paternal grandfathers was associated with metabolic and cardiovascular disease in grandchildren (Kaati et al., 2002; Pembrey et al., 2006). In rodents, transgenerational effects induced by endocrine disruptors (Anway et al., 2005) and nutrition disruption (Anderson et al., 2006; Carone et al., 2010; Ng et al., 2010) were suggested to be male-mediated. Considering that sperm can transmit haploid epigenetic information in addition to genetic factors, thus allowing pure epigenetic transmission with fewer confounding factors (Krawetz, 2005), these data strongly support that parental environment-induced changes of the cellular/physical function are not just a maternal concern: the father’s nutritional and metabolic status also merits attention for optimizing the health of his children and grandchildren.

The differences arising from the paternal and maternal germline with respect to the transmission of transgenerational effects probably result from differences in the epigenetic reprogramming process between the male and female germlines and also from differences between the maternal and paternal genomes post fertilization (Fig. 1) (Kobayashi et al., 2013), although this hypothesis requires further experimental evidence.

(2) Gender-specific effects on progeny

Similar to the phenomenon of sex differences that occur in most non-communicable diseases, including metabolic diseases, diabetes, hypertension, cardiovascular disease, and psychiatric and neurological disorders (Champagne, 2013; Saavedra-Rodriguez & Feig, 2013), parental environments exert gender/sex-specific effects on their progeny. The most compelling gender-specific transgenerational effects were induced by food supply in the previously discussed Overkalix population study. Specifically, the mortality rate of men born in the target years was linked to the food supply of only their paternal grandfather during mid-childhood, whereas the mortality rate of the women studied was associated solely with the paternal grandmother’s food supply (Pembrey et al., 2006). Also, sex-specific effects have been reported in model systems of epigenetic inheritance. For example, a paternal HFD increased body mass, adiposity, impaired glucose tolerance, and insulin sensitivity only in female F1 rats (Ng et al., 2010). In addition, CUMS was found to affect the emotional traits of progeny in a sex-switch manner, with the altered phenotypes in F1 male, then F2 female, and subsequent F3 male (Franklin et al., 2010).

The potential mechanisms mediating such gender-specific transgenerational effects have rarely been investigated and remain largely unknown. It has been suggested that chromosomal differences between the sexes, including both sex steroid-dependent and sex steroid-independent effects, as well as differences in epigenetic plasticity during gamete maturation might potentially contribute to the observed sex-specific epigenetic inheritance (Champagne, 2013). Moreover, differential epigenetic processes in the placenta, with respect to the abundance of X-linked genes involved in placentogenesis and early unequal gene expression by the sex chromosomes between males and females, might exert an early impact, i.e., beginning at conception, and thus might set the context for events in later life (Gabory et al., 2013). In addition, some sex-specific epigenetic inheritance effects have been described in mice that are incompatible with XY transmission (Champagne, 2013; Saavedra-Rodriguez & Feig, 2013).

V. DISCUSSION

Emerging evidence supports the idea that both maternal and paternal transmission to progeny of the non-genetic effects of ancestral environments represents a valid phenomenon.
Such transmission is proposed to occur mostly through alterations in the epigenomes of germ cells and is no longer dependent on parental environment exposure, maternal in utero environment, or genetic effects on germ cells. However, the data presented here raise fundamental questions regarding the potential mechanisms responsible for the described phenomena.

(1) Remaining gaps between phenotypic adaptation and epigenetic mechanisms

Despite advances in understanding the epigenetic biochemistry and developmental mechanisms involved in transgenerational inheritance, the means by which developmental programming effects can be passed between generations remain poorly understood. In particular, the germline can serve as a vector for transmitting information from parental experience across generations. However, most epigenetic marks are inherent in a tissue-specific manner in the progeny. Thus, a long series of differentiation steps exist between sperm and specific tissues, including many stages during which cell type-specific epigenetic information is established. For example, it remains unclear what defines the specificity of the response to parental experiences in the gamete genome, or how the epigenetic change in the gamete escapes reprogramming. Additionally, the mechanism by which epigenetic change in sperm is replicated during embryonic development is not known, nor how it directs the establishment of the specific epigenetic change in a particular tissue in response to given environments across generations.

