

## REVIEW

# Neurobiological disease etiology and inheritance: an epigenetic perspective

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## ABSTRACT

Epigenetic marks in mammals are essential to properly control the activity of the genome. They are dynamically regulated during development and adulthood, and can be modulated by environmental factors throughout life. Changes in the epigenetic profile of a cell can be positive and favor the expression of advantageous genes such as those linked to cell signaling and tumor suppression. However, they can also be detrimental and alter the functions of important genes, thereby leading to disease. Recent evidence has further highlighted that some epigenetic marks can be maintained across meiosis and be transmitted to the subsequent generation to reprogram developmental and cellular features. This short review describes current knowledge on the potential impact of epigenetic processes activated by environmental factors on the inheritance of neurobiological disease risk. In addition, the potential adaptive value of epigenetic inheritance, and relevant current and future questions are discussed.

**KEY WORDS:** Diseases, Epigenetics, Inheritance, Neurobiology

## Introduction: the concept of epigenetic inheritance

Inheritance or heritability is defined as the transfer of phenotypic traits from parents to offspring through genetic and epigenetic processes. Genetic processes involve genes and DNA sequences that are passed on to future generations through gametes. Epigenetic processes involve an ensemble of mitotically and/or meiotically heritable changes in gene activity that, in contrast, do not implicate any change in DNA sequence, but can nonetheless be passed on to future generations through gametes (Allis et al., 2007; Russo et al., 1996). This distinction is important because many common human diseases have an observable heritable component (revealed, for example, through twin studies) that cannot be entirely explained with classical Mendelian inheritance, which governs genetic transmission (Altshuler et al., 2008; Eichler et al., 2010; Manolio et al., 2009). Epigenetic factors, though long ignored because of their lack of accordance with classical Mendelian inheritance (Jablonka and Raz, 2009), are now accepted to contribute to this ‘missing heritability’ (Eichler et al., 2010; Manolio et al., 2009). Moreover, transgenerational transmission by epigenetic mechanisms has now been documented (Franklin and Mansuy, 2010a; Jablonka and Raz, 2009). Some of the best examples involve stress as a possible transgenerational risk factor for depression, diet as a risk factor for cardiovascular diseases and diabetes, and exposure to environmental toxins as a risk factor for cancer, anxiety disorders and social dysfunction (Bygren, 2013; Jakovcevski and Akbarian, 2012; Webster et al., 2013). This review takes a close look at recent

evidence for mechanisms of epigenetic inheritance in the context of neurobiological disease, an exciting and important field of research that is still in its infancy.

## Molecular processes mediating epigenetics

Epigenetic mechanisms constitute the interface between genes and the environment, and primarily include DNA methylation, histone post-translational modifications (PTMs) and non-coding RNAs (ncRNAs). These processes can be dynamically regulated by external factors, such as diet and salient life events, providing a means by which cells with identical genetic information can acquire and maintain a specific molecular identity. Thus, while epigenetic processes are plastic in the sense that they can be modified by environmental experiences, once induced, epigenetic marks can also persist throughout the lifetime of a cell or an organism, and be passed on to future generations (transgenerational epigenetic inheritance). They are therefore an important consideration in the context of complex human neurobiological disorders.

DNA methylation in mammals is induced by the addition of a methyl residue to the fifth position of the pyrimidine ring of cytosines, and occurs primarily in dinucleotide CpG sequences (Lister et al., 2013; Tost, 2009). By altering the three-dimensional structure of DNA, cytosine methylation determines which DNA-binding molecules can associate with the DNA at specific loci, and thus has a potent and precise impact on gene expression. Similarly, histone PTMs are covalent modifications induced on histone proteins that mediate DNA packaging in nucleosomes, and control the level of transcription by altering the accessibility of DNA to transcription factors and enhancers. Several histone PTMs are possible, including acetylation, methylation, phosphorylation, sumoylation and ubiquitination (Jenuwein and Allis, 2001). Together with DNA methylation, histone PTMs constitute an epigenetic code that can regulate gene expression by remodeling the structure of DNA.

