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## Transgenerational Epigenetic Inheritance: myths and mechanisms

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### Abstract

Since the human genome was sequenced, the term “epigenetics” is increasingly being associated with the hope that we are more than just the sum of our genes. Might what we eat, the air we breathe, or even the emotions we feel, influence not only our genes but those of descendants? The environment can certainly influence gene expression, and can lead to disease, but trans-generational consequences are another matter. While the inheritance of epigenetic characters can certainly occur - particularly in plants – how much is due to the environment and the extent to which it happens in humans, remains unclear.

### Introduction

The notion that heredity is influenced by the environment has figured prominently in evolutionary thinking for centuries, as Luther Burbank famously stated “heredity is only the sum of all past environment” (in “The Training of the Human Plant.” *Century* magazine (1906)). But with the re-discovery of genetics, conventional wisdom had it that selection acts on phenotypic variation, via genetic variation that is itself blind to environmental cues. Further, according to Weismann's principle of the germplasm (1892), somatic cells are separated from germ cells and thus no mechanisms were thought to exist for germ cells to be modified by the environment. Over the last few years, the “re-discovery” of epigenetics and its underlying mechanisms, has re-opened this old debate, giving rise to the concept of transgenerational inheritance of epigenetic variation, and even of acquired traits (Box 1).

In principle, epigenetic inheritance and germline reprogramming are two sides of the same coin. Germline reprogramming facilitates totipotency of the zygote, a corner stone of

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developmental biology since the concept of “epigenesis” was first proposed (Aristotle, *On the Generation of Animals*; Harvey (1651) *Exercitationes de generatione animalium*, Wolff (1759) *Theoria Generationis*). Reprogramming is required to remove epigenetic signatures acquired during development, or imposed by the environment, so that subsequent elaboration of the body plan in the embryo properly reflects the genetic blueprint characteristic of each species. If germline reprogramming fails, epigenetic marks can be retained, and could be transmitted from one generation to the next. As with classical (i.e. DNA sequence) mutations, most epigenetic “mutations” (epialleles) are either neutral or deleterious, frequently involving the unleashing of transposable elements and other genomic parasites. But transgenerational epigenetic inheritance has also the potential to be adaptive, and in some cases, might even respond to environmental challenges, with major implications for heredity, breeding and evolution.

Epigenetic inheritance is relatively common in plants. The plant germline arises from somatic cells exposed to developmental and environmental cues (Box 2), and many plant species can be propagated clonally, with no germline passage at all. It is perhaps no accident that the inheritance of acquired traits was first proposed by botanists, most famously by Jean-Baptiste Lamarck, and most infamously by Trofim Denisovich Lysenko. The potential implications for mammalian development, and for human health, were quickly realized and in recent years many potential examples of epigenetic inheritance have been documented. However such studies often concern inter- rather than trans-generational effects (Figure 1) and rarely exclude DNA sequence changes as the underlying cause for heritability. Although inter-generational effects (such as maternal effects) certainly occur in mammals, the degree to which they can be transmitted in the absence of the initial trigger remains unclear. In mammals efficient reprogramming occurs in the early embryo and in the germ line (Box 2). These two rounds of epigenetic erasure leave little chance for inheritance of epigenetic marks, whether programmed, accidental or environmentally induced (Fig. 2A). Thus, although transmission of acquired states can occur in some animals (such as nematodes), proof that transgenerational inheritance has an epigenetic basis is generally lacking in mammals. Indeed, evolution appears to have gone to great lengths to ensure the efficient undoing of any potentially deleterious bookmarking that a parent's lifetime experience may have imposed.

In this review, we will examine the mechanisms underlying epigenetic inheritance and germline reprogramming (Box 3). Several comprehensive reviews of epigenetic inheritance in plants (Schmitz and Ecker, 2012; Weigel and Colot, 2012) and animals (Daxinger and Whitelaw, 2012; Jablonka and Raz, 2009; Lim and Brunet, 2013) have been published recently, so we will focus on aspects that are shared – and for which parental effects and DNA sequence mutations have been excluded as far as possible. We also examine the limited evidence for adaptive inheritance of environmentally induced epigenetic traits, and consider the implications for evolution, plant breeding and human health.

## RNA interference and transcriptional silencing

What are the factors that specify transposons and transgenes, but not essential genes, for trans-generational silencing? Building on classical work in maize, recent work in

*Arabidopsis*, *Drosophila* and *C.elegans* suggests that small RNA may be an essential component of the trigger that targets heritable silencing. RNA interference, which requires transcription, can initiate and maintain a more permanent form of transcriptional silencing, passed from generation to generation in the absence of the small RNA trigger. Many of the clues to this mechanism have come from fission yeast, in which RNAi guides histone modifications including methylation of histone H3 lysine-9 (H3K9) and the demethylation of H3K4. In fission yeast, histone modification is achieved by co-transcriptional recruitment of RNAi transcriptional silencing (RITS) and histone modification complexes via binding of small RNA to PolII-dependent non-coding RNA precursors. Spreading of these complexes along the chromosome occurs by interaction with H3K9me2 itself, and by interaction with DNA polymerase and the replisome during S phase. RNAi promotes the release of RNA polymerase II and prevents DNA damage and defects in heterochromatin repair (Castel and Martienssen, 2013; Keller and Buhler, 2013).

### RNA dependent DNA methylation

In plants, as in fission yeast, it was quickly realized that RNAi was linked to transcriptional silencing and was likely responsible for the previously described process of RNA directed DNA methylation (RdDM) (Eun et al., 2012; Law and Jacobsen, 2010; Slotkin and Martienssen, 2007). In *Arabidopsis*, genes required for RdDM encode factors associated with the large subunits of RNA polymerases PolIV and PolV, which are closely related to PolII (Eun et al., 2012; Haag and Pikaard, 2011; Law and Jacobsen, 2010). For example, RNA DEPENDENT RNA POLYMERASE 2 is required for 24nt siRNA biogenesis, and is associated with PolIV, while Argonaute proteins that bind 24nt siRNA are associated with PolV, as are chromatin remodelers, histone methyltransferases and DNA binding proteins, suggesting a link with chromatin as well as RNAi (Haag and Pikaard, 2011; Law and Jacobsen, 2010). The de novo DNA methyltransferases DRM1 and DRM2 (homologs of mammalian Dnmt3), the chromomethyltransferase CMT2 and several histone H3 lysine 9 methyltransferases (SUVH homologs of Su Var 3–9) are also required for RdDM, but the direct link between RNAi, and DNA and histone methylation remains unknown (Law and Jacobsen, 2010; Stroud et al., 2014; Zemach et al., 2013). RdDM can correct transgenerational defects in transposon methylation that arise in *ddm1*. These transposons retain or acquire siRNA in *ddm1* mutants, and RNAi is required for restoration of silencing and methylation when DDM1 is reintroduced (Creasey et al., 2014; Ito et al., 2011; Mirouze et al., 2009; Teixeira et al., 2009; Zemach et al., 2013).

### Paramutation

Around the same time that McClintock discovered transposable element silencing, R. Alexander Brink, Ed Coe Jr. and Marcus Rhoades reported the first examples of transgenerational gene silencing by “paramutation” in maize (Chandler, 2007; Hollick, 2012). Individual alleles at 3 different color gene loci gave rise to epialleles with reduced pigmentation. These epialleles silenced other alleles in heterozygotes, more or less permanently. Silencing was allele-specific, dose-dependent, temperature dependent, and occurred shortly after fertilization. Numerous examples have now been described in plants (Arteaga-Vazquez and Chandler, 2010), and powerful genetic screens in maize uncovered a central role for RNAi (Chandler, 2007; Hollick, 2012): *mop1* (*mediator of paramutation 1*)

and *mop2* encode orthologs of *RNA DEPENDENT RNA POLYMERASE 2* and the large subunit of RNA polymerase IV, respectively, while *rmr6* (*required to maintain repression6*) encodes the second largest subunit of PolIV. Accessory factors, such as the chromatin remodeler RMR1, the plant specific RMR2, and the DNA binding protein CBBP (Barbour et al., 2012; Brzeska et al., 2010) likely interact with paramutated loci. DNA methylation is found at most paramutable loci, and there are up to 2,000 such loci in maize (Eichten et al., 2013; Regulski et al., 2013), but DNA methylation changes are modest and may not be responsible for silencing (Chandler, 2010). The promoters of paramutable genes usually contain transposons and repeats (Chandler, 2007; Erhard et al., 2013; Hollick, 2012), which act *in trans* as the apparent source of small RNA (Arteaga-Vazquez et al., 2010). Inverted repeats can also drive transgenerational silencing of transposons by RdDM in maize (Figure 4A). This co-ordinate silencing of transposons, reminiscent of paramutation, also depends on *RDR2* (in both maize and *Arabidopsis*), and might operate by a similar mechanism (Lisch, 2012, 2013; Mari-Ordonez et al., 2013).

RNA interference in *C. elegans* has long-term multigenerational consequences that resemble paramutation (Figure 4B). In worms inactive transgene arrays containing viral and reporter genes heritably silence active arrays (Rechavi et al., 2011). Like paramutation, maintenance of silencing depends on endogenous RNA dependent RNA polymerase, encoded by *rrf1*, that is responsible for the generation of 22Gs, which are endogenous 22nt small RNA that preferentially begin with 5' guanosine triphosphate (G). piRNA (piwi interacting small RNA), known as 21Us in *C.elegans*, can trigger endogenous secondary 22Gs that bind nuclear, non-catalytic, worm-specific argonautes (WAGOs). These 22Gs silence transgenes and endogenous genes for more than 20 generations (Ashe et al., 2012; Buckley et al., 2012; Burton et al., 2011; Gu et al., 2012; Shirayama et al., 2012). Again, silent transgenes silence other transgenes in a dominant fashion, resembling paramutation (Ashe et al., 2012).

An important example of paramutation that depends on piRNA was also reported in *Drosophila*. Tandem arrays of P element transposons that contain reporter genes exhibit a *trans*-silencing effect (TSE) in that they can silence similar arrays on other chromosomes, but only when transmitted through the maternal germline. Like paramutation in maize, TSE in *Drosophila* is non-allelic and somewhat unstable, but is maintained for >50 generations (de Vanssay et al., 2012). TSE requires Aubergine (a metazoan piwi protein), but not RNA dependent RNA polymerase (which is absent from *Drosophila*), or Dicer-2, which is required for siRNA biogenesis. Such arrays generate large amounts of piRNA, but only when they are silenced, resembling heterochromatic repeat arrays in *S. pombe* (Castel and Martienssen, 2013; Keller and Buhler, 2013) and *Arabidopsis* (Stroud et al., 2014) in this respect. Again, silent target arrays are potent silencers themselves, thus fulfilling the definition of paramutation via maternal piRNA (de Vanssay et al., 2012).

In mammals, very few paramutation-like phenomena have been reported. The best known is the murine *Kit<sup>tm1Alf</sup>* allele (Rassoulzadegan et al., 2006), whereby a *LacZ* insertion at the *Kit* locus produces a *Kit-LacZ* fusion (resulting in non-functional Kit protein) that leads to melanocyte defects. Wild type progeny from *Kit* heterozygous parents (and some control progeny) displayed the *Kit* phenotype – but this transmission disappeared after a few generations (F4). The transgenerational phenotype was linked to RNA transmission based

on microinjection experiments, and the tRNA methyltransferase Dnmt2 was implicated (Kiani et al., 2013), although the molecular basis for such trans-generational inheritance remains unclear.