In particular, although DNA methylation represents a candidate mark for epigenetic inheritance, the means by which the un-erased DNA methylation pattern is re-established in the specific tissues of inheriting progeny is a critical question requiring further investigation. Furthermore, it remains unknown whether a biochemical mechanism exists that links parental exposures to discrete changes at particular sites in chromatin in specific tissues or how these discrete chromatin states are directly inherited or re-established in the inheriting progeny. As ncRNAs exhibit greater flexibility compared with DNA methylation and histone modification and can be released from a given cell and systematically distributed, it remains to be determined what is the relative importance of these regulators in delivering signals from a tissue that senses the experience to the germ cells, or how this epigenetic information is maintained through generations.

(2) Limitations on epigenetic techniques and breeding strategies

The methods that have been used for examining the epigenetic modifications underlying acquired effects in mammals are summarized in Table 2. In most studies described herein, identification of the transcriptional alterations induced by parental environments is usually limited to functional candidate approaches by purposely examining a number of candidate genes. In addition, target-specific bisulfite methylation sequencing and ChIP coupled with quantitative real-time polymerase chain reaction (ChIP-qPCR) methods represent the most-developed and widespread techniques used for examining epigenetic modifications in response to environmental cues. However, the introduction of next-generation sequencing technologies has facilitated whole-genome bisulfite sequencing (WGBS) (Cokus et al., 2008; Beck, 2010) and ChIP-sequencing (ChIP-Seq) (Park, 2009), which currently represent the most powerful and unbiased strategies for quantitative genome-wide detection of epigenetic modifications.

In addition, most studies performed to date and discussed herein have been performed on heterogeneous cell populations; thus, the temporal and spatial epigenetic dynamics of individual cells have not been described. Alternatively, following the establishment of approaches to profile transcriptomes at the single-cell level (Tang, Lao & Surani, 2011), DNA-methylation (Guo et al., 2013) and chromatin modification via ChIP-Seq (Rotem et al., 2015) can be measured at the resolution of single cells. Therefore, various environmental factor-induced epigenetic changes could be investigated at the single-cell level (Siklenka et al., 2015; Chen et al., 2016; Sharma et al., 2016). In the future, the examination of environment-induced epigenetic changes in a single sperm or an egg, rather than in tens of thousands of sperm or heterogeneous cell populations, may more precisely decipher the relationships between acquired phenotypes and their epigenetic marks.

Furthermore, to exclude maternal or genetic effects on germ cells, cross-fostering and IVF are classic methods currently used in transgenerational studies. Additionally, the nuclear transplantation of tRNA has also recently been utilized (Chen et al., 2016; Sharma et al., 2016). We expect that the manipulation and transfer of specific epigenetic marks in a sperm or an egg, thereby changing the traits of the offspring, may provide more causal evidence for non-genetic transgenerational inheritance. In addition, the availability of animal models mimicking epigenetic alterations generated through the newly emerging technologies, such as the clustered, regularly interspaced, short palindromic repeats (CRISPR)/Cas9 systems (Rusk, 2014), will provide keys towards understanding the molecular mechanisms underlying epigenetic inheritance. Such a strategy would allow researchers to follow parental environment-induced physiological and cellular changes in a tissue-/cell-specific manner and during both normal development and through germline transmission.

(3) Exposure window for parental environment-induced epigenetic inheritance

It has been suggested that exposure to environmental cues during a relatively highly sensitive period might have a greater impact on an individual than exposure at other times. In support of this view, the mortality study in the Overkalix population indicated that there was a 32-year difference in the survival of grandchildren whose paternal grandfathers were either overfed or poorly fed during their mid-childhood
Table 2. Epigenetic techniques and specific tissues involved in epigenetic inheritance in mammals