In contrast to DNA methylation and histone PTMs, ncRNAs do not affect the structure of DNA, but nonetheless can strongly impact transcriptional processes. In particular, several forms of small ncRNAs, such as microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs), have post-transcriptional effects on protein synthesis by directly controlling translation. As many targets of small ncRNAs are transcription factors [for example, of miR-124 (Millan, 2011)], or part of the epigenetic machinery [for example, of miR-132 (Lv et al., 2013)], they can have a profound impact on gene regulation. Long ncRNAs can directly co-regulate transcription by helping guide transcription factors to specific loci of the genome [for example, Evf-2 (Feng et al., 2006)].

The epigenetic profile of an individual changes considerably over the course of a lifetime, depending on environmental conditions such as diet, exposure to chemicals, toxicants or drugs, and salient life events (Franklin and Mansuy, 2010b; Murgatroyd et al., 2009; Robison and Nestler, 2011). Transgenerational epigenetic effects

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occur when an environment induces epigenetic changes that can be observed in the subsequent generation. However, true epigenetic inheritance implies that transmission involves the germline and is across at least three generations in the absence of the initial trigger as the third generation is the first not to carry any cell directly exposed to stress including the germ cell from which it originates. When maintained in the offspring, epigenetic changes can provide an adaptive advantage and allow the offspring to be better prepared for similar conditions (Harper, 2005; Jablonka and Raz, 2009). But at the same time, they can also render an individual more sensitive to environmental conditions and confer risk factors for diseases. As many epigenetic changes are associated with a predisposition to cancer or psychiatric disorders, correcting epigenetic aberrations may represent a potential avenue of treatment.

### Epigenetic transgenerational inheritance of behavior: environmental triggers

Epigenetic inheritance refers to the transmission of the effects of the exposure of one generation to specific environmental factors or conditions, and does not require that these conditions be present in subsequent generations. It is proposed to implicate epigenetic marks embedded in germ cells as a molecular trace of the experience. The transfer of such epigenetic traces across generations occurs through sexual reproduction, and is the most potent mode of transmission that can affect multiple generations. There is now substantial evidence to support the involvement of epigenetic transmission in animal models and in several human diseases (Table 1). This review describes their potential role in neurobiological diseases, as one of the most recent and currently least understood contexts for epigenetic transgenerational inheritance.

### Transmission involving the germline

Substantial evidence demonstrates that acquired traits can be transmitted not only via classical Mendelian heredity but also through germline-dependent epigenetic marks. Transmission of epigenetic information via the germline depends on the establishment of epigenetic marks in germ cells of the parent generation, and on their maintenance across meiosis and in the embryo after fertilization. Germline transmission has primarily been demonstrated through male germ cells because they are easier to examine (more abundant than female germ cells) and studies can be designed to circumvent the influence of maternal care and *in utero* development (Champagne, 2008). Still, epigenetic transmission through females has also been documented in rodents (e.g.

Rassoulzadegan et al., 2006; Wagner et al., 2008; Weiss et al., 2011), but little is known about how this transmission takes place. Critical factors to affirm germline transmission and exclude any maternal contribution include evaluation of maternal investment during early postnatal periods [which can vary depending on the fitness of the mated male (Curley and Mashoodh, 2010; Gowaty et al., 2007)], or control of the intrauterine environment by, for instance, *in vitro* fertilization (IVF), although this procedure itself can alter the epigenome (Grace and Sinclair, 2009).

### Transposable elements in mice

In mammals, one of the first instances of non-genomic inheritance of specific traits through the germline was observed in *agouti variable yellow* ( $A^{vy}$ ) mice. In these mice, the *agouti* gene carries a transposable element (intracisternal A particle, IAP) that can be differentially silenced by DNA methylation, and introduces variability in expression and phenotype (metastable epialleles) (Morgan et al., 1999). When hypomethylated,  $A^{vy}$  interferes with *agouti* expression and leads to a yellow coat (*agouti*-brown in wild-type mice), while an intermediate level of DNA methylation results in a patchy yellow fur (Wolff et al., 1998). Methylation can be shifted from low to high by administration of a methyl donor to gestating females, which leads to epiallele silencing (Wolff et al., 1998). Differential methylation can be passed to the offspring through the mother (Morgan et al., 1999). While this transmission occurs through pseudoagouti females across two generations (Cropley et al., 2006), it is not observed in females carrying a weakly methylated allele (yellow or mottled phenotype) (Cropley et al., 2007; Waterland et al., 2007). Such instances of epigenetic inheritance are exemplary and although no comparable retroviral insert exists in the *Agouti-related protein* gene in humans (Rosenfeld, 2010), transposable elements may contribute to epigenetic inheritance in humans as well, as they are very common in the genome (Lane et al., 2003).

