Transgenerational Epigenetic Effects

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Abstract

Transgenerational epigenetic effects include all processes that have evolved to achieve the nongenetic determination of phenotype. There has been a long-standing interest in this area from evolutionary biologists, who refer to it as non-Mendelian inheritance. Transgenerational epigenetic effects include both the physiological and behavioral (intellectual) transfer of information across generations. Although in most cases the underlying molecular mechanisms are not understood, modifications of the chromosomes that pass to the next generation through gametes are sometimes involved, which is called transgenerational epigenetic inheritance. There is a trend for those outside the field of molecular biology to assume that most cases of transgenerational epigenetic effects are the result of transgenerational epigenetic inheritance, in part because of a misunderstanding of the terms. Unfortunately, this is likely to be far from the truth.

INTRODUCTION

Transgenerational epigenetic effects and transgenerational epigenetic inheritance are not the same, but a novice to the discipline would find this hard to understand. The situation has arisen because the word epigenetic has changed its meaning over the past fifty years. In the phrase 'transgenerational epigenetic effects,' epigenetic is being used in its broader (and original) sense to include all processes that have evolved to achieve the nongenetic determination of phenotype. Waddington, who coined the word epigenetics, was interested in how gene expression patterns are modified during differentiation and development. He was a developmental biologist, with no particular interest in transgenerational events. However, evolutionary biologists have studied the transgenerational nongenetic determination of phenotype for centuries. This has been termed soft inheritance, non-Mendelian inheritance, parental effects, and fetal programming, among others. Although in many instances these phrases refer to different phenomena, including both physiological and behavioral (intellectual) processes, they all involve a transfer of nongenetic information across generations; i.e., they are transgenerational epigenetic effects. Although in most cases the underlying molecular mechanisms are not understood, modifications to the chromosomes that pass to the next generation through the gametes are sometimes involved. The latter has to-date been called 'transgenerational epigenetic inheritance.' Here, the word epigenetic refers to mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in gene sequence. This narrower definition of epigenetics has recently become widely accepted among molecular biologists.

We have learned a considerable amount about epigenetic (in its more recent sense) modifications, including both the methylation of the cytosine residue of DNA and the modification of the chromatin proteins that package the DNA. In general these marks are established in early development and are stable through rounds of mitosis. Recent evidence shows that the establishment of epigenetic state can be influenced by environmental factors (33, 40, 129). To ensure the totipotency of the zygote and to prevent perpetuation of abnormal epigenetic states, most gene regulatory, i.e., epigenetic, information is not transferred between generations. Several mechanisms have evolved to erase the marks, including germline and somatic reprogramming of DNA methylation and chromatin proteins. However, we know that at some loci the epigenetic marks are not cleared. Examples of this include genomic imprinting in mammals, mating type switching in yeast, and paramutation in plants. Although exceptional with respect to their resistance to reprogramming, these examples can be considered part of normal development, and they are not dependent on environmental cues. Two issues that we now need to address are firstly, the extent of this resistance to transgenerational epigenetic reprogramming and secondly, whether or not epigenetic marks established in response to environmental cues are also resistant.

There is a current trend for those outside the field of molecular biology to assume that all cases of transgenerational epigenetic effects are the result of transgenerational epigenetic inheritance, in part because of a misunderstanding of the terms. This is misleading. When discussing transgenerational epigenetic effects, care must be taken not to make assumptions about the underlying mechanisms. In an attempt to improve this situation, we propose that the term 'transgenerational epigenetic inheritance' be replaced by 'gametic epigenetic inheritance,' which is a more precise description of the event. In this review we discuss the current knowledge in the broad area of non-Mendelian inheritance and attempt to highlight those cases where gametic epigenetic inheritance is known to occur.

