Leading Edge
Review
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Transgenerational Epigenetic Inheritance: Myths and Mechanisms

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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 transgenerational consequences are another matter. Although 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 remain 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" (Burbank, 1906). But, with the rediscovery 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 "rediscovery" of epigenetics and its underlying mechanisms has reopened 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 cornerstone of developmental biology since the concept of "epigenesis" was first proposed (Aristotle, *On the Generation of Animals*; Harvey, 1651; Wolff, 1759). 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 also has 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 transgenerational effects (Figure 1) and rarely exclude DNA sequence changes as the underlying cause for heritability. Although intergenerational 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 germline (Box 2). These two rounds of epigenetic erasure leave little chance for inheritance of epigenetic marks, whether programmed, accidental, or environmentally induced (Figure 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

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 toward 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, 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 transgenerational is often used rather broadly to describe all nonsequence-based effects that can be transmitted from one generation to the next. However, it is important to distinguish parental (or intergenerational) effects, such as the impact of in utero exposure to particular nutritional, hormonal, or stress/toxin environments on the developing embryo and its germline (which will eventually produce grandchildren), from truly transgenerational 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 (Figure 2). In the mouse germline and also early postfertilization, 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 germline, has revealed sophisticated mechanisms that enable DNA methylation imprints to resist the postfertilization wave of reprogramming (Messerschmidt, 2012) (Figure 2). In early zebrafish embryos, the paternal methylome is stably inherited without changing state during early development, whereas 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, whereas the maternal epigenome undergoes extensive remodeling, 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 (Figure 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 is 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 (Figure 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 posttranslational modifications, which may also contribute to loss of heterochromatin (Jacob et al., 2014; Schoft et al., 2009). Companion cell reprogramming results in transposon activation and the accumulation of small RNA in the gametes (Figure 2B), which reinforces both imprinting and transposon silencing in the germline (Hsieh et al., 2009; Slotkin et al., 2009).

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.

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 λ repressor (McClintock, 1961), parallels that are still popular today (Ptashne, 2013).

Subsequently, a variety of molecular mechanisms has 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 transposable elements (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