### Stress exposure during different periods of life

Stressful events can strongly impact an individual's development, physiology and behavior, and are major risk factors for mental health disorders later in life and across generations (Heim et al., 2008; Perepletchikova and Kaufman, 2010). Several studies in humans have documented inheritance of the effects of early experiences. Descendants of holocaust survivors have a higher prevalence of depression and anxiety disorders (Yehuda et al., 2008). Likewise, children of women with post-traumatic stress disorder

**Table 1. Transgenerational epigenetic inheritance relevant to neurobiological disease**

Environmental event	Primary phenotype in F2 to F4	Relevant human disorder	Implicated epigenetic mechanism	Reference
<i>In utero</i> stress	Demasculinization	Sex-biased neuro-developmental disorders	miRNA	(Morgan and Bale, 2011; Ward, 1972)
Early-life stress	Behavioral despair, reduced anxiety	Depression anxiety disorders	DNA methylation	(Franklin et al., 2010; Weiss et al., 2011)
Poor maternal care	Poor maternal care	Relevant for child neglect/abuse	DNA methylation	(Roth et al., 2009)
Stress in adulthood	Higher anxiety	Anxiety spectrum disorders	Not determined	(Dietz et al., 2011)
Enrichment	Enhanced plasticity and contextual fear memory	Memory-deficit disorders	Not determined	(Arai et al., 2009)
Toxins (vincosolin)	♂ higher anxiety, ♀ lower anxiety	Anxiety spectrum disorders	DNA methylation	(Jirtle and Skinner, 2007; Skinner et al., 2008)
Toxins (BPA)	Increased social interaction	Autism spectrum disorders	Not determined	(Wolstenholme et al., 2012)
Drug abuse (cocaine)	Drug resilience	Addiction	DNA methylation, histone PTMs	(Maze et al., 2010; Novikova et al., 2008)

miRNA, microRNA; BPA, bis-phenol A; PTMs, post-translational modifications.

(PTSD), a pathology induced by severe stress, are more often affected by PTSD (this occurs to a lesser extent in children from PTSD men) and have increased susceptibility to a lower level of plasma cortisone like their parents (Yehuda et al., 2007). These effects may be transmitted through the parental germline, through differences in parenting, or may be linked to a genetic predisposition. In general, the intrinsic difficulty of analyzing human populations renders it exceedingly problematic to delineate the potential contribution of epigenetic mechanisms in the transgenerational effects of traumatic stress (Nadeau, 2009). For this reason, animal models of traumatic stress have been developed.

In doing so, it has become apparent that whether experienced in prenatal, early postnatal or adult life, traumatic stress can have detrimental consequences on subsequent generations. *In utero*, early mouse embryos subjected to stress (by stress applied to the gestating dam) develop high sensitivity to stress and have abnormal sexual development characterized by physical demasculinization of males. When bred to normal non-stressed females, these abnormally developed males give rise to male offspring with a similar, though muted, abnormal development (Morgan and Bale, 2011). In particular, these second-generation male offspring exhibit expression profiles within important neurodevelopmental genes reminiscent of females, including some that encode miRNAs. In particular, miR-322, miR-574 and miR-873 were all found to be reduced in the brain of second-generation males, while beta-glycan, a target common to all three of these miRNAs was increased (Morgan and Bale, 2011). This transgenerational inheritance of demasculinization has been proposed to involve epigenetic transmission associated with epigenetic marks introduced during the initial altered *in utero* environment as placental functions are highly susceptible to maternal stress and are subjected to epigenetic dysregulation (Monk et al., 2012). Prenatal stress also causes a demasculinization of behaviors in male mice, reducing copulatory behavior and increasing lordosis (Ward, 1972). It remains unknown whether these behavioral phenotypes are, like physical phenotypes, also passed on to subsequent generations.