SOFT INHERITANCE

Mendelian genetics is built on the inheritance of stable traits and the evolution of such traits

Transgenerational epigenetic effects: phenotypes present in successive generations that are not genetically determined

Transgenerational epigenetic inheritance or gametic epigenetic inheritance: a phenotype present in successive generations that is nongenetically determined and results from epigenetic modifications passed via the gametes that escape reprogramming

Hard inheritance: essentially Mendelian; hereditary material remains constant between generations (except for rare random mutations)

Soft inheritance: the generation of a new phenotype is less rigidly determined and shows a more rapid response to environment

Parental effects: effects on the phenotype of offspring that are not determined by the offspring's own genotype but by the genotype or environmental experience of its parents

Paramutation: an interaction between two alleles of a locus, resulting in a heritable epigenetic change of one allele induced by the other allele

occurs slowly as a result of rare genetic mutation, selection, and drift. The slow reactivity of this 'hard inheritance' is not ideal for an organism or population to thrive in a dynamic environment. Another more pliable system, which fine tunes the next generation to their future environment, would be an advantage. Ernst Mayr (1904–2005) (77, 78) first proposed the term 'soft inheritance' to describe this type of system. Soft inheritance would be especially suited to adaptation to fluctuations in nutrition, predation, or disease, which occur relatively unpredictably and may endure for more than one generation. Soft inheritance is adaptive in the sense used by evolutionary biologists, i.e., advantageous to the individual or species. The ability of epigenetic mechanisms to perpetuate gene expression patterns relatively stably, and to retain the capacity to react to environmental cues, makes them ideal for facilitating soft inheritance. The largest barrier to such a system is the resetting of epigenetic marks between generations.

The notion of soft inheritance is still viewed by some as controversial. This is mainly due to its association with the rejected evolutionary ideas of Jean-Baptiste Lamarck (1744–1829). His pre-Darwinian work proposed a mechanism for the transformation of species through the inheritance of characters that are acquired during the lifetime of an organism. According to Lamarck, both environment and behavior direct organic change in an organism's form and guide adaptation through the generations. A major problem with Lamarckian evolution, as pointed out initially by August Weismann in the nineteenth century, is the separation of germline and soma. How could environmentally induced epigenetic adaptations in somatic lineages be transmitted to the germline? The precise time point of germline separation from somatic tissues varies among species. In mammals, primordial germ cells (PGCs) are derived from the epiblast and arise in the posterior primitive streak during gastrulation. So, there is an extremely short period for epigenetic alterations to be included in the germline. In contrast, in plants there is no early separation of germline and soma and the gametes are derived from vegetative tissue after most development is complete. This may provide plants with a greater opportunity for soft inheritance than mammals.

It should be emphasized that the examples of soft inheritance described in this review, although Lamarckian in their environmental determination, involve short-term adaptations that supplement the evolutionary processes of Darwin and Mendel. Thus, they are distinct from Lamarck's proposed overall mechanism of evolution.

Adaptation: a phenotypic change that is ultimately beneficial to the reproductive success of an organism

Adaptive Parental Effects in Plants and Insects

Parental effects are defined as effects on the phenotype of offspring that are not determined by the offspring's genotype but instead are determined by the genotype or environmental experience of the parents. These effects can be paternal or maternal and have been reported in a range of multicellular organisms. Such effects fit within the confines of transgenerational epigenetic effects. Some are known to involve gametic epigenetic inheritance, others are not. Some are classified as adaptive by evolutionary biologists, others are not. Adaptive parental effects are examples of soft inheritance and are extensively reviewed elsewhere (48, 85). Here we limit our discussion of adaptive parental effects to a few of the better-understood cases.

Numerous examples of adaptive maternal effects exist in plants. A range of environmental stimuli acting on the mother, including predation (2), competition (97), soil type (106, 107), temperature (68), light (48, 108, 121), and nutrient availability (80, 108, 120, 121), has been found to induce changes to F1 phenotype. For example, offspring of *Polygonum persicaria* grown under low light allocate proportionately more resources to shoot growth than those of parents with higher light exposures (121). Conversely, offspring of limited-nutrient plants allocate proportionately more to root growth than genetically similar individuals with nutrient-rich parents (121). Wild

radishes (*Raphanus raphanistrum*) produce physical spines and insect-repellent chemicals in response to predation by caterpillars (2). These adaptations provide protection against further attack. Agrawal and colleagues (2) showed that seedlings from parent plants that had been damaged by caterpillars develop phenotypic features (spines) more like their parents than seedlings from unexposed plants. These changes to the F1 are associated with reduced predation from caterpillars. The authors noticed shifts in the profiles of the defensive chemicals in the seeds from predated mothers, suggesting a possible mechanism for the transgenerational inheritance.