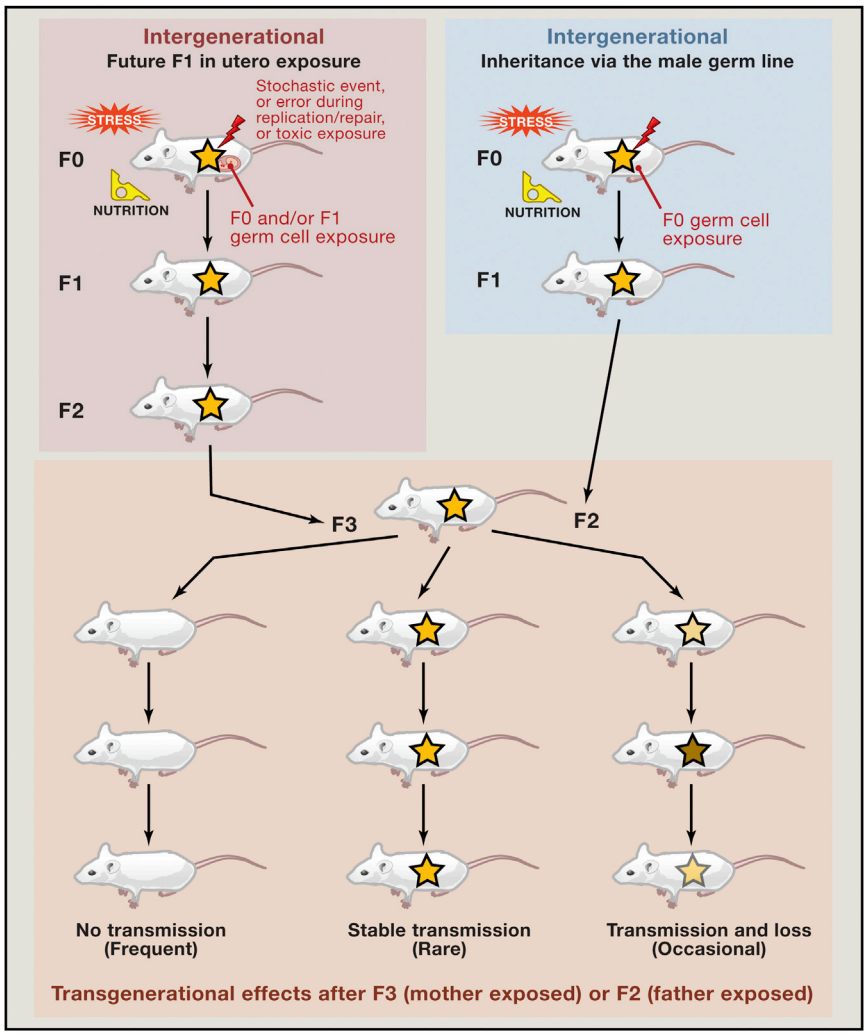

Figure 1. Transgenerational and Intergenerational Epigenetic Effects

Epigenetic changes in mammals can arise sporadically or can be induced by the environment (toxins, nutrition, and stress). In the case of an exposed female mouse, if she is pregnant, the fetus can be affected in utero (F1), as can the germline 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 transgenerational 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 germline (future F1) are exposed; the F1 is thus considered as intergenerational. Only F2 and subsequent generations can be considered for evidence of transgenerational inheritance.

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 that these mechanisms can rescue methylation defects to some extent. Epigenetic variants in garden varieties, such as peloric flowers in toad flax and nonripening tomatoes, also have unstable phenotypes associated with methylation changes near genes, in a nearby transposon in at least one case (Cubas et al., 1999; Manning et al., 2006).

In perhaps the most comprehensive studies to date, heritable hypomethylated chromosomal segments have been propagated for eight 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, quantititative genetics, and epigenetic profiling, the phenotypic consequences of this epigenetic inheritance could be determined, with many phenotypes displaying very high levels of epigenetic heritability (Colomé-Tatché et al., 2012; Cortijo et al., 2014). Several of the differentially methylated regions (DMR) behave as bona fide epigenetic quantitative trait loci (QTL^{epi}) 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 et al., 2013) 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 $Agouti^{vy}$ and $Axin^{Fu}$ (axin fused). Transcription originating in an intracisternal A particle (IAP) retrotransposon inserted 100 kb upstream of the agouti gene (*A*) causes ectopic expression of agouti protein, resulting in yellow fur, obesity, diabetes, and increased susceptibility to tumors (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