Likewise, stress experienced in early postnatal life can have a detrimental impact involving epigenetic processes. In the mouse, unpredictable maternal separation combined with maternal stress in the first 2 weeks of life triggers severe depressive symptoms in the animals in adulthood, and alters their response to novelty, risk-assessment behaviors and social behaviors (Franklin et al., 2011; Franklin et al., 2010; Weiss et al., 2011). These distorted behaviors are associated with alterations in several molecular pathways, including serotonergic signaling via reduced serotonin receptor 5HT1A expression in the dorsal raphe and increased serotonin release in the frontal cortex (Franklin et al., 2011). These stress-induced symptoms in the first generation persist in the following two generations and are transmitted through both females (Weiss et al., 2011) and males (Franklin et al., 2010). In females, the phenotypes are not reversed by cross-fostering, indicating that they are independent of maternal care. Indeed, transmission to the third generation suggests epigenetic inheritance as the germ cells giving rise to this generation are never exposed to stress, and therefore must carry a trace of the initial stress in their epigenome (as a change in DNA sequence in all animals is highly unlikely). Consistently, in the sperm of males exposed to stress when pups and in the brain of their progeny, DNA methylation is altered at the promoter region of several genes relevant for behavior, including corticotrophin releasing factor receptor 2 (CRFR2) and cannabinoid receptor 1 (CB1). These genes have well-described roles in stress and emotionality and may contribute to the observed behavioral phenotypes.

Similarly, evidence for the alteration of DNA methylation and transgenerational epigenetic inheritance has been reported following early stress in rats. Pups raised by a mother with poor and abusive maternal behaviors have increased DNA methylation at the promoter of the brain-derived neurotrophic factor (BDNF) gene and impaired BDNF mRNA expression in the prefrontal cortex. Changes to the prefrontal cortex are especially relevant here because this brain region is associated with human episodes of early-life stress (Fumagalli et al., 2007; Lee and Hoaken, 2007). In this model, although induced by the mother, DNA methylation of the BDNF gene could not be completely reversed by cross-fostering, implying a contribution from epigenetic processes independent of maternal care (Roth et al., 2009). Importantly, changes in BDNF gene expression in the prefrontal cortex were rescued by infusion of the DNA-methyl transferase inhibitor zebularine.

Even stress in adulthood can induce subtle detrimental effects on subsequent generations through epigenetic processes. In one study, chronic social defeat [achieved by co-housing a subordinate adult C57Bl/6 male mouse and an aggressive adult CD1 male with a partition and allowing 10 min day<sup>-1</sup> of direct physical interaction (Berton et al., 2006)] led to a generalized social avoidance phenotype, where chronic social defeat mice, and their offspring, avoided even non-aggressive stranger mice (Dietz et al., 2011). The offspring of socially defeated mice also demonstrated increased anxiety, as observed by a decrease in the time spent in the open arms of an elevated plus maze, and behavioral despair, as observed by a decreased latency to adopt an immobile floating posture in the Porsolt swim test (Dietz et al., 2011). Interestingly, production of offspring through *in vitro* fertilization rescued some of the behavioral phenotypes, but the decreased latency to immobility in the swim test persisted in both male and female offspring, suggesting a potential epigenetic mode of transgenerational inheritance involving germ cells. The epigenetic transmission in this model may involve deregulation of the neuroendocrine system as male offspring of socially defeated mice have decreased basal levels of vascular endothelial growth factor (VEGF). This result is of particular interest as VEGF is associated with stress in humans, contributes to hippocampal neurogenesis, and may have a role in major depression (Clark-Raymond and Halaris, 2013). Female offspring have a highly variable level of VEGF, rendering the detection of differences more arduous. Hormone levels in offspring generated through *in vitro* fertilization were not measured.

Together, these animal models of traumatic stress at various stages of development have begun to provide exciting clues to the potential mechanisms of transgenerational epigenetic inheritance of neurobiological diseases.