## Histone modification

Similarities between RNAi-mediated heterochromatic silencing in *S.pombe* and paramutation in *C. elegans* and *Drosophila*, neither of which have DNA methylation, suggest that histone modifications might also be important for transgenerational inheritance (Castel and Martienssen, 2013). WAGO/NRDE/HRDE mediated silencing in worms (Figure 4B), Piwi mediated silencing in *Drosophila*, and RdDM in plants all result in histone H3 K9 methylation and depend on it for their transgenerational effects (Burkhart et al., 2011; Gu et al., 2012; Huang et al., 2013; Law and Jacobsen, 2010; Le Thomas et al., 2013; Rozhkov et al., 2013; Shirayama et al., 2012; Sienski et al., 2012). Differences between yeast, plants and worms include differing dependence of H3K9me2 (and transcriptional silencing) on argonaute catalytic activity (Ashe et al., 2012; Irvine et al., 2006; Qi et al., 2006; Shirayama et al., 2012), reflecting the multiplicity of argonautes in metazoans and plants. Another important difference is that, in addition to histone methylation, plants also deploy DNA methylation downstream of RNAi, although RdDM of transposons can take several generations to take effect (Law and Jacobsen, 2010; Mari-Ordonez et al., 2013; Teixeira et al., 2009). For example, although RdDM is highly active in pollen (Box 2), it does not silence transposons in the vegetative nucleus (Calarco et al., 2012; Ibarra et al., 2012; Slotkin et al., 2009). It seems likely therefore that histone modifications may play an important role in transgenerational inheritance even in plants, especially in the germline.

Screens for loss of transgenerational germline silencing were performed in *C.elegans* (Ashe et al., 2012; Buckley et al., 2012) leading to the identification of *HRDE-1* (heritable defective RNAi) as *WAGO-9*, an argonaute related to *NRDE-3*, and H3K9 methyltransferases SET-24 and -25 (Figure 4B). Further, even though the *NRDE* genes were identified in a screen for somatic silencing defects, *NRDE1* to *NRDE4* were also required for transgenerational germline silencing, and displayed *hrde* phenotypes. *HRDE-1* binds 22G secondary endo-siRNA from several thousand genes, pseudogenes and cryptic loci, resembling *NRDE-3* in this respect, but only in the germline. Also like *NRDE-2/3/4*, *HRDE-1* is required for H3K9 methylation at many of these loci in the germline (Buckley et al., 2012).

Remarkably, both *nrde* and *hrde* mutants exhibit progressive loss of fertility of both male and female gametes, as well as loss of gametogenesis itself, after many generations of inbreeding (Buckley et al., 2012). These defects are fully restored when crossed to wild-type indicating they are unlikely due to accumulating chromosomal abnormalities. This was not the first time such a phenotype had been observed: mutants in *LSD1*, the enzyme responsible for demethylation of H3K4 have a very similar progressive loss of fertility, such that later generations have few if any offspring compared to early generations (Katz et al., 2009). In another study, mutants in *SET1*, the enzyme responsible for H3K4 methylation, display a progressive loss of longevity so that each generation of worms has a shorter and shorter lifespan (Benayoun and Brunet, 2012). In each case, H3K4me2, a mark associated

with actively transcribed genes seems to be involved. One idea is that the inability to reprogram this transcriptional histone mark in the germline results in aberrant memory of transcription that increases longevity on the one hand but reduces fertility on the other. Thus, the balance between fertility (germline immortality) and longevity (somatic mortality) may be one of the most profound consequences of epigenetic transgenerational inheritance (Lim and Brunet, 2013).

In some ways, mutants in *ddm1* in Arabidopsis resemble mutants in *lsd1* in worms, as they both display elevated levels of H3K4me2, especially in heterochromatin (Lippman et al., 2004). Interestingly, *ddm1* mutants also progressively lose fertility over generations of inbreeding (Kakutani et al., 1996), and lose it much more rapidly in double mutants in which both transcriptional and post-transcriptional silencing are lost (Creasey et al., 2014; Mathieu et al., 2007). Thus it is possible that RNAi and histone modification also play a role in germline immortality in plants.

## Germline reprogramming and imprinting

A major barrier to transgenerational epigenetic inheritance is germline reprogramming, during which histone variants and their modifications, as well as small RNAs and DNA methylation, are all reset (Box 2). In mammals reprogramming occurs both in the germline and in the zygote immediately after fertilization (Figure 2A). Imprinted loci succumb to germ line reprogramming but resist the post-zygotic phase. The mechanisms that maintain the DNA methylation of imprint control regions (ICRs), in the face of global demethylation in the zygote have recently started to be unraveled. On the one hand, specific factors (PGC7/Stella/Dppa3) prevent demethylation by binding H3K9me2 and blocking Tet3 activity (which can convert 5-mC to 5-hydroxyl-mC) on the maternal genome, as well as at imprinted loci in the paternal genome (Nakamura et al., 2012). Also, the DNA binding factor Zfp57 together with Kap1/Trim28 are critical for post-fertilization maintenance of maternal and paternal methylation imprints (Li et al., 2008; Messerschmidt et al., 2012). In the germ line, where all known imprints appear to be erased, the efficiency of DNA methylation reprogramming of the epigenome has been comprehensively assessed in two recent studies in the mouse (Hackett et al., 2012; Seisenberger et al., 2012). Genome wide DNA methylation profiling revealed that although the bulk of the genome (including imprinted loci) becomes demethylated in primordial germ cells, a number of loci (4730) that escape this demethylation (showing >40% 5mC) in PGCs were found to be predominately repeat-associated – in particular IAPTR1 elements, which are the most active and mobile (thus potentially mutagenic) repeat elements that may thus need to be silenced even during germ line reprogramming. In addition to these IAPs, 233 single-copy loci with >40% 5mC, were found. Why these loci are particularly prone to escape reprogramming is still not clear but they could represent prime candidates for possible trans-generational inheritance in mammals.

In *C.elegans*, the germline undergoes characteristic alterations in histone modifications that result in meiotic silencing of unpaired DNA, which efficiently silences most transgene arrays in the germline, as well as the X chromosome in males, and depends on the RdRP *ego-1*, responsible in part for 22Gs (Kelly and Aramayo, 2007). piRNA (piwi interacting



small RNA), known as 21Us, can also trigger endogenous secondary 22Gs that bind nuclear, non-catalytic, worm-specific argonautes (WAGOs) and silence transposons and some endogenous genes (Ashe et al., 2012; Buckley et al., 2012; Burton et al., 2011; Gu et al., 2012; Shirayama et al., 2012). Germline genes are thought to be protected from silencing by another argonaute, CSR-1 that binds the same 22G siRNA. This has led to the idea that piRNA scan the genome to silence foreign, non-self DNA, while CSR-1 22Gs prevent silencing, perhaps by restricting siRNA access to WAGO in the germline (Shirayama et al., 2012). A similar scanning mechanism has been proposed in ciliates which recognize transposons and other insertion sequences that are present in zygotic genomes, but not in the maternal genome, via small RNA (Chalker and Yao, 2011).

In plants, the extent of germline reprogramming of DNA methylation has been examined by whole genome bisulphite sequencing in pollen cell types (Calarco et al., 2012; Ibarra et al., 2012). In sperm cells and their microspore progenitors, more than 80% of mC residues are retained, including all those in a symmetric (CG or CHG, where H is A,C,T) sequence context, but asymmetric CHH methylation is specifically reduced (Figure 2B). As mCHH is guided by small RNA this allows for reprogramming of this epigenetic mark after fertilization (Jullien et al., 2012), when the majority of 24nt heterochromatic siRNA is provided by the maternal genome (Mosher et al., 2009). This results in transgenerational maternal silencing of otherwise active retrotransposons by RNA guided DNA methylation (Mari-Ordonez et al., 2013; Reinders et al., 2013), and may be related to “self-non-self” distinction in *Tetrahymena*, *C. elegans* and *Drosophila* (Brennecke et al., 2008; Chalker and Yao, 2011; de Vanssay et al., 2012; Shirayama et al., 2012).

24nt and 21nt siRNA pathways antagonize each other in plants (Creasey et al., 2014; Jauvion et al., 2012; Mari-Ordonez et al., 2013), reminiscent of WAGO and CSR-1 22Gs in *C. elegans*, and may participate in scanning for “non-self” transposons in pollen (Slotkin et al., 2009). 21nt secondary siRNA (epigenetically activated siRNA or easiRNA) are triggered by miRNA (Creasey et al., 2014), and target transposons that are strongly activated in the vegetative nucleus (Figure 2), accompanied by down regulation of DDM1 (Calarco et al., 2012; Ibarra et al., 2012; Slotkin et al., 2009). 21nt easiRNA accumulate in sperm cells, where they recognize these same transposons in the germline (Slotkin et al., 2009) and could contribute to reduced RdDM. After fertilization, methylation levels are restored in the embryo (Figure 2) but remain low in the endosperm, an extrabryonic tissue in the seed that is the product of fertilization of the central cell nucleus (companion to the egg cell) with a second sperm cell. The endosperm also acts a source of mobile small RNA that may reinforce RdDM in the embryo (Hsieh et al., 2009). Imprinting in flowering plants is largely restricted to the endosperm, and in sperm cells maternally expressed imprinted genes are protected from reprogramming by 24nt siRNA from the vegetative nucleus that triggers RdDM in sperm (Calarco et al., 2012; Ibarra et al., 2012). For this reason, imprinting of a subset of imprinted Maternally Expressed Genes (MEGs) depends on RdDM (Vu et al., 2013). However, small RNA has not yet been implicated in resistance to reprogramming in mammals.

## Epigenetic variation and the adaptive inheritance of acquired traits

DNA sequence change (mutation) can be a slow process, and is therefore not ideal for an organism or population to survive in a dynamic environment. Epigenetic mechanisms, modulated by environmental cues, have been proposed to enable 'soft inheritance', permitting adaptation to fluctuating environments and nutrition (Richards, 2006). The question is, can epigenetic inheritance truly represent such soft inheritance, given the resetting of epigenetic marks between generations? In plants, evidence for heritable epigenetic variation is more than half a century old, and likely reflects the high heritability and limited reprogramming of epigenetic variants in the germline, so that epialleles can be propagated for literally hundreds of years (Cubas et al., 1999). Many, perhaps most of these epialleles are induced by transposons that bring nearby genes under their control (Slotkin and Martienssen, 2007). In animals, by contrast, there are relatively few examples of heritable epigenetic variation at individual genes, but there are many examples of quantitative epigenetic traits that appear to respond to environmental, and especially nutritional cues experienced by former generations. For example in the nematode *C. elegans*, exposure to an olfactory cue early in development affects behaviour when encountering the chemical in adulthood, a process known as olfactory imprinting, and this behavior can then be transmitted over more than 40 generations (Remy, 2010). Worms that have been imprinted not only exhibit a more robust ability to migrate toward the chemical, but also lay significantly more eggs. Although the mechanisms remain unclear, olfactory imprinting provides a memory of a favorable environment that can be passed onto multiple generations (Remy, 2010). It is possible therefore that the very short generation time, acute exposure to the environment, and the abundance of small RNA have predisposed *C. elegans*, like plants, to dispense with germline reprogramming to some extent and indulge in transgenerational inheritance.