Paternal effects in plants have also been described, but less frequently than maternal effects (68, 106). This may be because seedlings are likely to grow up in an environment more similar to their mothers' than their fathers', because seed dispersal is limited compared with that of pollen. Further reasons could include the larger maternal (2n) than paternal (n) nuclear contribution to the endosperm (the organ providing nutrients to the developing embryo) and the maternal origin of the seed coat.

In addition to their work on wild radishes, Agrawal and colleagues (2) examined the defensive responses of the water flea, *Daphnia*, which is subject to predation by other insects. When females are exposed to chemical signals associated with the presence of predators, they develop a protective helmet, which renders them less vulnerable to attack. Females exposed to these signals lay eggs that, as neonates, develop the same defense as their mothers—even in the absence of the predator-related signals. Subsequent maternal broods, initiated after the mothers were transferred to signal-free environments, also show enhanced defenses as neonates. The effect diminishes by the second generation, though subtle grandparental effects are evident. The average helmet size of neonates whose mothers, but not grandmothers, were exposed to the predation cue is not as large as those whose mothers and grandmothers were exposed to the signal.

Another study in *Daphnia* investigated their ability to produce dormant eggs in response to cues for the forthcoming food supply (3). Alekseev and Lampert (6) manipulated the photoperiod of mothers to mimic conditions that would predict poor food availability, even though the mother had ample food. The daughters of females exposed to short days (which stimulate dormant egg production) were more likely to produce dormant eggs than daughters of mothers exposed to long days.

The relevance of the findings in *Daphnia* to events in higher animals is somewhat tempered by the fact that *Daphnia* reproduce mainly parthenogenetically. Soft inheritance may be more important to asexual reproducers that cannot adapt to environmental changes by obtaining new genetic information through sexual reproduction.

In the cases of the adaptive parental effects described above, we can be relatively confident that the information is transferred via the gametes.

Adaptive Parental Effect in Nonhuman Vertebrates

The best-characterized case of adaptive transgenerational epigenetic effects in mammals is that of the maternally transmitted responses to stress in rats. Similar to the maternal effect observed in the radish and *Daphnia*, these responses are thought to represent an inducible defense mechanism (140). In times of increased environmental stress, such as when more predators are present, there is less time for maternal care in the form of postnatal maternal licking/grooming and arched-back nursing (LG-ABN). Low levels of LG-ABN in the first week after birth cause offspring to be more fearful; the theory is that their increased watchfulness will increase their survival chances. In contrast, the offspring of high LG-ABN mothers are less fearful. These behavioral traits persist into adulthood, when a female will usually display the same behavior as her mother, thus perpetuating the trend. Cross fostering pups from one mother type to the other in the first week of