#### Exposure to environmental toxicants

Phenotypes induced by environmental toxicants can also be transmitted across generations through epigenetic mechanisms. In rats, for example, repeated high-dose exposure of gestating females to vinclozolin, a crop fungicide that attenuates endocrine signaling, has severe transgenerational effects likely mediated by DNA methylation (Jirtle and Skinner, 2007). Behaviorally, exposure to vinclozolin affects anxiety levels of individuals of the third generation, as measured by altered performance in the light–dark box (Skinner et al., 2008). In addition, epigenetic transgenerational inheritance following vinclozolin exposure also increases the risk of tumor formation, kidney disease and immune dysfunction (Anway et al., 2005). Interestingly, anxiety levels in male and female mice changed in opposite directions, whereby male mice actually became less anxious (Skinner et al., 2008), potentially reflecting the muting

effects of vinclozolin on sexual development. Vinclozolin persistently alters DNA methylation in multiple genes in sperm. This effect is maintained down to the third generation in at least 16 genes (Guerrero-Bosagna et al., 2010), and has a profound impact on gene expression in the hippocampus and amygdala (Skinner et al., 2008), brain regions important for cognitive functions, mood regulation and anxiety. Vinclozolin also reduces DNA methylation at several paternally imprinted genes, but increases it at some maternally imprinted genes (Stouder and Paoloni-Giacobino, 2010). Some of the alterations involve a consensus DNA sequence that is more prevalent in the promoter region of the altered genes, suggesting that this sequence may have increased susceptibility to perturbations in DNA methylation by the toxic drug during development. The effects of vinclozolin across generations appear to be complex and sex dependent. Whether other mechanisms besides DNA methylation are involved remains unclear and it is also not known how the DNA methylation profile can be maintained and transmitted across generations. The high dose of vinclozolin used in these studies and the reported difficulty in reproducing some of these findings suggest that the effects observed may be contingent upon specific conditions (Inawaka et al., 2009; Schneider et al., 2008).

Other substances, including bis-phenol A (BPA) can also have transgenerational effects on disease risk (Manikkam et al., 2013; Salian et al., 2009; Wolstenholme et al., 2012), and induce epigenetically mediated alterations in behavior (Wolstenholme et al., 2012). Exposing gestating female mice to a dose of BPA that induces plasma levels similar to those found in humans was sufficient to reduce social interactions in the first generation (Wolstenholme et al., 2012). Interestingly, the second and fourth generations, which were never directly exposed to BPA, had increased social interactions compared with control animals. This relatively low dose of BPA was also sufficient to reduce expression of arginine vasopressin in the embryonic (E18.5) brain of both the first and fourth generations, which could directly contribute to the observed behavioral phenotype given arginine vasopressin's known role in social cognition and motivation (McCall and Singer, 2012). In addition, the embryonic brain of males from the fourth generation had a significant decrease in oxytocin, another neuropeptide involved in social interactions (Benarroch, 2013), although there was no difference in the first generation exposed to BPA (Wolstenholme et al., 2012). In this study, BPA did not have any effect on general anxiety as evaluated on the elevated plus maze (Wolstenholme et al., 2012), highlighting the specificity of the behavioral phenotype.

#### Drug and alcohol abuse

Cocaine is a strong epigenetic dysregulator that affects chromatin remodeling in the brain, in particular in reward circuits (Kumar et al., 2005; Maze et al., 2010; Renthal et al., 2009). It alters histone PTMs and the associated machinery, and was recently shown to impact PTMs in sperm and participate in the transmission of a drug-resilient phenotype to the next generation (Novikova et al., 2008; Vassoler et al., 2013). Chronic consumption of alcohol is another strong epigenetic disruptor. In human or rodents, alcohol abuse in mothers during pregnancy or in fathers just prior to conception dramatically alters the epigenome of the offspring and affects multiple genes and loci, including retroviral sequences and imprinted genes normally silenced (partially or fully) by DNA methylation (Knezovich and Ramsay, 2012; Ponomarev et al., 2012). In mice for instance, A<sup>vy</sup> methylation is increased and agouti expression is higher in the offspring of mothers consuming alcohol (Kaminen-Ahola et al., 2010). DNA methylation is also reduced at

the paternally imprinted growth-related gene H19 in sperm of the offspring and in the brain of their own progeny (F2) (Stouder et al., 2011), suggesting transgenerational transmission. This corroborates data in humans showing that moderate and heavy drinkers have lower H19 DNA methylation in sperm (Ouko et al., 2009). As both retroviral sequences and imprinted genes can maintain their profile of DNA methylation during gametogenesis and fertilization, they may contribute to the inheritance of the effects of alcohol.