The degree to which germline reprogramming and transgenerational inheritance have contributed to potentially adaptive epigenetic variation in plants has been explored by genome-wide profiling of DNA methylation in natural and inbred populations. These studies have revealed the extent of DNA methylation cycling and paramutation-like behavior, and their contribution to epigenetic variation (Becker et al., 2011; Becker and Weigel, 2012; Eichten et al., 2013; Greaves et al., 2012; Li et al., 2013; Regulski et al., 2013; Schmitz and Ecker, 2012; Schmitz et al., 2011). Cycling contributes to the limited epigenetic variation found in individual inbred populations (Becker et al., 2011; Schmitz et al., 2011), while DNA methylation at most retrotransposons is more faithfully maintained. There are also a few hundred conserved targets of RdDM that never lose methylation (Schmitz and Ecker, 2012), resembling a sort of epigenetic selective sweep (Vaughn et al., 2007). Interestingly, these regions are demethylated in the vegetative nucleus of the pollen grain, along with imprinted genes (Calarco et al., 2012; Ibarra et al., 2012), and reinforce silencing in sperm cells via mobile 24nt small RNA (Figure 2). Some of these genes are required for pollen tube growth providing a plausible evolutionary origin (Schmitz and Ecker, 2012). Paramutation has also contributed to epigenetic variation in natural populations, and sometimes occurs between non-allelic positions leading to hybrid incompatibility reminiscent of the Dobzhansky Bateson Muller effect (Durand et al., 2012). Examples include PAI2, a non-essential gene which is heritably silenced by an unlinked inverted



repeat in a subset of *Arabidopsis* accessions (Enke et al., 2011; Schmitz and Ecker, 2012) and *AtFOLT1* an essential gene which can be paramutated by non-allelic epialleles resulting in inviable transgressive phenotypes in hybrids (Durand et al., 2012).

While heritable epigenetic variation clearly exists in nature, it is very important to distinguish random epivariation acted on by selection, from adaptive epigenetic variation induced by the environment. These two forms of transgenerational inheritance may well be related, but this assumption is not yet justified. For example, transgenerational viral reporter gene silencing in *C. elegans* may be related to an adaptive antiviral response, triggered by viral infection, but no such antiviral response has been explicitly demonstrated with this heterotypic virus (Rechavi et al., 2011). Further, it is only when individuals that are truly genetically identical exhibit a range of phenotypes that are heritable – that these can truly be attributed to epivariation. When the genes underlying the particular trait are not known, it is almost impossible to rule out DNA sequence mutation. For example, outbred rats exposed to the fungicide vinclozolin *in utero* exhibited diminished male fertility over three to four generations of offspring, transmitted through the male germline (Anway et al., 2005). However, no effects were observed with another strain of inbred rats raising the possibility that genetic variation was responsible for the effect (Schneider et al., 2008).

Clearly epigenetic variation can respond to the environment. However, whether this has any impact on adaptive fitness is far from clear. For example, in *Drosophila* heat-shock or osmotic stress-induced *white* gene derepression can be inherited maternally and paternally over several generations, before returning to the normal state (Seong et al., 2011). In mice, *Agouti*<sup>vy</sup> mothers can modulate the coat color phenotype of their progeny through a specific diet of methyl donors, but this effect is only transmitted over two generations and is lost by the third (Daxinger and Whitelaw, 2012) indicating that the influence of diet is not stable nor truly transgenerational (Box 1). However, genetic variation at the *Agouti* locus can come under very rapid adaptive selection for coat color “camouflage” (Linnen et al., 2013), raising the question as to whether some haplotypes may be prone to epigenetic variation as well.

In plants, there is no question that environmental cues such as temperature can have transgenerational effects – on paramutation (Brink et al., 1968), and on transposon activity (Slotkin and Martienssen, 2007) which is often temperature sensitive and can be inherited when remethylation by RdDM is abolished (Ito et al., 2011). Attempts to demonstrate adaptive epigenetic change in plants have focused on biotic and abiotic stress, and have proved much more problematic. Plant breeders often note that the introduction of a foreign variety appears to involve a process of adaptation, such that seeds, and clonal propagules (cuttings) become progressively more adapted to new climates, and new pathogen loads (Holeski et al., 2012). However, attempts to experimentally demonstrate adaptive epigenetic variation in stress tolerance have so far met with very limited success (Slaughter et al., 2012), and intergenerational maternal effects on seeds, similar to maternal effects in mammals (Figure 1), are hard to rule out (Pecinka and Mittelsten Scheid, 2012).

Perhaps the best known epigenetic environmental cue in plants is the influence of temperature and season on flowering time (Andres and Coupland, 2012; Ream et al., 2012; Song et al., 2012). *Arabidopsis* and related brassicas are known as “winter annuals” and

encode a floral repressor, the FLC MADS box transcription factor, that prevents flowering in embryos and young plants. Prolonged periods of cold (more than a few weeks) experienced in winter result in stable epigenetic silencing of FLC. This process, called vernalization, involves PHD-containing proteins, Polycomb Repressive Complex 2 and antisense transcription. The cold-induced epigenetic silencing allows flowering to occur when photoperiod is long again in the following spring. While the mechanism of cold sensing remains unclear, long-term silencing of FLC is achieved through trimethylation of H3K27. In principle, this memory of winter could be retained in the next generation, but instead it is robustly reset in the germline and early embryo (Sheldon et al., 2008). Up-regulation of FLC even in plants that have not experienced cold suggests the resetting process may be part of the genome-wide epigenetic reprogramming that occurs during embryogenesis (Song et al., 2012). This resetting does not seem to involve DNA methylation, but histone modification and replacement undergo drastic changes in the germline and could be responsible (Ingouff et al., 2007; Schoft et al., 2009). Lysenko contributed significantly to the discovery of this cold induced phenomenon in wheat and other cereals before the molecular basis of vernalization was known. However, he famously and unfortunately went on to propose that early flowering, induced by prolonged cold, could be inherited as an acquired trait. This led to disastrous attempts to rapidly breed high yielding wheats that could be planted in the Spring.

Thus, while the notion of adaptive epigenetic inheritance retains considerable appeal, concrete evidence from model systems is still lacking. Lysenko and Burbank were both followers of Lamarck in that they believed that the inheritance of acquired traits should underlie evolution, and it is often forgotten that Darwin himself considered Lamarck's hypothesis sound. Darwin even proposed the existence of “gemmules”, pieces of information that could arise in somatic cells under environmental challenge, modify the germline and confer some advantage on the progeny in the next generation. A molecular basis for such signals has long eluded geneticists, but RNA interference is a modern-day candidate with renewed appeal. This is because small RNA signals are highly mobile, being transmitted through the gut in *C.elegans*, through the vasculature and plasmodesmata in plants, and through exosomes and even serum in mammals. At least in *C. elegans*, these small RNAs or their derivatives can enter the germline, and mediate heritable transcriptional silencing in subsequent generations, using histone modification mechanisms analogous to fission yeast. One can easily imagine a scenario in which, for example, pathogen infection in one generation might give rise to small RNA that are inherited in the next, conferring some level of resistance. However, such inheritance of adaptive resistance has not yet been demonstrated, despite tantalizing clues in both plants and animals (Hilbricht et al., 2008; Rechavi et al., 2011; Yu et al., 2013).

### Implications for human health

Given the medical and public health implications (Jirtle and Skinner, 2007), numerous studies have examined the potential for epigenetic inheritance of nutritional metabolic risk in human and mouse populations. It has been proposed that alterations in paternal diet (high-fat or low-protein diets) or else a prior history of intrauterine exposure to maternal caloric restriction can result in increased metabolic risk in offspring (also known as Barker's

theory (Hales and Barker, 2013)). Nutritional conditions during uterine development may have effects later in life, and influence the occurrence of adult metabolism and diseases. Thus, under poor nutritional conditions, the fetal environment could modify the development of the embryo to prepare the offspring for a future environment with low resources during adult life ("thrifty" phenotype). For example during the Dutch famine at the end of WWII, individuals exposed to famine during gestation had a poorer glucose tolerance than those born the year before the famine. Studies have found increased neonatal adiposity among the grandchildren of women who had been undernourished during pregnancy. Furthermore, offspring of prenatally undernourished fathers, but not mothers, were heavier and more obese than offspring of fathers and mothers who had not been undernourished prenatally (Painter et al., 2008; Veenendaal et al., 2013). No evidence of transgenerational effects of grandmaternal under-nutrition during gestation were found, but the increased adiposity in the offspring of prenatally undernourished fathers might lead to chronic disease rates in the future.

Recent studies in rodent models have focused on nutritional effects transmitted via the paternal lineage (as this avoids the confounding effects of in utero variations). Mice fed a high cholesterol diet passed on gene expression differences, and modest DNA methylation differences, to their paternal offspring (Carone et al., 2010; Radford et al., 2012). The sons of mothers calorically restricted during pregnancy transmit metabolic phenotypes to offspring with altered transcript profiles evident prior to onset of disease (Radford et al., 2012). Such paternal-lineage risk is likely to be conferred via sperm, although whether this is via alterations in chromatin, small RNAs or other agents is currently unclear (Ferguson-Smith and Patti, 2011; Rando, 2012). No global alterations in sperm methylation have been noted so far. Furthermore, most paternal RNAs are thought to be degraded shortly after fertilization and although some histones may persist in sperm chromatin (Brykczynska et al., 2010; Hammoud et al., 2009) most are rapidly replaced upon fertilization. Another study (Padmanabhan et al., 2013) found that a mutation in folate metabolism (methionine synthase reductase (*Mtrr*)) led to epigenetic instability and transgenerational effects on development. Although epigenetic inheritance may contribute to these effects, as shown by altered DNA methylation profiles, mutations induced under these conditions could not be excluded, as folate metabolism regulates nucleotide biosynthesis pathways and hence might have an impact on genetic mutation/DNA repair mechanisms. Furthermore, epigenetic instability might lead to reactivation of TEs and insertional mutations.

Even though epidemiological studies and animal models provide support for the "thrifty phenotype" hypothesis, most of the studies so far concern inter-generational (parental or grand-parental exposure) rather than truly trans-generational inheritance (Figure 1) - and in most of the epidemiological studies, it has been difficult to rule out other effects (Box 3) such as the influence of post-natal nutritional environment, and the use of cohorts where important covariates are missing. Nevertheless, it is clear that different nutritional cues during infancy and childhood can have adverse effects during adult life, and exposure to pollutants, alcohol and tobacco can affect fetal programming. Such phenomena have now been put under the umbrella of DOHaD 'developmental origins of health and disease' which proposes that a wide range of environmental conditions during embryonic development and

early life determine susceptibility to disease during adult life (Hochberg et al., 2011). Whether such effects result in *bone fide* transgenerational epigenetic inheritance over multiple generations seems unlikely given the robust reprogramming found in the mammalian germ line. Further investigations will clearly be needed using well controlled experiments in mammalian models and large, well characterized cohorts in epidemiological studies.