| Parental experiences                              | Rodent models         | Transcriptome profiling                                                                 | DNA methylation profiling                                                                 | Histone modification profiling | small RNA-Seq profiling | References                  |
|--------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|--------------------------------|-------------------------|---------------------------|
| Nutrition-induced epigenetic inheritance         | Paternal low-protein diet | Microarray profiles in liver and epididymis                                             | RRBS-Seq in liver; McDIP-Seq in sperm                                                    | H3K27me3 ChIP in sperm         | Small RNA-Seq in liver  | Carone et al. (2010)     |
|                                                  | Paternal high-fat diet  | Microarray profiles in pancreatic islets                                               | —                                                                                        | —                              | —                       | Ng et al. (2010)         |
|                                                  | Paternal diabetes      | Microarray analyses in pancreatic islets                                               | MeDIP-Seq in pancreatic islets and sperm                                                 | —                              | —                       | Wei et al. (2014)        |
| In utero under-nourishment                       | —                     | —                                                                                      | MeDIP-Seq of F1 sperm, F2 brain and liver at embryonic day 16.5                          | —                              | —                       | Radford et al. (2014)    |
| Paternal low-protein diet                        | RNA-Seq of single embryo | —                                                                                      | —                                                                                        | small RNA-Seq in sperm         | small RNA-Seq in sperm  | Sharma et al. (2016)     |
| Paternal high-fat diet                            | Single cell transcriptome; RNA-Seq of embryo  | —                                                                                      | —                                                                                        | —                              | —                       | Chen et al. (2016)       |
| Sperm from lean and obese men                    | —                     | Bisulfite-Seq of sperm                                                                 | —                                                                                        | —                              | —                       | Donkin et al. (2016)     |
| Parental stress-induced epigenetic inheritance   | CUMS                   | Pyrosequencing of CpG islands in sperm                                                 | —                                                                                        | —                              | —                       | Franklin et al. (2010)   |
|                                                   | —                     | Expression of 93 neurodevelopment-related genes in the brain of postnatal day 1       | —                                                                                        | —                              | 239 miRNAs in the brain of postnatal day 1 using the Taqman Array miRNA array in sperm | Morgan & Bale (2011)     |
|                                                   | —                     | Affymetrix gene chip mouse gene analysis of whole brain                                 | —                                                                                        | —                              | —                       | Rodgers et al. (2013)    |
|                                                   | —                     | —                                                                                      | —                                                                                        | —                              | —                       | Gapp et al. (2014)       |
|                                                   | —                     | RNA-Seq of adult PVN; single-cell technology examining gene expression in zygotes      | —                                                                                        | —                              | —                       | Rodgers et al. (2015)    |
| Genetic manipulation                             | Overexpressing KDM1A during spermatogenesis | RNA analysis from two-cell embryos; RNA analysis of sperm                               | RRBS-Seq and Sequenom MassARRAY methylation analysis of sperm                           | H3K4me2 ChIP-Seq in sperm      | —                       | Siklenka et al. (2015)   |

Bisulfite-Seq, bisulfite sequencing; ChIP-Seq, chromatin immunoprecipitation (ChIP) combined with sequencing; CUMS, chronic and unpredictable maternal separation; H3K27me3, histone H3 lysine 27 trimethylation; KDM1A, lysine (K) demethylase 1A; McDIP-Seq, methylated DNA immunoprecipitation sequencing; miRNAs, microRNAs; MassARRAY, a system based on mass spectrum analysis for qualitative and quantitative detection of DNA methylation; PVN, paraventricular nucleus; RNA-Seq, RNA sequencing; RRBS-Seq, reduced representation bisulfite sequencing; sncRNA, small non-coding RNA.
years (9–12 years of age for boys and 8–10 years for girls) but not during later childhood (Bygren, Kaati & Edvinsson, 2001). This exposure-sensitive period to nutrition was confirmed in two of three independent cohorts in a subsequent analysis. For example, diabetes mortality increased following paternal grandfather exposure to a surfeit of food during mid-childhood (Kaati et al., 2002). With respect to specific windows of sensitivity for maternal nutrition exposure, it has been reported that the period around conception might be especially sensitive to famine exposure and is associated with diverse phenotypic outcomes (Kyle & Pichard, 2006). In particular, more DNA methylation changes were found when famine exposure occurred during the periconception period (Tobi et al., 2009). In addition, considering that a number of major epigenetic transitions, such as the erasure of previous imprints and the establishment of male-specific cytosine methylation patterns, occur during the last week of mouse gestation (Popp et al., 2010), the period when primordial germ cells develop represents another key period for exposure, although much remains to be learned regarding the plasticity of the epigenome and the ability of spermatogonial stem cells to respond to environmental alterations.