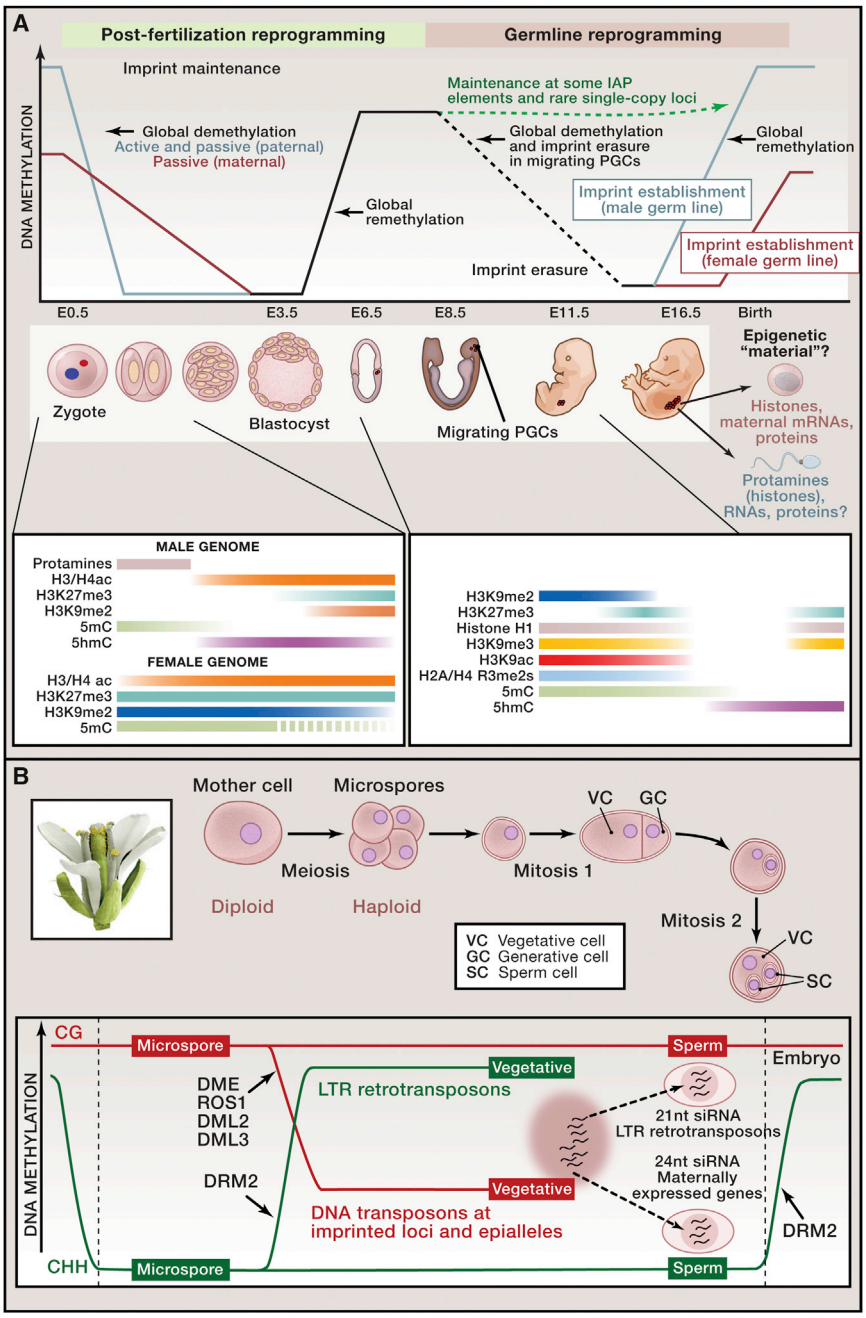

Figure 2. Germline Reprogramming of DNA Methylation in Mice and Plants

(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 germline, 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 germline. 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 germline, deamination by AID, BER, and passive demethylation has been implicated in reprogramming, but the processes are still poorly understood.

(B) In plants, meiocytes differentiate from somatic cells, and the germline undergoes two to three sterotypical 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. 21 nt epigenetically activated siRNA and a subset of 24 nt 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).

A^{vy} allele is passed through the female germline. Parent-of-origin effects probably arise because the resistance of IAPs to epigenetic reprogramming differs between the male and female germline and also between maternal and paternal genome 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; Eun et al., 2012). In humans, even if transposons are not directly involved, several potential epialleles (e.g., 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 germline (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 transgenerational 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 in which transposons clearly impact imprinting in mammals is the mouse *Rasgrf1* locus, where noncoding RNA and the PIWI-interacting RNA (piRNA) pathway are required for de novo methylation of the promoter DMR (Watanabe et al., 2011). A retrotransposon sequence

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 germline 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 germline. 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 and 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 transgenerational 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, and H3K9 methyltransferases, have been implicated in transgenerational inheritance in *C. elegans*.

Noncoding and Coding RNA

RNAs of multiple types have been implicated in epigenetic inheritance across generations. These include maternal stores of mRNAs and long noncoding lncRNAs, as well as small RNAs that interfere with transcription (siRNAs and 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), SNPs, de novo TE insertions, etc., could provide a DNA basis for inheritance that otherwise appears epigenetic.

Behavioral 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 (Théodorou, 2013).

Metabolites

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 cofactors for chromatin modification or RNA processing, for example.