## Conclusions

In conclusion, in plants and in some animals such as nematodes, transgenerational epigenetic inheritance is well-documented and relatively common. Epialleles may even form the basis of some complex traits in plants, where epigenetic inheritance is usually, if not always associated with transposable elements, viruses or transgenes and may be a by-product of aggressive germ line defense strategies. In mammals epialleles can also be found, but are extremely rare, presumably due to robust germ-line reprogramming. How epialleles arise in nature is still an open question but environmentally induced epigenetic changes are rarely transgenerationally inherited, let alone adaptive, even in plants. Thus, although much attention has been drawn to the potential implications of transgenerational inheritance for human health, so far there is little support. On the other hand, the human transmission of culture and improved habits is clearly Lamarckian. To quote SJ Gould (*The Panda's thumb*, 1980) “Human cultural evolution, in strong opposition to our biological history, is Lamarckian in character. What we learn in one generation, we transmit directly by teaching and writing.” In this and other respects, perhaps it is premature to compare humans to plants (as Burbank did) in terms of their capacity to recall past environments, in this generation and the next.

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## References

- Andres F, Coupland G. The genetic basis of flowering responses to seasonal cues. *Nat Rev Genet.* 2012; 13:627–639. [PubMed: 22898651]
- Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science.* 2005; 308:1466–1469. [PubMed: 15933200]
- Arteaga-Vazquez M, Sidorenko L, Rabanal FA, Shrivastava R, Nobuta K, Green PJ, Meyers BC, Chandler VL. RNA-mediated trans-communication can establish paramutation at the b1 locus in maize. *Proc Natl Acad Sci U S A.* 2010; 107:12986–12991. [PubMed: 20616013]
- Arteaga-Vazquez MA, Chandler VL. Paramutation in maize: RNA mediated trans-generational gene silencing. *Curr Opin Genet Dev.* 2010; 20:156–163. [PubMed: 20153628]
- Ashe A, Sapetschnig A, Weick E-M, Mitchell J, Bagijn MP, Cording AC, Doebley A-L, Goldstein LD, Lehrbach NJ, Le Pen J, et al. piRNAs Can Trigger a Multigenerational Epigenetic Memory in the Germline of *C. elegans*. *Cell.* 2012; 150:88–99. [PubMed: 22738725]

- Barbour JE, Liao IT, Stonaker JL, Lim JP, Lee CC, Parkinson SE, Kermicle J, Simon SA, Meyers BC, Williams-Carrier R, et al. required to maintain repression2 is a novel protein that facilitates locus-specific paramutation in maize. *Plant Cell*. 2012; 24:1761–1775. [PubMed: 22562610]
- Becker C, Hagmann J, Muller J, Koenig D, Stegle O, Borgwardt K, Weigel D. Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature*. 2011; 480:245–249. [PubMed: 22057020]
- Becker C, Weigel D. Epigenetic variation: origin and transgenerational inheritance. *Curr Opin Plant Biol*. 2012; 15:562–567. [PubMed: 22939250]
- Benayoun BA, Brunet A. Epigenetic memory of longevity in *Caenorhabditis elegans*. *Worm*. 2012; 1:77–81. [PubMed: 24058828]
- Bird A. Perceptions of epigenetics. *Nature*. 2007; 447:396–398. [PubMed: 17522671]
- Brennecke J, Malone CD, Aravin AA, Sachidanandam R, Stark A, Hannon GJ. An Epigenetic Role for Maternally Inherited piRNAs in Transposon Silencing. *Science (New York, NY)*. 2008; 322:1387–1392.
- Brink RA, Styles ED, Axtell JD. Paramutation: directed genetic change. Paramutation occurs in somatic cells and heritably alters the functional state of a locus. *Science*. 1968; 159:161–170. [PubMed: 5634904]
- Brykczynska U, Hisano M, Erkek S, Ramos L, Oakeley EJ, Roloff TC, Beisel C, Schubeler D, Stadler MB, Peters AH. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nat Struct Mol Biol*. 2010; 17:679–687. [PubMed: 20473313]
- Brzeska K, Brzeski J, Smith J, Chandler VL. Transgenic expression of CBBP, a CXC domain protein, establishes paramutation in maize. *Proc Natl Acad Sci U S A*. 2010; 107:5516–5521. [PubMed: 20212123]
- Buckley BA, Burkhardt KB, Gu SG, Spracklin G, Kershner A, Fritz H, Kimble J, Fire A, Kennedy S. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. *Nature*. 2012; 489:447–451. [PubMed: 22810588]
- Burkhardt KB, Guang S, Buckley BA, Wong L, Bochner AF, Kennedy S. A pre-mRNA-associating factor links endogenous siRNAs to chromatin regulation. *PLoS Genetics*. 2011; 7:e1002249. [PubMed: 21901112]
- Burton NO, Burkhardt KB, Kennedy S. Nuclear RNAi maintains heritable gene silencing in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:19683–19688. [PubMed: 22106253]
- Calarco JP, Borges F, Donoghue MT, Van Ex F, Jullien PE, Lopes T, Gardner R, Berger F, Feijo JA, Becker JD, et al. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell*. 2012; 151:194–205. [PubMed: 23000270]
- Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, et al. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell*. 2010; 143:1084–1096. [PubMed: 21183072]
- Castel SE, Martienssen RA. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nat Rev Genet*. 2013; 14:100–112. [PubMed: 23329111]
- Chalker DL, Yao MC. DNA elimination in ciliates: transposon domestication and genome surveillance. *Annu Rev Genet*. 2011; 45:227–246. [PubMed: 21910632]
- Champagne FA, Curley JP. Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neurosci Biobehav Rev*. 2009; 33:593–600. [PubMed: 18430469]
- Chandler VL. Paramutation: from maize to mice. *Cell*. 2007; 128:641–645. [PubMed: 17320501]
- Chandler VL. Paramutation's properties and puzzles. *Science*. 2010; 330:628–629. [PubMed: 21030647]
- Colome-Tatche M, Cortijo S, Wardenaar R, Morgado L, Lahouze B, Sarazin A, Etcheverry M, Martin A, Feng S, Duvernois-Berthet E, et al. Features of the *Arabidopsis* recombination landscape resulting from the combined loss of sequence variation and DNA methylation. *Proc Natl Acad Sci U S A*. 2012; 109:16240–16245. [PubMed: 22988127]
- Cortijo S, Wardenaar R, Colome-Tatche M, Gilly A, Etcheverry M, Labadie K, Caillieux E, Hospital F, Aury J-M, Wincker P, et al. Mapping the epigenetic basis of complex traits. *Science*. 2014. In Press DOI: 10.1126/science.1248127

- Creasey KM, Zhai J, Borges F, Van Ex F, Regulski M, Meyers BC, Martienssen R. miRNAs trigger widespread epigenetically-activated siRNAs from transposons in Arabidopsis. *Nature*. 2014 In Press.
- Cubas P, Vincent C, Coen E. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature*. 1999; 401:157–161. [PubMed: 10490023]
- Daxinger L, Whitelaw E. Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nat Rev Genet*. 2012; 13:153–162. [PubMed: 22290458]
- de Vanssay A, Bouge AL, Boivin A, Hermant C, Teyssset L, Delmarre V, Antoniewski C, Ronsseray S. Paramutation in *Drosophila* linked to emergence of a piRNA-producing locus. *Nature*. 2012; 490:112–115. [PubMed: 22922650]
- Dietz DM, Laplant Q, Watts EL, Hodes GE, Russo SJ, Feng J, Oosting RS, Vialou V, Nestler EJ. Paternal transmission of stress-induced pathologies. *Biol Psychiatry*. 2011; 70:408–414. [PubMed: 21679926]
- Durand S, Bouche N, Perez Strand E, Loudet O, Camilleri C. Rapid establishment of genetic incompatibility through natural epigenetic variation. *Curr Biol*. 2012; 22:326–331. [PubMed: 22285031]
- Eichten SR, Briskine R, Song J, Li Q, Swanson-Wagner R, Hermanson PJ, Waters AJ, Starr E, West PT, Tiffin P, et al. Epigenetic and genetic influences on DNA methylation variation in maize populations. *Plant Cell*. 2013; 25:2783–2797. [PubMed: 23922207]
- Enke RA, Dong Z, Bender J. Small RNAs Prevent Transcription-Coupled Loss of Histone H3 Lysine 9 Methylation in *Arabidopsis thaliana*. *PLoS Genetics*. 2011; 7:e1002350. [PubMed: 22046144]
- Erhard KF Jr, Parkinson SE, Gross SM, Barbour JE, Lim JP, Hollick JB. Maize RNA polymerase IV defines trans-generational epigenetic variation. *Plant Cell*. 2013; 25:808–819. [PubMed: 23512852]
- Eun C, Lorkovic ZJ, Sasaki T, Naumann U, Matzke AJ, Matzke M. Use of forward genetic screens to identify genes required for RNA-directed DNA methylation in *Arabidopsis thaliana*. *Cold Spring Harb Symp Quant Biol*. 2012; 77:195–204. [PubMed: 23197303]
- Ferguson-Smith AC, Patti ME. You are what your dad ate. *Cell Metab*. 2011; 13:115–117. [PubMed: 21284975]
- Gehring M, Bubbs KL, Henikoff S. Extensive demethylation of repetitive elements during seed development underlies gene imprinting. *Science*. 2009; 324:1447–1451. [PubMed: 19520961]
- Greaves IK, Groszmann M, Ying H, Taylor JM, Peacock WJ, Dennis ES. Trans chromosomal methylation in *Arabidopsis* hybrids. *Proc Natl Acad Sci U S A*. 2012; 109:3570–3575. [PubMed: 22331882]
- Gu SG, Pak J, Guang S, Maniar JM, Kennedy S, Fire A. Amplification of siRNA in *Caenorhabditis elegans* generates a transgenerational sequence-targeted histone H3 lysine 9 methylation footprint. *Nature Genetics*. 2012; 44:157–164. [PubMed: 22231482]
- Gutierrez-Marcos JF, Dickinson HG. Epigenetic reprogramming in plant reproductive lineages. *Plant Cell Physiol*. 2012; 53:817–823. [PubMed: 22505692]
- Haag JR, Pikaard CS. Multisubunit RNA polymerases IV and V: purveyors of non-coding RNA for plant gene silencing. *Nat Rev Mol Cell Biol*. 2011; 12:483–492. [PubMed: 21779025]
- Hackett JA, Surani MA. Beyond DNA: programming and inheritance of parental methylomes. *Cell*. 2013; 153:737–739. [PubMed: 23663772]
- Hackett JA, Zyllicz JJ, Surani MA. Parallel mechanisms of epigenetic reprogramming in the germline. *Trends Genet*. 2012; 28:164–174. [PubMed: 22386917]
- Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Int J Epidemiol*. 2013; 42:1215–1222. [PubMed: 24159065]
- Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. Distinctive chromatin in human sperm packages genes for embryo development. *Nature*. 2009; 460:473–478. [PubMed: 19525931]
- Hilbricht T, Varotto S, Sgaramella V, Bartels D, Salamini F, Furini A. Retrotransposons and siRNA have a role in the evolution of desiccation tolerance leading to resurrection of the plant *Cratogeomys plantagineum*. *New Phytol*. 2008; 179:877–887. [PubMed: 18482228]
- Hitchins MP, Rapkins RW, Kwok CT, Srivastava S, Wong JJ, Khachigian LM, Polly P, Goldblatt J, Ward RL. Dominantly inherited constitutional epigenetic silencing of MLH1 in a cancer-affected