#### Exposure to enriched conditions

Stimulating environments providing sensory, motor, social and cognitive stimuli improve cognitive performance in humans and animals (Petrosini et al., 2009). The long-term cognitive benefits induced by stimulating environments involve epigenetic mechanisms (Sweatt, 2009). For example, in mice exposed to enriched conditions, acetylation and methylation of H3 and H4 are increased in the hippocampus and cortex, an effect that can be mimicked pharmacologically with histone deacetylase (HDAC) inhibitors (Fischer et al., 2007). Epigenetic marks induced by environmental enrichment can also be transmitted to the following generation. For example, a 2 week exposure of female mice to enriched conditions (postnatal weeks 2–4) was found to enhance synaptic plasticity in the offspring through a maternal care-independent mechanism (Arai et al., 2009). However, the same enrichment procedure in males did not induce any transgenerational effect on memory or plasticity. Additionally, enriching the environment of learning-impaired *ras-grf* knockout mice rescued the deficit in hippocampal long-term potentiation as well as the cognitive defect in contextual fear conditioning that would otherwise be present in their offspring (Arai et al., 2009). These observations point to a profound beneficial impact of transgenerational epigenetic processes on future generations. Mechanistically, the augmented long-term hippocampal plasticity in the first and second generation following environmental enrichment was accompanied by a cAMP/p38 MAP kinase signaling cascade that is not normally recruited in the form of long-term potentiation examined. How this pathway is newly recruited is not known, but may involve persistent modifications such as DNA methylation, in a manner similar to how exposure to stressful conditions in early life leads to a hypermethylation and reduction of expression of the MeCP2 and CB1 genes (Franklin et al., 2010).

#### Potential mechanisms of germline transmission

The mechanisms of inheritance of epigenetic marks in the germline are complex and still poorly understood. While DNA methylation and histone PTMs in gametes have long been viewed as too labile for transgenerational transmission, recent evidence has shown that they can persist in the germline and be transferred from parents to offspring. Recent data also suggest that non-coding RNAs in germ cells may contribute to transgenerational transfer of information (Johnson et al., 2011). The main modes of epigenetic transgenerational inheritance are discussed below.

#### DNA methylation

In sperm cells, DNA methylation is established in successive steps and is dynamically regulated in the developing embryo. Shortly after fertilization, it is erased in the pre-implantation embryo through DNA demethylation and/or hydroxymethylation (Iqbal et al., 2011). This DNA 'reprogramming' is key to ensure totipotency, DNA methylation being later re-established during development in a cell-specific manner (Feng et al., 2010). Although this reprogramming affects the whole genome, some loci can retain parental DNA

profiles, in some cases, across several generations. First, sex-specific imprints, established during oogenesis and gametogenesis to silence one of the parental (maternal or paternal) alleles, maintain their profile of DNA methylation (Abramowitz and Bartolomei, 2012). Second, DNA methylation can also be maintained across embryonic development at IAPs, common retrovirus-like long-terminal repeat transposable elements in the mouse genome (Lane et al., 2003). Genes carrying such repeat-rich sequences might therefore be targets for transgenerational epigenetic regulation (Ruden et al., 2008). Further, over 200 non-imprinted genes keep their DNA methylation in the promoter region in the pre-implantation and post-implantation embryo; in particular, methylation present on DNA from the oocyte is retained (Borgel et al., 2010). More genes may have a similar feature, not only in the promoter but perhaps also at more restricted regions such as individual CpG, or transcription factor or enhancer binding sites, and thereby strongly impact expression programs in the developing embryo. These multiple modes of maintenance suggest that DNA methylation can be a vector for transgenerational transmission.