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 noncoding RNA. This mechanism is 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).

RNA Interference and Transcriptional Silencing

What are the factors that specify transposons and transgenes, but not essential genes, for transgenerational 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. RNAi, 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 cotranscriptional recruitment of

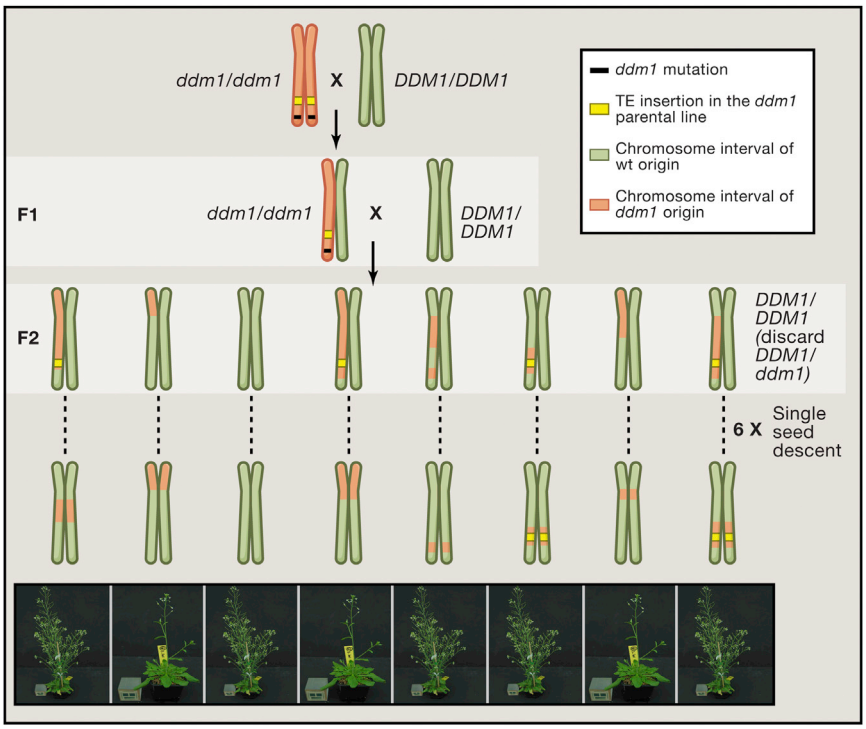

Figure 3. Transgenerational Inheritance of Hypomethylated DNA in Epigenetic Recombinant Inbred Lines, Known as epi-RILs

Arabidopsis plants homozygous for *ddm1* 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 each epi-RIL). Adapted from Cortijo et al. (2014).

RNAi transcriptional silencing (RITS) and histone modification complexes via binding of small RNA to PolII-dependent noncoding 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 Bühler, 2013).

RNA-Dependent DNA Methylation

In plants, as in fission yeast, it was also 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 24 nt siRNA biogenesis and is associated with PolIV, whereas Argonaute proteins that bind 24 nt 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, 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 three 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, and 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, whereas *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