(4) Potential implications of transgenerational inheritance for human health

Although transgenerational epigenetic inheritance is well documented and relatively common in experimentally controlled species such as nematodes, the preponderance of transgenerational inheritance that is not based on DNA sequence alterations rarely has been reported in rodents and is even less frequent in humans, presumably owing to their robust germline reprogramming after fertilization and during development. In particular, considering that species survive and adapt to diverse environments during evolution and thereby generate specific epigenomes themselves, the conservation and prevalence of the mechanism underlying epigenetic inheritance in response to ecological challenges in nematodes and flies requires further investigation in humans. For example, mammalian progeny are known to be affected by maternal diet (Pembrey et al., 2014), but whether the similar 22G-RNAs in C. elegans play a role in the epigenetic inheritance observed following starvation in humans requires further investigation. Similarly, environmental alterations are able to regulate human longevity transgenerationally (Pembrey et al., 2006), so whether the H3K4 regulators identified in C. elegans are involved in transgenerational lifespan phenotypes in humans represents a promising area of study. In addition to examining the relative importance of these mechanisms in humans, whether they influence the rapid evolution of phenotypes observed in human populations remains to be determined. Notably, humans can transmit culture and habits through direct learning, teaching and education, which are typically Lamarckian phenomena. In this respect, we must be careful when drawing conclusions regarding the potential molecules responsible for epigenetic inheritance in human health. The question of how humans recall past experiences and environments may require a different perspective on data collection and analysis.

(5) Evidence for the interpretation of epigenetic inheritance as adaptive or pathological responses

Notably, most documented evidence for epigenetic inheritance is pathological. For instance, exposure to dietary challenge, including acute sugar dietary intervention (Ost et al., 2014), protein restriction (Carone et al., 2010; Sharma et al., 2016), in utero undernourishment (Pembrey et al., 2006; Gluckman et al., 2007; Radford et al., 2014), high-fat diet (Ng et al., 2010; Chen et al., 2016), and folate deficiency (Padmanabhan et al., 2013), all exhibited broad metabolic defects on offspring. Moreover, parental traumatic experiences, including unpredictable maternal separation (Franklin et al., 2010; Gapp et al., 2014), chronic variable stress (Rodgers et al., 2013, 2015), and chronic paternal social defeat stress ( Dietz et al., 2011), also resulted in behavioural and emotional disorders in the offspring. Nevertheless, phenotypic outcomes should not be assumed to be purely detrimental, and there are protective ‘adaptations’, which potentially lessen the risk of adverse outcomes in successive generations. For example, the transgenerational inheritance of antiviral effect in worms (Rechavi et al., 2011) may serve as an effective defence mechanism and provide adaptive benefits for future generations. The mechanism capable of fixing the epigenetically driven phenotypic changes and thus shortening the duration of epigenetic inheritance (Houri-Ze’evi et al., 2016) may reduce detrimental responses in offspring. The long-term silencing of foreign transcripts from one generation to the next (Conine et al., 2010; Ashe et al., 2012; Shirayama et al., 2012) may help to protect bona fide germline transcripts in the progeny. Moreover, the generation-to-generation acquisition of nurturing behaviour ( Weaver et al., 2004) and the inheritance of odour sensitivity ( Dias & Ressler, 2014) may provide the offspring with the ability to respond specifically and efficiently to odour and predator threat.

VI. CONCLUSIONS

(1) Identification of the epigenetic and molecular links between parental experience and the epigenomes in response to new challenges and of the relationship among the epigenomes of gametes and developing embryos as well as those of specific tissues in response to experience has represented a formidable challenge. Thus, delineating a common or several fundamental mechanism(s) capable of explaining the different modes of non-genetic inheritance might constitute the most essential issue for future investigation.

(2) More precise epigenetic techniques at both the genome-wide level and single-cell resolution are required for the profiling and screening of epigenetic alterations among generations. This will likely provide more comprehensive
Parental experience-induced epigenetic inheritance

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