#### Histone PTMs and protamines

Histones and protamines in sperm may contribute to the maintenance of epigenetic codes across generations (Jenkins and Carrell, 2012), possibly through PTMs. To allow for the chromatin compaction that accompanies spermatogenesis, most histones are replaced by protamines. Following fertilization, protamines associated with the male genome are then removed, and are replaced essentially by maternal histones. Some paternal histones are, however, retained in sperm cells [1–2% in mice and up to 15% in humans (Brykczynska et al., 2010; Hammoud et al., 2009; Johnson et al., 2011)]. Further, paternal histones can also retain their PTMs. For example, in human sperm, dimethylated histone 3 lysine 4 (H3K4me2) and trimethylated histone 3 lysine 27 (H3K27me3) were shown to be retained at multiple loci with clustered genes important for development (Hammoud et al., 2009). In addition, H3K27me3 was found to be specifically enriched at the promoters for developmental genes that are repressed in early embryos. Indeed, the presence of H3K27me3 at transcription start sites of developmental genes in sperm correlates with gene expression in the early embryo (Brykczynska et al., 2010; Hammoud et al., 2009), suggesting that H3K27me3 likely contributes to paternal epigenetic transmission (Brykczynska et al., 2010). H3K27me3 may also influence the regulation of DNA methylation as histones in sperm are preferentially retained at CpG islands around transcription start sites. This may account for the establishment of DNA methylation-free regions in the early embryo as histone PTMs and DNA methyltransferases are linked (Ooi and Bestor, 2008; Vavouri and Lehner, 2011). It is conceivable that, like histones, protamines also play a role in the inheritance of epigenetic marks, but there is no evidence available for this. Recent studies have, however, begun to demonstrate that diet can shape histone PTMs in sperm cells (Carone et al., 2010). Specifically, a low-protein diet in mice causes a 4-fold decrease in the amount of H3K27me3 within the promoter regions of the monoamine oxidase gene. Future research will have to demonstrate whether and how these alterations can impact the oocyte and the developing embryo upon fertilization.

#### Small ncRNAs in sperm

Small ncRNAs, including miRNAs and piRNAs, are abundant in sperm. In human, about 24,000 short ncRNA molecules are delivered to the oocyte upon fertilization by a single sperm cell (Kawano et al., 2012; Krawetz et al., 2011; Peng et al., 2012),

representing a delivery of 10–20 fg RNA (Johnson et al., 2011). Therefore, sperm RNA constitutes a direct mediator for the transfer of information from one generation to the next. miRNAs may be particularly important in this respect as about 20% of known mouse miRNAs are introduced into the oocyte during fertilization (Amanai et al., 2006). At the same time, it has been noted that endogenous miRNAs in the oocyte are less effective at repressing reporter mRNA translation (Ma et al., 2010), suggesting that (at least early on) paternal miRNAs might produce only a muted effect. However, the level of oocyte-induced reporter mRNA repression demonstrated in this study was fairly modest (~40%), and no effect was observed once the oocyte was fully grown. As some miRNAs are stable, with a half-life of over 24 h (Winter and Diederichs, 2011), which is within the maturation time of a fertilized oocyte, it is conceivable that paternally derived miRNAs regulate translation immediately upon fertilization or at later stages of embryonic development. Accordingly, individual sperm-borne miRNAs, miR-135a and miR-34c, have recently been shown to regulate key genes in the mouse zygote essential for normal embryonic development (Liu et al., 2012; Pang et al., 2011). Specifically, inhibition of miR-135a via microinjection of a synthetic antagomir reduced the percentage of zygotes developing to the two-cell stage (Pang et al., 2011). This effect could be almost completely rescued by co-injection of an antibody against Siah1, a ubiquitination/proteasome pathway protein, which is a direct target of miR-135a. Similarly, inhibition of miR-34a (which is only present in sperm) dramatically delayed mouse zygote development (Liu et al., 2012). Moreover, sperm loaded with pre-miR-212 produced zygotes with a 75% increase in mature miR-212 transcript (Liu et al., 2012), supporting the existence of a functional miRNA processing machinery in the developing embryo. piRNAs have also been detected in mature sperm (over 1000 in human sperm) (Kawano et al., 2012; Krawetz et al., 2011) and may play a role in germ cell development, especially in the silencing of transposons by guiding DNA methylation (Aravin et al., 2008; Law and Jacobsen, 2010), but it is not known whether they are also delivered to the oocyte.