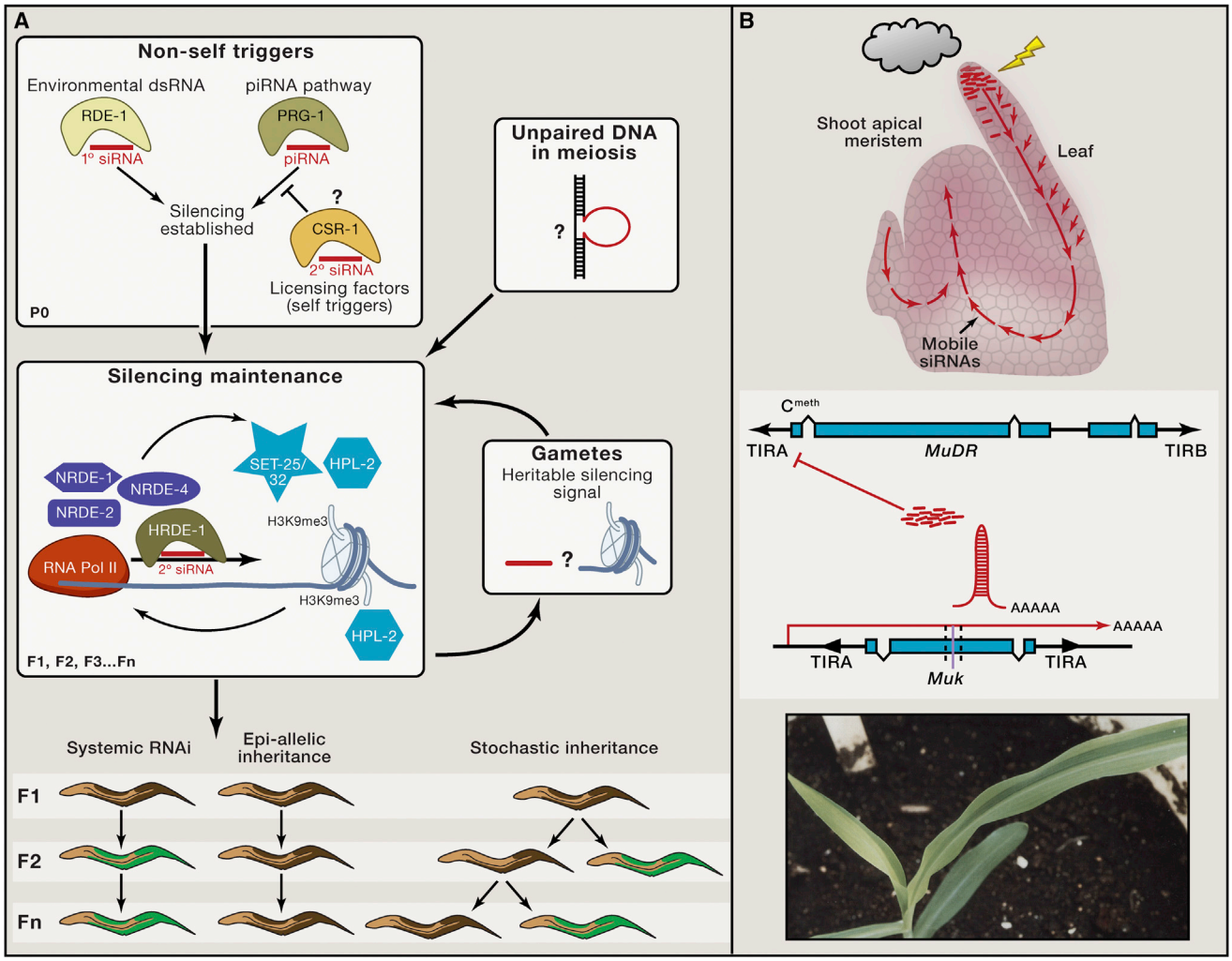

Figure 4. Mechanisms for Transgenerational Inheritance

(A) 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 epiallelic 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. Courtesy of Ashe et al. (2012).

(B) 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 RNAs from roots and leaves are mobile and can re-enter the meristem and, eventually, the flowers. In maize, small RNAs 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, and 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).

transgenerational silencing of transposons by RdDM in maize (Figure 4B). This coordinate 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; Marí-Ordóñez et al., 2013).