- family is linked to a single nucleotide variant within the 5'UTR. *Cancer Cell*. 2011; 20:200–213. [PubMed: 21840485]
- Hochberg Z, Feil R, Constancia M, Fraga M, Junien C, Carel JC, Boileau P, Le Bouc Y, Deal CL, Lillycrop K, et al. Child health, developmental plasticity, and epigenetic programming. *Endocr Rev*. 2011; 32:159–224. [PubMed: 20971919]
- Holeski LM, Jander G, Agrawal AA. Transgenerational defense induction and epigenetic inheritance in plants. *Trends Ecol Evol*. 2012; 27:618–626. [PubMed: 22940222]
- Hollick JB. Paramutation: a trans-homolog interaction affecting heritable gene regulation. *Curr Opin Plant Biol*. 2012; 15:536–543. [PubMed: 23017240]
- Hsieh TF, Ibarra CA, Silva P, Zemach A, Eshed-Williams L, Fischer RL, Zilberman D. Genome-wide demethylation of Arabidopsis endosperm. *Science*. 2009; 324:1451–1454. [PubMed: 19520962]
- Huang XA, Yin H, Sweeney S, Raha D, Snyder M, Lin H. A major epigenetic programming mechanism guided by piRNAs. *Dev Cell*. 2013; 24:502–516. [PubMed: 23434410]
- Ibarra CA, Feng X, Schoft VK, Hsieh TF, Uzawa R, Rodrigues JA, Zemach A, Chumak N, Machlicova A, Nishimura T, et al. Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science*. 2012; 337:1360–1364. [PubMed: 22984074]
- Ingouff M, Hamamura Y, Gourgues M, Higashiyama T, Berger F. Distinct dynamics of HISTONE3 variants between the two fertilization products in plants. *Curr Biol*. 2007; 17:1032–1037. [PubMed: 17555967]
- Irvine DV, Zaratiegui M, Tolia NH, Goto DB, Chitwood DH, Vaughn MW, Joshua-Tor L, Martienssen RA. Argonaute slicing is required for heterochromatic silencing and spreading. *Science (New York, NY)*. 2006; 313:1134–1137.
- Ito H, Gaubert H, Bucher E, Mirouze M, Vaillant I, Paszkowski J. An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature*. 2011; 472:115–119. [PubMed: 21399627]
- Jablonka E, Raz G. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q Rev Biol*. 2009; 84:131–176. [PubMed: 19606595]
- Jauvion V, Rivard M, Bouteiller N, Elmayan T, Vaucheret H. RDR2 partially antagonizes the production of RDR6-dependent siRNA in sense transgene-mediated PTGS. *PLoS One*. 2012; 7:e29785. [PubMed: 22242179]
- Jiang L, Zhang J, Wang JJ, Wang L, Zhang L, Li G, Yang X, Ma X, Sun X, Cai J, et al. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell*. 2013; 153:773–784. [PubMed: 23663777]
- Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet*. 2007; 8:253–262. [PubMed: 17363974]
- Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, Bulski A, Albuissou J, Heredia F, Audigier P, et al. Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet*. 2009; 5:e1000530. [PubMed: 19557164]
- Jullien PE, Susaki D, Yelagandula R, Higashiyama T, Berger F. DNA methylation dynamics during sexual reproduction in Arabidopsis thaliana. *Curr Biol*. 2012; 22:1825–1830. [PubMed: 22940470]
- Kakutani T, Jeddeloh JA, Flowers SK, Munakata K, Richards EJ. Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. *Proc Natl Acad Sci U S A*. 1996; 93:12406–12411. [PubMed: 8901594]
- Katz DJ, Edwards TM, Reinke V, Kelly WG. A C. elegans LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. *Cell*. 2009; 137:308–320. [PubMed: 19379696]
- Keller C, Buhler M. Chromatin-associated ncRNA activities. *Chromosome Res*. 2013; 21:627–641. [PubMed: 24249576]
- Kelly WG, Aramayo R. Meiotic silencing and the epigenetics of sex. *Chromosome Res*. 2007; 15:633–651. [PubMed: 17674151]
- Kiani J, Grandjean V, Liebers R, Tuorto F, Ghanbarian H, Lyko F, Cuzin F, Rassoulzadegan M. RNA-mediated epigenetic heredity requires the cytosine methyltransferase Dnmt2. *PLoS Genet*. 2013; 9:e1003498. [PubMed: 23717211]

- Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet.* 2010; 11:204–220. [PubMed: 20142834]
- Le Thomas A, Rogers AK, Webster A, Marinov GK, Liao SE, Perkins EM, Hur JK, Aravin AA, Toth KF. Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state. *Genes Dev.* 2013; 27:390–399. [PubMed: 23392610]
- Li Q, Eichten SR, Hermanson PJ, Springer NM. Inheritance Patterns and Stability of DNA Methylation Variation in Maize Near-Isogenic Lines. *Genetics.* 2013
- Li X, Ito M, Zhou F, Youngson N, Zuo X, Leder P, Ferguson-Smith AC. A maternal-zygotic effect gene, *Zfp57*, maintains both maternal and paternal imprints. *Dev Cell.* 2008; 15:547–557. [PubMed: 18854139]
- Lim JP, Brunet A. Bridging the transgenerational gap with epigenetic memory. *Trends Genet.* 2013; 29:176–186. [PubMed: 23410786]
- Linnen CR, Poh YP, Peterson BK, Barrett RD, Larson JG, Jensen JD, Hoekstra HE. Adaptive evolution of multiple traits through multiple mutations at a single gene. *Science.* 2013; 339:1312–1316. [PubMed: 23493712]
- Lippman Z, Gendrel A-V, Black M, Vaughn MW, Dedhia N, Richard McCombie W, Lavine K, Mittal V, May B, Kasschau KD, et al. Role of transposable elements in heterochromatin and epigenetic control. *Nature.* 2004; 430:471–476. [PubMed: 15269773]
- Lisch D. Regulation of transposable elements in maize. *Curr Opin Plant Biol.* 2012; 15:511–516. [PubMed: 22824142]
- Lisch D. How important are transposons for plant evolution? *Nat Rev Genet.* 2013; 14:49–61. [PubMed: 23247435]
- Manning K, Tor M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat Genet.* 2006; 38:948–952. [PubMed: 16832354]
- Mari-Ordonez A, Marchais A, Etcheverry M, Martin A, Colot V, Voinnet O. Reconstructing de novo silencing of an active plant retrotransposon. *Nat Genet.* 2013; 45:1029–1039. [PubMed: 23852169]
- Martienssen R, Barkan A, Taylor WC, Freeling M. Somatically heritable switches in the DNA modification of Mu transposable elements monitored with a suppressible mutant in maize. *Genes Dev.* 1990; 4:331–343. [PubMed: 2159936]
- Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszkowski J. Transgenerational stability of the Arabidopsis epigenome is coordinated by CG methylation. *Cell.* 2007; 130:851–862. [PubMed: 17803908]
- McClintock B. Some parallels between gene control systems in maize and in bacteria. *Am Nat.* 1961; 95:265–277.
- Messerschmidt DM. Should I stay or should I go: protection and maintenance of DNA methylation at imprinted genes. *Epigenetics.* 2012; 7:969–975. [PubMed: 22869105]
- Messerschmidt DM, de Vries W, Ito M, Solter D, Ferguson-Smith A, Knowles BB. Trim28 is required for epigenetic stability during mouse oocyte to embryo transition. *Science.* 2012; 335:1499–1502. [PubMed: 22442485]
- Mirouze M, Lieberman-Lazarovich M, Aversano R, Bucher E, Nicolet J, Reinders J, Paszkowski J. Loss of DNA methylation affects the recombination landscape in Arabidopsis. *Proc Natl Acad Sci U S A.* 2012; 109:5880–5885. [PubMed: 22451936]
- Mirouze M, Reinders J, Bucher E, Nishimura T, Schneeberger K, Ossowski S, Cao J, Weigel D, Paszkowski J, Mathieu O. Selective epigenetic control of retrotransposition in Arabidopsis. *Nature.* 2009; 461:427–430. [PubMed: 19734882]
- Mosher RA, Melnyk CW, Kelly KA, Dunn RM, Studholme DJ, Baulcombe DC. Uniparental expression of PolIV-dependent siRNAs in developing endosperm of Arabidopsis. *Nature.* 2009; 460:283–286. [PubMed: 19494814]
- Nakamura T, Liu YJ, Nakashima H, Umehara H, Inoue K, Matoba S, Tachibana M, Ogura A, Shinkai Y, Nakano T. PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature.* 2012; 486:415–419. [PubMed: 22722204]