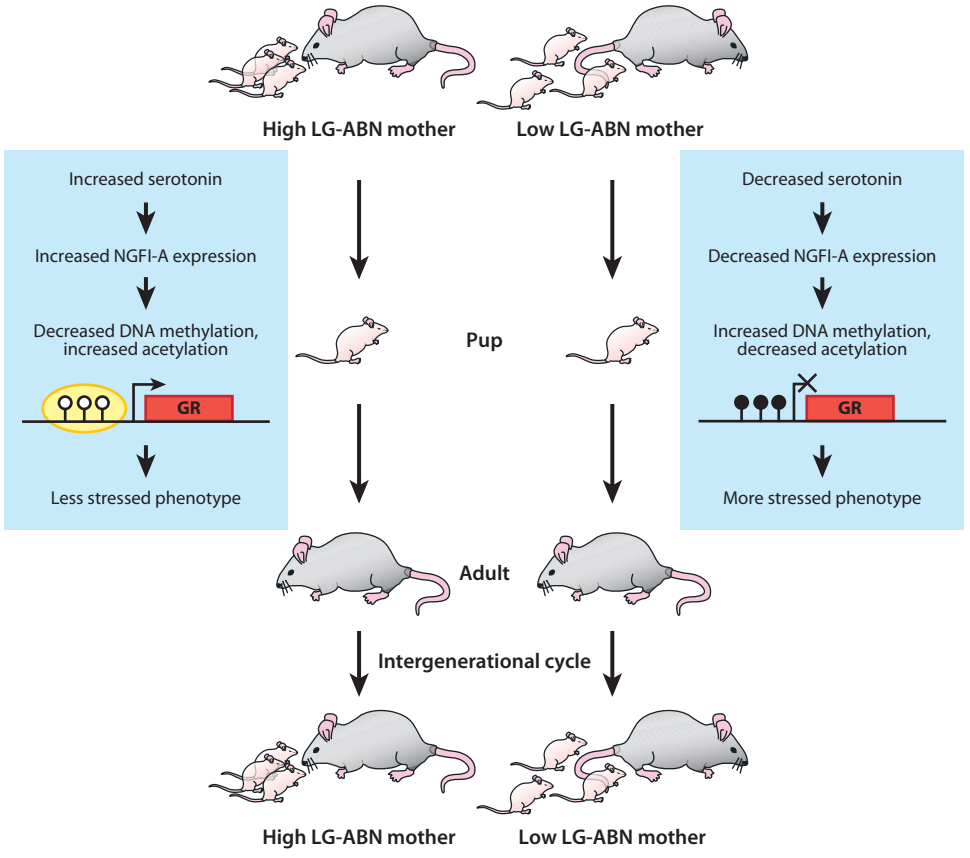

Figure 1

Transgenerational inheritance of mothering style and stress in rat. Mothering style as characterized by licking/grooming (LG) and arched-back nursing (ABN) is perpetuated across generations by a cascade of molecular events set in the the first week of life. High LG-ABN mothering results in a high serotonergic tone in the hippocampus of the pups, leading to increased expression of the transcription factor nerve growth factor inducible protein A (NGFI-A). Binding of NGFI-A to the promoter of the glucocorticoid receptor (GR) gene stimulates DNA hypomethylation, histone acetylation, and increased expression of GR. Higher glucocorticoid receptor numbers in the hippocampus are associated with reduced stress levels. The epigenetic marks maintain the GR expression state into adulthood and in females will determine the level of LG-ABN mothering, thus perpetuating the phenotype. Open circle lollipops are unmethylated CpGs, filled lollipops are methylated CpGs, and yellow ovals are nerve growth factor inducible protein A (NGFI-A) (131, 132).

life causes pups to have the stress type of their adoptive mothers (47). Therefore, in this case the adaptive maternal effect is epigenetic (in its broader sense) but not gametic.

A remarkable feature of this case is that many aspects of the mechanism have been uncovered, revealing an elegant multilevel process that involves behavioral, physiological, cellular, and molecular events (**Figure 1**). Indeed, epigenetic modifications to the regulatory elements of some relevant genes have been detected. Stress responses in mammals are mediated through the hypothalamic-pituitary-adrenal (HPA) axis and involve the action of glucocorticoid hormones. The reduced fearfulness of high LG-ABN rats is the result of an increase in the number of glucocorticoid receptors in the hippocampus. High LG-ABN mothering results

Epigenetic modifications: chromatin and DNA modifications that influence genome function but do not change the underlying DNA sequence

in a high serotonergic tone in the hippocampus of the pups, leading to activation of cAMP and increased expression of the transcription factor nerve growth factor inducible protein A (NGFI-A). Increased binding of NGFI-A to the promoter of the glucocorticoid receptor (GR) gene is associated with DNA hypomethylation, histone acetylation, and increased expression of GR. This increase in expression, in turn, results in more glucocorticoid receptors in the hippocampus. The epigenetic marks appear to maintain the GR expression state for the rest of the rat's life (131, 132).