RNA interference in *C. elegans* has long-term multigenerational consequences that resemble paramutation (Figure 4A). In worms, inactive transgene arrays containing viral and reporter genes heritably silence active arrays (Rechavi et al., 2011). Resembling paramutation, maintenance of silencing depends on an endogenous RNA-dependent RNA polymerase, encoded by *rrf1*, that is responsible for the generation of 22Gs, which are endogenous 22 nt small RNA that preferentially begin with 5′ guanosine triphosphate (G). piRNA, known as 21Us in *C. elegans*, can trigger endogenous secondary 22Gs that bind nuclear, noncatalytic, worm-specific argonautes (WAGOs). These 22Gs can direct silencing of 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 nonallelic 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 Bühler, 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^{tm1Alf} allele (Rassoulzadegan et al., 2006), whereby a *LacZ* insertion at the *Kit* locus produces a *Kit-LacZ* fusion (resulting in nonfunctional 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 transgenerational 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 4A), Piwi-mediated silencing in *Drosophila*, and RdDM in plants all result in histone H3K9 methylation and depend on it, to some extent, 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; Marí-Ordóñez 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 an H3K9 methyltransferase SET-25, as well as a putative histone methyltransferase, set-32 (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 that 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 an H3K4 methyltransferase complex caused a heritable increase in longevity for multiple generations after the normal activities of the factors were restored (Greer et al., 2011). In each case, H3K4 methylation, 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 posttranscriptional 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 germline reprogramming but resist the postzygotic 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-meC to 5-hydroxyl-meC) 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, is critical for postfertilization maintenance of maternal and paternal methylation imprints (Li et al., 2008; Messerschmidt et al., 2012). In the germline, 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 (4,730) that escape this demethylation (showing >40% 5 mC) 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 germline reprogramming. In addition to these IAPs, 233 single-copy loci with >40% 5 mC were found. Why these loci are particularly prone to escape reprogramming is still not clear, but they could represent prime candidates for possible transgenerational 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*, which is responsible in part for 22Gs (Kelly and Aramayo, 2007). piRNA, known as 21Us, can also trigger endogenous secondary 22Gs that bind nuclear, noncatalytic 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, whereas 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 that 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 24 nt 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 (Marí-Ordóñez 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).

24 nt and 21 nt siRNA pathways antagonize each other in plants (Creasey et al., 2014; Jauvion et al., 2012; Marí-Ordóñez 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). 21 nt 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 downregulation of DDM1 (Calarco et al., 2012; Ibarra et al., 2012; Slotkin et al., 2009). 21 nt 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 extrambryonic 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 24 nt 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 the following: 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 behavior 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 et al., 2013; Schmitz et al., 2011). Cycling contributes to the limited epigenetic variation found in individuals (Becker et al., 2011; Schmitz et al., 2011), whereas DNA methylation at most retrotransposons is more faithfully maintained. There are also a few hundred conserved targets of RdDM that never lose methylation in inbred populations (Schmitz et al., 2013), resembling a sort of epigenetic selective sweep (Vaughn et al., 2007). Interestingly, many of these same 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 24 nt small RNA (Figure 2). Some of these genes are required for pollen tube growth providing a plausible evolutionary origin (Schmitz et al., 2013). Paramutation has also contributed to epigenetic variation in natural populations and sometimes occurs between nonallelic positions, leading to hybrid incompatibility reminiscent of the Dobzhansky-Bateson-Muller effect (Durand et al., 2012). Examples include PAI2, a nonessential gene that is heritably silenced by an unlinked inverted repeat in a subset of *Arabidopsis* accessions (Enke et al., 2011; Schmitz et al., 2013), and AtFOLT1, an essential gene that can be paramutated by nonallelic epialleles, resulting in inviable transgressive phenotypes in hybrids (Durand et al., 2012).

Although 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*^{vy} 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 or 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 (Andrés and Coupland, 2012; Ream et al., 2012; Song et al., 2012). Some *Arabidopsis* species 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 plant homeodomain (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. Although 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). Upregulation of FLC, even in plants that have not experienced cold, suggests that 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, although 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. In *The Variation of Animals and Plants under Domestication* (1868), 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 RNAs 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 may 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 undernutrition during gestation was 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 low-protein diet passed on a high-cholesterol phenotype, with 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 intergenerational (parental or grandparental exposure) rather than truly transgenerational 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 postnatal 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 bona fide transgenerational epigenetic inheritance over multiple generations seems unlikely given the robust reprogramming found in the mammalian germline. 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 byproduct of aggressive germline defense strategies. In mammals, epialleles can also be found but are extremely rare, presumably due to robust germline 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 S.J. Gould (Gould, 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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