- Padmanabhan N, Jia D, Geary-Joo C, Wu X, Ferguson-Smith AC, Fung E, Bieda MC, Snyder FF, Gravel RA, Cross JC, et al. Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. *Cell*. 2013; 155:81–93. [PubMed: 24074862]
- Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DI, Roseboom TJ. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. *BJOG*. 2008; 115:1243–1249. [PubMed: 18715409]
- Pecinka A, Mittelsten Scheid O. Stress-induced chromatin changes: a critical view on their heritability. *Plant Cell Physiol*. 2012; 53:801–808. [PubMed: 22457398]
- Potok ME, Nix DA, Parnell TJ, Cairns BR. Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell*. 2013; 153:759–772. [PubMed: 23663776]
- Ptashne M. Epigenetics: core misconception. *Proc Natl Acad Sci U S A*. 2013; 110:7101–7103. [PubMed: 23584020]
- Qi Y, He X, Wang X-J, Kohany O, Jurka J, Hannon GJ. Distinct catalytic and non-catalytic roles of ARGONAUTE4 in RNA-directed DNA methylation. *Nature*. 2006; 443:1008–1012. [PubMed: 16998468]
- Radford EJ, Isganaitis E, Jimenez-Chillaron J, Schroeder J, Molla M, Andrews S, Didier N, Charalambous M, McEwen K, Marazzi G, et al. An unbiased assessment of the role of imprinted genes in an intergenerational model of developmental programming. *PLoS Genet*. 2012; 8:e1002605. [PubMed: 22511876]
- Rando OJ. Daddy issues: paternal effects on phenotype. *Cell*. 2012; 151:702–708. [PubMed: 23141533]
- Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F. RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. *Nature*. 2006; 441:469–474. [PubMed: 16724059]
- Raval A, Tanner SM, Byrd JC, Angerman EB, Perko JD, Chen SS, Hackanson B, Grever MR, Lucas DM, Matkovic JJ, et al. Downregulation of death-associated protein kinase 1 (DAPK1) in chronic lymphocytic leukemia. *Cell*. 2007; 129:879–890. [PubMed: 17540169]
- Ream TS, Woods DP, Amasino RM. The molecular basis of vernalization in different plant groups. *Cold Spring Harb Symp Quant Biol*. 2012; 77:105–115. [PubMed: 23619014]
- Rebollo R, Miceli-Royer K, Zhang Y, Farivar S, Gagnier L, Mager DL. Epigenetic interplay between mouse endogenous retroviruses and host genes. *Genome Biol*. 2012; 13:R89. [PubMed: 23034137]
- Rechavi O, Minevich G, Hobert O. Transgenerational Inheritance of an Acquired Small RNA-Based Antiviral Response in *C. elegans*. *Cell*. 2011
- Regulski M, Lu Z, Kendall J, Donoghue MT, Reinders J, Llaca V, Deschamps S, Smith A, Levy D, McCombie WR, et al. The maize methylome influences mRNA splice sites and reveals widespread paramutation-like switches guided by small RNA. *Genome Res*. 2013; 23:1651–1662. [PubMed: 23739895]
- Reinders J, Mirouze M, Nicolet J, Paszkowski J. Parent-of-origin control of transgenerational retrotransposon proliferation in *Arabidopsis*. *EMBO Rep*. 2013; 14:823–828. [PubMed: 23835507]
- Remy JJ. Stable inheritance of an acquired behavior in *Caenorhabditis elegans*. *Curr Biol*. 2010; 20:R877–878. [PubMed: 20971427]
- Richards EJ. Inherited epigenetic variation--revisiting soft inheritance. *Nat Rev Genet*. 2006; 7:395–401. [PubMed: 16534512]
- Rozhkov NV, Hammell M, Hannon GJ. Multiple roles for Piwi in silencing *Drosophila* transposons. *Genes Dev*. 2013; 27:400–412. [PubMed: 23392609]
- Schmitz RJ, Ecker JR. Epigenetic and epigenomic variation in *Arabidopsis thaliana*. *Trends Plant Sci*. 2012; 17:149–154. [PubMed: 22342533]
- Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA, Libiger O, Schork NJ, Ecker JR. Transgenerational epigenetic instability is a source of novel methylation variants. *Science*. 2011; 334:369–373. [PubMed: 21921155]

- Schneider S, Kaufmann W, Buesen R, van Ravenzwaay B. Vinclozolin--the lack of a transgenerational effect after oral maternal exposure during organogenesis. *Reprod Toxicol*. 2008; 25:352–360. [PubMed: 18485663]
- Schoft VK, Chumak N, Mosiolek M, Slusarz L, Komnenovic V, Brownfield L, Twell D, Kakutani T, Tamaru H. Induction of RNA-directed DNA methylation upon decondensation of constitutive heterochromatin. *EMBO Rep*. 2009; 10:1015–1021. [PubMed: 19680290]
- Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, Popp C, Thienpont B, Dean W, Reik W. The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Mol Cell*. 2012; 48:849–862. [PubMed: 23219530]
- Seisenberger S, Peat JR, Hore TA, Santos F, Dean W, Reik W. Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. *Philos Trans R Soc Lond B Biol Sci*. 2013; 368:20110330. [PubMed: 23166394]
- Seong KH, Li D, Shimizu H, Nakamura R, Ishii S. Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell*. 2011; 145:1049–1061. [PubMed: 21703449]
- Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ. Resetting of FLOWERING LOCUS C expression after epigenetic repression by vernalization. *Proc Natl Acad Sci USA*. 2008; 105:2214–2219. [PubMed: 18250331]
- Shirayama M, Seth M, Lee H-C, Gu W, Ishidate T, Conte D, Mello CC. piRNAs Initiate an Epigenetic Memory of Nonsilencing RNA in the *C. elegans* Germline. *Cell*. 2012
- Sienski G, Donertas D, Brennecke J. Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression. *Cell*. 2012; 151:964–980. [PubMed: 23159368]
- Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B. Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. *Plant Physiol*. 2012; 158:835–843. [PubMed: 22209872]
- Slotkin RK, Martienssen R. Transposable elements and the epigenetic regulation of the genome. *Nat Rev Genet*. 2007; 8:272–285. [PubMed: 17363976]
- Slotkin RK, Vaughn M, Borges F, Tanurdzi M, Becker JD, Feijó JA, Martienssen RA. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell*. 2009; 136:461–472. [PubMed: 19203581]
- Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, Meissner A. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature*. 2012; 484:339–344. [PubMed: 22456710]
- Song J, Angel A, Howard M, Dean C. Vernalization - a cold-induced epigenetic switch. *J Cell Sci*. 2012; 125:3723–3731. [PubMed: 22935652]
- Stroud H, Do T, Du J, Zhong X, Feng S, Johnson L, Patel DJ, Jacobsen SE. Non-CG methylation patterns shape the epigenetic landscape in Arabidopsis. *Nat Struct Mol Biol*. 2014; 21:64–72. [PubMed: 24336224]
- Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, Ciaudo C, Cruaud C, Poulain J, Berdasco M, Fraga MF, et al. A role for RNAi in the selective correction of DNA methylation defects. *Science*. 2009; 323:1600–1604. [PubMed: 19179494]
- Theodorou V. Susceptibility to stress-induced visceral sensitivity: a bad legacy for next generations. *Neurogastroenterol Motil*. 2013; 25:927–930. [PubMed: 24251872]
- Vaughn MW, Tanurdzic M, Lippman Z, Jiang H, Carrasquillo R, Rabinowicz PD, Dedhia N, McCombie WR, Agier N, Bulski A, et al. Epigenetic natural variation in Arabidopsis thaliana. *PLoS Biol*. 2007; 5:e174. [PubMed: 17579518]
- Veenendaal MV, Painter RC, de Rooij SR, Bossuyt PM, van der Post JA, Gluckman PD, Hanson MA, Roseboom TJ. Transgenerational effects of prenatal exposure to the 1944–45 Dutch famine. *BJOG*. 2013; 120:548–553. [PubMed: 23346894]
- Vu TM, Nakamura M, Calarco JP, Susaki D, Lim PQ, Kinoshita T, Higashiyama T, Martienssen RA, Berger F. RNA-directed DNA methylation regulates parental genomic imprinting at several loci in Arabidopsis. *Development*. 2013; 140:2953–2960. [PubMed: 23760956]
- Walter J, Hutter B, Khare T, Paulsen M. Repetitive elements in imprinted genes. *Cytogenet Genome Res*. 2006; 113:109–115. [PubMed: 16575169]

- Watanabe T, Tomizawa S.i. Mitsuya K, Totoki Y, Yamamoto Y, Kuramochi-Miyagawa S, Iida N, Hoki Y, Murphy PJ, Toyoda A, et al. Role for piRNAs and Noncoding RNA in de Novo DNA Methylation of the Imprinted Mouse Rasgrf1 Locus. *Science (New York, NY)*. 2011; 332:848–852.
- Weigel D, Colot V. Epialleles in plant evolution. *Genome Biol*. 2012; 13:249. [PubMed: 23058244]
- Yu A, Lepere G, Jay F, Wang J, Bapaume L, Wang Y, Abraham AL, Penterman J, Fischer RL, Voinnet O, et al. Dynamics and biological relevance of DNA demethylation in Arabidopsis antibacterial defense. *Proc Natl Acad Sci U S A*. 2013; 110:2389–2394. [PubMed: 23335630]
- Zemach A, Kim MY, Hsieh PH, Coleman-Derr D, Eshed-Williams L, Thao K, Harmer SL, Zilberman D. The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell*. 2013; 153:193–205. [PubMed: 23540698]

### Box 1: Definitions of Transgenerational Epigenetics

The term epigenetics was originally coined by Conrad Hal Waddington in 1942 to describe the bridge between genotype and phenotype during development. Subsequently, the definition shifted towards the notion of heritability, in part due to studies on DNA methylation and its potential role as a memory mark for propagating cell identity via control of gene expression states. Although more recent definitions range from the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states (Bird, 2007), to environmental influences on gene expression and chromatin states, here we employ the term in the more conservative sense that concerns the perpetuation of gene expression and function across cell divisions without changes in DNA sequence.

The term trans-generational is often used rather broadly to describe all, non-sequence based effects that can be transmitted from one generation to the next. However, it is important to distinguish parental (or inter-generational) effects, such as the impact of *in utero* exposure to particular nutritional, hormonal or stress/toxin environments on the developing embryo and its germ line (which will eventually produce grandchildren), from truly trans-generational effects (Figure 1) that are found in generations that were not exposed to the initial signal or environment that triggered the change (Daxinger and Whitelaw, 2012; Lim and Brunet, 2013)(Ferguson-Smith and Patti, 2011).



## Box 2: Germline and early embryonic reprogramming in animals and plants

Strategies for reprogramming parental epigenomes vary considerably in vertebrates and plants (Fig. 2). In the mouse germ line and also early post-fertilisation, the two parental genomes undergo extensive DNA demethylation via both active and passive mechanisms, leading to equivalent hypomethylated states in early cleavage stages accompanied by dynamic changes in histone modification (Hackett and Surani, 2013; Smith et al., 2012). The study of genomic imprinting, which represents a paradigm of epigenetic erasure and resetting in the germ line, has revealed sophisticated mechanisms that enable DNA methylation imprints to resist the post-fertilisation wave of reprogramming (Messerschmidt, 2012) (Figure 2). In early zebrafish embryos, the paternal methylome is stably inherited without changing state during early development, while the maternal methylome undergoes demethylation of oocyte-specific hypermethylated regions and de novo methylation of oocyte-specific hypomethylated regions (Jiang et al., 2013; Potok et al., 2013). How the zebrafish paternal methylome is protected from remodeling during development, while the maternal epigenome undergoes extensive remodelling is unclear. In humans and mice, certain genes are protected from protamine replacement in sperm, preserving key histone variants and their modifications (Brykczynska et al., 2010; Hammoud et al., 2009).

In flowering plants, meiocytes (gamete progenitors) differentiate within floral organ primordia that arise from postembryonic stem cells in shoot and floral meristems (Fig. 2B). These stem cells remain more or less undifferentiated from early embryogenesis until floral determination, but also give rise to somatic branches and leaves, and can sometimes be replaced by surrounding cells. For this reason the plant germline is poorly defined, and potentially subject to somatic modification. Epigenetic inheritance is widespread in plants, in part because germline reprogramming of DNA methylation is limited to asymmetric cytosines (or CHH, where H=A, C, T) in sperm cells. CHH methylation is regained after fertilization guided by maternal small RNA and further propagated in the embryo (Calarco et al., 2012; Ibarra et al., 2012). Hence, unlike mammals, there is no overt germline reprogramming of CG methylation. However, reprogramming in germline companion cells (the vegetative nucleus in pollen and the central cell in the ovule) coincides with loss of chromatin remodelers (Fig. 2B), and variants of histone H3 largely replace canonical variants in both pollen cell types (Ingouff et al., 2007; Schoft et al., 2009). Some of these variants cannot undergo key post translational modifications, which may also contribute to loss of heterochromatin (Schoft et al., 2009). Companion cell reprogramming results in transposon activation and the accumulation of small RNA in the gametes (Fig. 2B) which reinforces both imprinting and transposon silencing in the germline (Hsieh et al., 2009; Slotkin et al., 2009).