In addition to alterations in hippocampal GR expression, enhanced maternal LG-ABN behavior results in increased hippocampal neuronal survival, synaptogenesis, and improved cognitive performance under stressful conditions (72, 73, 133). Microarray expression analysis identified over three hundred genes with differing hippocampal expression patterns in offspring of low LG-ABN compared with high LG-ABN mothers. Furthermore, intracerebroventricular infusions of the histone deacetylase inhibitor trichostatin A (TSA) or the methyl-donor L-Methionine modified the expression, consistent with epigenetic regulation (134). So, the modulation of GR is likely to be part of a larger group of adaptive effects that result from maternal nurturing. To our knowledge, this is the only study that links an adaptive maternal effect to an epigenetic change; it suggests that, at least in this case, the epigenetic marks are the molecular memory that confers persistence of the phenotype into adulthood.

Exposure to hormones in utero, in the egg, or even postnatally has been proposed to facilitate adaptation to the future environment in a number of vertebrate species. In birds, the maternally determined level of androgens deposited in egg yolk influences the offspring's embryonic development, postnatal growth, competitiveness in the nest, and dispersal distances from the nest (51). Injection of testosterone into eggs recapitulates the effects on dispersal patterns (126). Elevated in utero androgen exposure also increases dispersal distance in voles (59). However, the pleiotropic effects of changes in hormone level make it hard to know whether this process is truly adaptive, in the sense of being advantageous. The same issue has emerged over claims of adaptive significance in relation to physiological and behavioral changes caused by food availability in a number of vertebrate species. The most widely publicized example of this is fetal programming in humans.

Fetal Programming in Humans

Fetal programming or developmental origins of adult disease are terms used to describe extensive and permanent effects of the environment experienced by fetuses and neonates. David Barker (14) reported an inverse relationship between birth weight and the risk of hypertension, cardiovascular disease, and type 2 diabetes in adulthood. The effect seems to be exacerbated when the individual is well nourished postnatally (45, 46). As a consequence of these observations, Barker proposed that adverse effects in utero induce compensatory responses in the fetus. The cellular, physiological, and metabolic responses are thought to represent adaptations made by the fetus to prepare for postnatal life. This is called the thrifty phenotype hypothesis (10). According to this hypothesis, the increased levels of insulin resistance in offspring of starved mothers, rather than being an inevitable consequence of a poor early environment, is actually deliberately induced because it will confer an advantage later in life. Increased insulin resistance causes energy conservation and reduced somatic growth to allow the offspring a better chance of survival in an environment where nutrition is poor. However, with insulin resistance, the higher blood plasma levels of fatty acids, insulin, and glucose become a problem if food becomes abundant.

Some evidence in support of this hypothesis comes from the offspring of women pregnant during two civilian famines of World War II, the Dutch Hunger Winter (1944–1945) and the Siege of Leningrad (1941–44) (15). In the former case, those who were starved prenatally

were found to have impaired glucose tolerance in adulthood; this was not observed in the latter case (116). So although in both situations the fetuses predicted a poor postnatal environment, it proved to be true only in the Leningrad case, where nutrition stayed poor in the subsequent years. Whether the postnatal effects of gestational undernutrition are truly adaptations, or developmental abnormalities that resemble them, remains unclear.

Fetal Programming Across More Than One Generation

Reports of multigenerational epigenetic effects in human populations are scant, in part because phenotypic records across generations have rarely been collected and in part because ruling out genetic and environmental confounders is extremely difficult. However, a few studies have been published.

Follow up work on the Dutch Hunger Winter initially suggested that mothers who were exposed to famine as fetuses delivered offspring (F2) of lower birth weight than those with no fetal exposure to famine (74). However, this study was flawed in a number of ways. In particular, birth weights in famine-exposed mothers were not measured directly, but were instead extrapolated from another group. A subsequent study by the same author found no significant effect of maternal fetal exposure to famine on the birth weights of the next generation (117).