In studies of a mouse mutant for the gene encoding the Kit receptor, *Kit tmlAlf1*, the characteristic white-tail phenotype of the mutant was unexpectedly maintained in wild-type homozygotes following mating of heterozygote mice, and also in subsequent generations. The *Kit*<sup>+</sup> alleles thus underwent an epigenetic modification (indicated by *Kit*<sup>\*</sup>) resulting in the expression of a modified phenotype similar to that of the mutant, and displaying reduced expression of *Kit* mRNA. Once established, this epigenetic regulation of gene transcription is transmitted through the germ line with high efficiency, both paternally and maternally, in non-Mendelian ratios. Remarkably, this epigenetic regulation could also be induced by injection of *Kit*-specific miRNAs, miR-221 and miR-222, directly into wild-type zygotes (Rassoulzadegan et al., 2006), and the paramutation is not unique to the *Kit* gene, but may rather be a more general phenomenon. Microinjection of miR-1, a miRNA known for its involvement in heart physiology, led to mice with hypertrophic hearts, and this phenotype was also passed on to subsequent generations (Wagner et al., 2008). The heart defects were linked to upregulation of *Cdk9*, a key effector of cardiac development with significant sequence homology to miR-1, and a similar pathology was induced upon injection of fragments of the *Cdk9* mRNA (Wagner et al., 2008).

Together, these genetic and molecular studies evidence a mechanism whereby RNA molecules may mediate paternal epigenetic transgenerational inheritance, though the underlying molecular mechanisms are still largely unknown.

## Conclusions

Evidence from different model systems has accumulated in the past years to demonstrate the existence and importance of epigenetic inheritance. This notion radically changes the classical view of heredity and adds an outstanding component to the importance of the interaction between genes (nature) and environmental factors (nurture). This broader view is not only a major conceptual step forward but also a key advance in the understanding of diseases and disease risk. But while the notion of epigenetic inheritance is now better accepted, its evolutionary relevance is still debated. However, it is clear that a primary advantage of epigenetic inheritance over classical Mendelian inheritance is that it can allow rapid adaptation to specific environmental conditions, and transfer of the adaptive responses to the following generation (Harper, 2005; Jablonka and Raz, 2009). Such adaptation is beneficial because it helps prepare future generations for environmental changes. However, it may lead to mismatch situations that can ultimately be detrimental when marked differences between the anticipated and the actual environment are experienced (Beldade et al., 2011).

The mechanisms underlying epigenetic inheritance of neurobiological disorders still remain poorly understood. Epigenetic inheritance likely involves several pathways that may operate in parallel or in synergy depending on the conditions. Similar molecular pathways and shared components might be recruited to regulate epigenetic profiles in different tissues and during different temporal windows, and when affected in germ cells they can lead to disease across generations (Bohacek and Mansuy, 2013). A better understanding of epigenetic inheritance also holds great promise for diagnostics and drug development. Profiling of epigenetic marks can give useful indications about disease susceptibility in humans. For instance, methylation of the glucocorticoid receptor gene was shown to be higher in the brain of suicide victims, and correlates with childhood abuse in these victims (McGowan et al., 2009). Thus, assuming such epigenetic changes are systematically altered in specific pathologies, and are consistent in brain and blood in patients, it may be conceivable to design diagnostic tools such as blood tests to detect them. Such detection may also be useful in the evaluation of drug treatments as, unlike genetic mutations and single-nucleotide polymorphisms, epigenetic aberrations are reversible. Drugs affecting DNA methylation, such as 5-azacytidine, which inhibits *de novo* DNA methyltransferases, or drugs that target histone PTMs, such as the histone deacetylase inhibitor trichostatin A, could be useful, although their lack of specificity can limit their benefit. Other types of drugs based on epigenetic modifiers may also be efficient (Hamm and Costa, 2011) and have better specificity. Finally, the study of transgenerational epigenetics is challenging because it requires difficult and long experimental designs, highly interdisciplinary expertise and technical innovations, and is still in its infancy. In the future, the development of more powerful and sensitive methods to detect and measure epigenetic marks, and of effective bioinformatics is expected to help move the field forward.

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