### BOX 3: Transgenerational Mechanisms

#### Epigenetic mechanisms

##### Self-sustaining feedback loops

the mRNA or protein product of a gene can stimulate its own transcription. Such feedback loops can clearly enable heritable states of altered gene expression without any need to evoke chromatin. However it is unlikely that such feedback loops alone would enable the propagation of states throughout the length of development and in the germ line of complex organisms.

##### Chromatin based mechanisms

DNA methylation is the best studied epigenetic mechanism for transgenerational inheritance, but is neither universal nor as stable as once thought, with dynamic changes during development and in the germ line. Its interplay with RNA interference in plants has provided some detailed mechanistic information on epigenetic inheritance. Histone variants, and histone (and protamine) modifications are all potential bearers of epigenetic information, and together with their “writer” and “reader” complexes, histones can perpetuate chromatin states. Polycomb and Trithorax group proteins (PcG, TrX), underlie ancestral memory strategies for maintaining gene activity in somatic cell lineages, but so far there is little evidence for PcG complexes as major players in trans-generational inheritance. On the other hand, Trx (COMPASS) complexes, responsible for histone H3 lysine 4 (H3K4) methylation, the Lysine Specific Demethylase (LSD1) of histone H3K4 and H3K9, as well as H3K9 methyltransferases, have been implicated in trans-generational inheritance in *C. elegans*.

##### Non-coding and coding RNA

RNAs of multiple types have been implicated in epigenetic inheritance across generations. These include maternal stores of mRNAs and long non-coding lncRNAs, as well as small RNAs that interfere with transcription (siRNAs, piRNAs), mRNA stability or translation (miRNA) via RNAi. Some of these small RNAs are strong candidates for triggering inheritance, as they guide DNA and histone modification in plants, animals and fungi.

##### Structural templating

prions, proteins that are propagated by changing the structure of normal proteins to match their own, have transgenerational effects in fungi but so far there is no evidence in plants or animals that prions can act to transmit information through meiosis. Chaperones such as Hsp90 can also mediate epigenetic variation, and may have transgenerational effects.

#### Confounding transgenerational mechanisms

##### Cryptic genetic variation

Many examples of transgenerational epigenetic inheritance are in fact likely to be DNA sequence based, although it may sometimes be difficult to pinpoint, even in supposedly genetically identical individuals. Several types of such cryptic sequence variation, including Copy Number Variants (CNVs), Single nucleotide polymorphisms (SNPs), *de*

*nov*o transposable element (TE) insertions, etc, could provide a DNA basis for inheritance that otherwise appears epigenetic.

### **Behavioural effects**

There are numerous reports of experience-driven heritable changes in the central nervous system (CNS) epigenome, involving maternal or paternal behavior, diet, exposure to drugs of abuse, and endocrine disruption. For example, maternal nurturing behavior of newborn pups apparently triggers DNA methylation changes in CNS glucocorticoid receptor genes that persist into adult offspring and result in behavioral changes (Champagne and Curley, 2009). Definitively determining whether experience-driven, acquired epigenetic changes can propagate through the germline and cause behavioral change in subsequent generations is clearly a very seductive but highly controversial topic (Lim and Brunet, 2013). Indeed, recent studies of social defeat phenotypes in males were linked to maternal provisioning (whereby mothers allocate resources to progeny depending on the quality of their mate) rather than epigenetic inheritance (Dietz et al., 2011). Careful experimental design is necessary to define the extent of heritability of experience-driven phenotypic changes, as well as underlying mechanisms. Cross-fostering and in vitro-fertilization can circumvent some of the issues in such studies, although they provide confounding factors of their own.

### **Microbiotic effects**

The intestinal flora – or microbiome – could also be a means of transmitting information across generations. Furthermore, given the recent links between metabolic and neurological diseases with the microbiome, apparent epigenetic inheritance linked with such phenotypes could in fact be due to transmission via bacterial populations (Theodorou, 2013).

### **Metabolites**

might also be transmitted from one generation to the next – and participate in bioenergetic feedback loops. These could be propagated over generations, and could also act as co-factors for chromatin modification or RNA processing for example.

### **Epigenetic inheritance of transposon and transgene silencing by DNA methylation**

Long before the terms “transgenerational” and “epigenetic” were in widespread use (Box 1), the first examples of epigenetic inheritance were described in plants. Following her discovery of transposable elements, Barbara McClintock recognized that *Activator* and *Suppressor Mutator* transposons in maize cycled between active and silent phases, and that these phases could be inherited across generations (McClintock, 1961). These transposons sometimes brought nearby color genes under their control, allowing the genetic identification of both *trans*-acting (transposase) and *cis*-acting (transposon) regulatory factors. For this reason McClintock drew parallels between transposons as “controlling elements”, and gene control by lambda repressor (McClintock, 1961), parallels that are still popular today (Ptashne, 2013).

Subsequently, a variety of molecular mechanisms have emerged that can result in transgenerational epigenetic inheritance of genes, transgenes and transposons (Box 3).

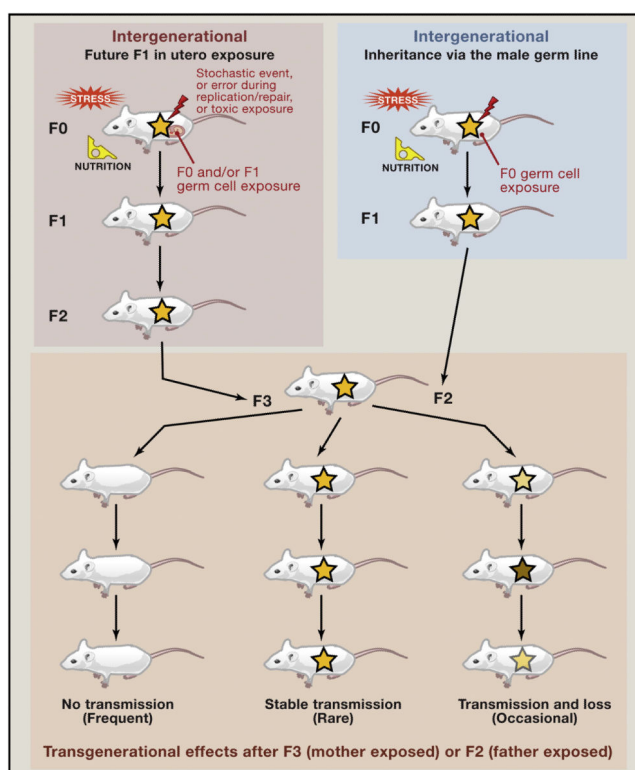
McClintock's "cycling" transposons were associated with changes in DNA methylation, as were epialleles at genes located nearby, which resulted in transgenerational leaf and seed color phenotypes (Lisch, 2012; Slotkin and Martienssen, 2007). At around the same time, silencing of transgenes and flower color genes was observed in petunia and tobacco, as well as in the model plant *Arabidopsis*, where genetic screens could be brought to bear (Law and Jacobsen, 2010). Some of the first silencing mutants isolated in *Arabidopsis* were in the maintenance DNA methyltransferase MET1 (DNA METHYLTRANSFERASE 1), the histone deacetylase HDA6, and the Snf2/swi2 chromatin remodeler DDM1 (DECREASE IN DNA METHYLATION 1) (Eun et al., 2012). Mutants in *met1* and *ddm1* had previously been isolated in molecular screens, and segregated unmethylated TEs and repeats in subsequent generations, independently of the causative mutation. Hypomethylated TEs neighboring genes resulted in epimutations such as *BONSAI* and *FWA* (*FLOWERING WAGENINGEN*) (Slotkin and Martienssen, 2007), and the penetrance of phenotypes observed in *ddm1* and *met1* mutants was greatly enhanced in double mutants with histone modification and RNAi (Creasey et al., 2014; Mathieu et al., 2007; Mirouze et al., 2009; Zemach et al., 2013), indicating these mechanisms can rescue methylation defects to some extent. Epigenetic variants in garden varieties, such as peloric flowers in toad flax and non-ripening tomatoes, also have unstable phenotypes associated with methylation changes near genes, in at least one case in a nearby transposon (Cubas et al., 1999; Manning et al., 2006).

In perhaps the most comprehensive studies to date, heritable hypomethylated chromosomal segments have been propagated for 8 or more generations in so called "epi RILs" (epigenetic Recombinant Inbred Lines). These are constructed by backcrossing *ddm1* and *met1* mutants and selfing wild type progeny by single seed descent (Johannes et al., 2009; Mirouze et al., 2012). Many of these hypomethylated segments, are inherited through meiosis and mitosis (Figure 3). By high throughput phenotyping, quantitative genetics and epigenetic profiling, the phenotypic consequences of this epigenetic inheritance could be determined, with many phenotypes displaying very high levels of epigenetic heritability (Colome-Tatche et al., 2012; Cortijo et al., 2014). Several of the differentially methylated regions (DMR) behave as *bona fide* epigenetic quantitative trait loci (QTL<sup>epi</sup>) accounting for up to 90% of the heritability for two complex traits, flowering time and primary root length (Cortijo et al., 2014). Up to 30% of these DMR exist in natural populations (Schmitz and Ecker, 2012), suggesting that transposon cycling is more prevalent than originally supposed.

A limited number of epialleles have also been described in mice. The expression of certain transgenes was found to be variable among littermates as was the tendency for active or inactive states to be inherited by the next generation. As the mice used were genetically inbred, it was deduced that the inheritance had an epigenetic basis (Daxinger and Whitelaw, 2012). A few *bona fide* cases of transgenerational inheritance at endogenous loci in mammals have also been identified. Importantly, these were associated with TEs, for example at *Agouti*<sup>vy</sup> and *Axin*<sup>Fu</sup> (axin fused). Transcription originating in an intra-cisternal A particle (IAP) retrotransposon inserted 100kb upstream of the agouti gene (*A*) causes ectopic expression of agouti protein, resulting in yellow fur, obesity, diabetes and increased susceptibility to tumours (Daxinger and Whitelaw, 2012).

Just like cycling transposons in maize,  $A^{vy}$  mice are epigenetic mosaics for IAP retrotransposon activity and DNA methylation: isogenic  $A^{vy}$  mice have coats varying from full yellow, through variegated yellow/agouti, to full agouti (pseudoagouti). The distribution of phenotypes among offspring is related to the phenotype of the dam; when an  $A^{vy}$  dam has the agouti phenotype, her offspring are more likely to be agouti; paternal transmission has no effect on phenotype (Daxinger and Whitelaw, 2012). This maternal epigenetic effect is not the result of a maternally contributed environment. Rather, it results from incomplete erasure of epigenetic modification when a silenced  $A^{vy}$  allele is passed through the female germ line. Parent-of-origin effects probably arise because the resistance of IAPs to epigenetic reprogramming differs between the male and female germ line and also between maternal and paternal genomes postfertilization (Figure 2A) – but no such difference is found with  $Axin^{Fu}$  which otherwise behaves in a similar fashion. Intriguingly, the first mutations found to suppress  $Agouti^{vy}$  in the mouse were in similar genes, and in some cases orthologous genes, to those found in similar screens in Arabidopsis, including DNA methyltransferases, histone deacetylases, chromatin remodelers and other ATPases responsible for chromatin compaction (Daxinger and Whitelaw, 2012; Law and Jacobsen, 2010). In humans, even if transposons are not directly involved, several potential epialleles (eg familial predisposition to cancer via the *MLH1*, or *DAPK1* loci) also turned out to be dependent on DNA sequence polymorphisms, so that aberrant gene silencing (epimutation) is established every generation, but is erased in the germ line (Hitchins et al., 2011; Raval et al., 2007).

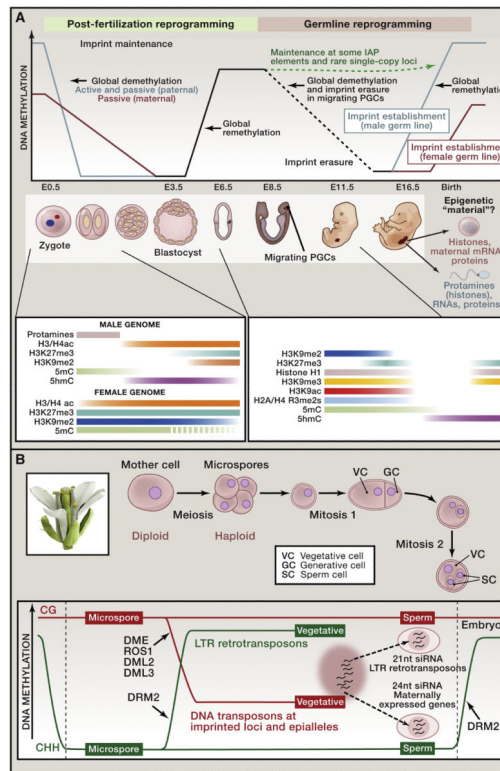
In both plants and animals, epigenetic inheritance of genes controlled by transposons may reflect a predisposition of transposons to DNA methylation and a resistance of transposons to reprogramming, leading to trans-generational epigenetic effects - and, in some cases parent-of-origin effects - providing a potential basis for the evolution of imprinting (Gehring et al., 2009; Walter et al., 2006). The only case so far where transposons clearly impact imprinting in mammals is the mouse *Rasgrf1* locus, where non-coding RNA and the PIWI-interacting RNA (piRNA) pathway are required for *de novo* methylation of the paternal differentially methylated region (DMR) (Watanabe et al., 2011). A retrotransposon sequence within the noncoding RNA is targeted by piRNAs which are generated from similar transposons elsewhere. A direct repeat in the DMR, which is required for methylation and imprinting of *Rasgrf1*, serves as a promoter for this non-coding RNA. This mechanism highly reminiscent of heterochromatic silencing in plants and fission yeast (see below), but the case for this imprinted gene is rather singular – raising the question of why most transposons and retroelement insertions in the mammalian genome do not induce imprinting or epimutations at nearby genes (Rebollo et al., 2012).



**Figure 1. Transgenerational and intergenerational epigenetic effects**

Epigenetic changes in mammals can arise sporadically, or can be induced by the environment (toxins, nutrition, stress). In the case of an exposed female mouse, if she is pregnant the fetus can be affected in utero (F1), as can the germ line of the fetus (the future F2). These are considered to be parental effects, leading to intergenerational epigenetic inheritance. Only F3 individuals can be considered as true trans-generational inheritance (see Box 1), in the absence of exposure. In the case of males in which an epigenetic change is induced, the individual (F0) and his germ line (future F1) are exposed; the F1 is thus considered as intergenerational. Only F2 and subsequent generations can be considered for evidence of trans-generational inheritance.

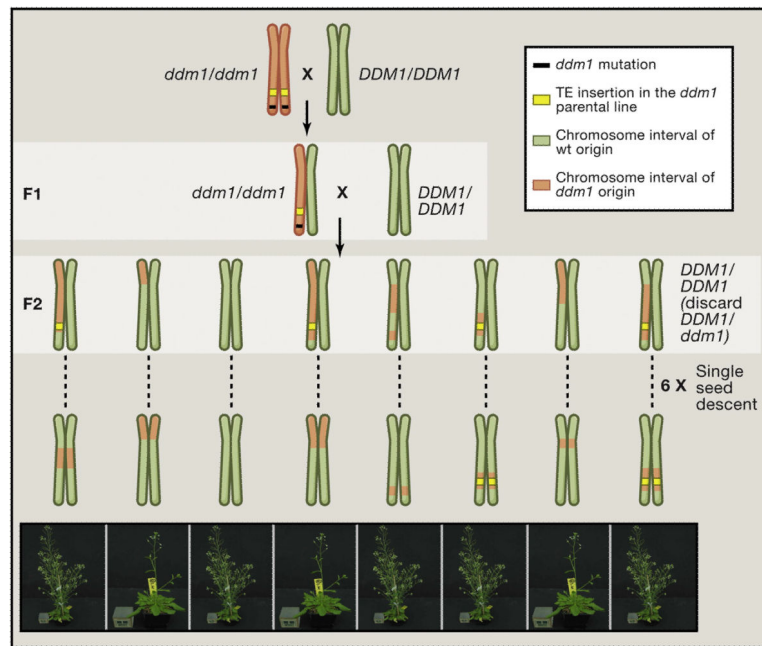




**Figure 2. Germline reprogramming of DNA methylation in mice and plants**

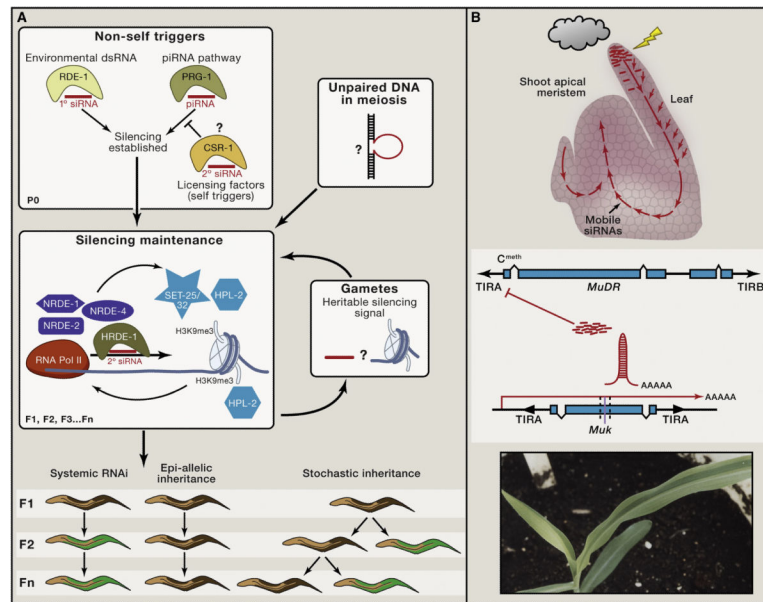
A. In mice there are at least two rounds of genome wide DNA methylation reprogramming. The first occurs just after fertilization, in the zygote and early cleavage stages, to erase gametic (sperm and oocyte) epigenomic marks. During this phase of reprogramming, genomic imprints are maintained. The other major reprogramming process occurs in the germ line where the paternal and maternal somatic programs are erased, together with imprints and the inactive X is reactivated. Subsequent to this, parent-specific imprints are laid down in the germ line. In each reprogramming window, a specific set of mechanisms regulates erasure and re-establishment of DNA methylation. Recent studies have uncovered roles for the TET3 hydroxylase and passive demethylation, together with base excision repair (BER) and the elongator complex, in methylation erasure from the zygote (Seisenberger et al., 2013). In the germ line, deamination by AID, BER and passive demethylation have been implicated in reprogramming, but the processes are still poorly understood.

B. In plants meiocytes differentiate from somatic cells, and the germline undergoes 2–3 stereotypical mitotic divisions after formation of the haploid microspore (pollen) and megaspore (ovule) (Gutierrez-Marcos and Dickinson, 2012). In pollen, symmetric CG and CHG methylation (H = A,C,T) is retained in the microspore and sperm cells, but CG methylation is lost from a few hundred imprinted and other genes in the companion vegetative cell nucleus. CHH methylation is sharply reduced in the microspore and sperm cells. 21nt epigenetically activated siRNA and a subset of 24nt siRNA arise in the vegetative nucleus but accumulate in sperm cells where they contribute to imprinting and epigenetic transposon control. Modified from Calarco et al. 2012 Cell. 2012 Sep 28;151(1):194–205.



**Figure 3. Transgenerational inheritance of hypomethylated DNA in epigenetic recombinant inbred lines (epi-RILs)**

Arabidopsis plants homozygous for *decrease in DNA methylation 1* lose heterochromatic (transposon and repeat) methylation in a heritable fashion, and were crossed to otherwise isogenic wild-type plants. Thus, the genome of the *ddm1/ddm1* parent is severely hypomethylated (red) relative to that of the wild type parent (green). Backcrossing of the F1 progeny to the wild type parental line was used to remove the *ddm1* mutation. Homozygous *DDM1/DDM1* lines were then self-crossed for six generations through single seed descent to generate recombinant inbred lines. Hundreds of parental differences in DNA methylation states across the genome were stably inherited in the epiRIL population, and account for most of the heritable variation observed for complex traits, such as flowering time (early and late flowering phenotype, illustrated below). Adapted from Cortijo et al. (2014) Science. 2014 Feb 6. [Epub ahead of print]



**Figure 4. Mechanisms for transgenerational inheritance**

A. In plants, the shoot apical meristem contains stem cells that give rise to leaves and flowers, in which meiocytes and gametes differentiate (Box 2). Small RNA from roots and leaves are mobile and can re-enter the meristem, and eventually the flowers. In maize, small RNA from “Mu-killer” are derived by transcription of a rearranged variant of the 5’ end of the MuDR element. The resulting transcript forms a hairpin, which is processed into small RNAs that target MuDR elements for DNA methylation. When nearby genes are controlled by *Mutator* transposons, MuDR methylation can be visualized as phenotypic sectors inherited by successive leaves, by flowers, and by seeds in the next generation (Lisch, 2012; Slotkin and Martienssen, 2007). Environmental triggers (drought, temperature, herbivory) can regulate transposon transcription in plants, and could hypothetically lead to transgenerational inheritance through similar mechanisms. Modified from (Lisch, 2012) and (Martienssen et al., 1990)

B. In *C.elegans*, triggers such as environmental RNAi and endogenous piRNAs lead to the establishment of a nuclear RNAi/ chromatin pathway. Maintenance of silencing requires nuclear RNAi factors, including the germline-specific nuclear Argonaute HRDE-1/ WAGO-9 and chromatin proteins such as the HP1 ortholog HPL-2 and the putative histone methyltransferases SET-25 and SET- 32. Silencing can be maintained into the F1 for multiple generations (F1–F5) or can become epi-allelic with multigenerational, nonstochastic inheritance. Silencing appears to be suppressed by a germline licensing pathway that recognizes bona fide germline transcripts (CSR-1 22G-RNA pathway) and enhanced through the recognition of unpaired DNA during meiosis (Ashe et al., 2012).