Studies on other cohorts have revealed some association between grandparental nutrition and grandchild (referred to as the proband in this work) phenotype. Extensive records of a population in Överkalix in Sweden, including yearly crop yields over multiple generations, revealed a link between grandparental and parental periods of low or high food availability with proband mortality and disease risk (27, 63, 64, 94). The work highlighted the possible importance of food availability during the paternal grandparental prepubertal slow growth periods (SGP), between age 8–10 in girls and 9–12 in boys. If the SGP of the paternal grandfather was a period of high food availability then male probands had reduced longevity (27, 94), an effect later shown to be related to increased risk of death by cardiovascular disease or diabetes (63). Also, abundant food in the SGP of the maternal grandmother was associated with an increased mortality in female probands (94).

No molecular data exist to explain the findings, but the involvement of epigenetic marks in the form of gametic epigenetic inheritance has been suggested (94). However, these studies reveal a complex process with sex- and age-specific variations. For example, in addition to the effect of food availability during paternal grandmaternal SGP on female probands, researchers also noted an effect of the food availability during a grandmother's first five years of life—but with an opposite influence on proband mortality. Moreover, the paternal grandfather to grandson effect was not seen in all cohorts (63). Independent replication in another cohort would be helpful. The possibility of societal confounders in these studies remains high and in the absence of molecular evidence, the conclusion that this is a case of gametic epigenetic inheritance seems unwarranted.

NONADAPTIVE TRANSGENERATIONAL EPIGENETIC EFFECTS

In all the examples cited so far, the unifying factor is the concept of an adaptive response to the environment and, in general, the studies have been carried out by behavioral psychologists, evolutionary biologists, or epidemiologists. Many other examples of transgenerational epigenetic effects exist that are not necessarily adaptive, such as the gametic transgenerational inheritance of epigenetic state at paramutated alleles or transgenes. In many of these cases the inherited phenotype is actually detrimental to the organism. These cases have taught us what little we know about the underlying molecular mechanisms of gametic transgenerational inheritance.

Table 1 Transgenerationally inherited epimutations and metastable epialleles

Locus/epiallele	Organism	Mechanism[a]	Phenotype	Stability	Parent of origin effect	Reference
a locus a-m2–7991A1	Maize	Methylation levels of a Spm transposon can switch between hyper and hypo to affect expression of the nearby a gene	Pigmentation and transposition	Metastable	Yes	(11)
b1locus B' epiallele	Maize	Paramutation-induced methylation	Reduced pigmentation	Stable	No	(115)
Lcyc	*Linaria vulgaris*	Epimutation. No apparent genetic mutation	Radially symmetrical flowers	Metastable	No	(36)
A^{vy}	Mouse	IAP-LTR promoter drives ectopic expression of a nearby gene	Coat color, diabetes, obesity	Metastable	Partially inherited down female line	(83)
$Axin^{Fu}$	Mouse	IAP-LTR promoter drives eptopic expression of a nearby gene	Kinked tail	Metastable	Partially inherited down male and female lines	(100)
MLH1	Human	Presence of an epimutation in all three germ layers suggests a germline origin	Colon cancer	Unknown	N/A	(57)

[a]Abbreviations: IAP, intracisternal A particle; LTR, long terminal repeat.

Nonadaptive Transgenerational Effects in Plants

The most famous example of gametic epigenetic inheritance in plants involves the peloric variant of toadflax (*Linaria*); in this case the modified phenotype is relatively stably inherited over many generations (36) (**Table 1**). Silencing of the *Lcyc* gene causes the symmetry of the flower to change from bilateral to radial. The silencing occurs not through mutation of the DNA sequence but through methylation of the promoter. This became one of the first reported cases of an epimutation. However, although this appears to be a clear case of gametic epigenetic inheritance, whether the phenotype is perpetuated across generations by cytosine methylation or by other epigenetic factors remains unknown. Similarly, DNA methylation of a transposon at the promoter of the *a* gene in maize influences the gene's expression and is stably transmitted through meiosis (11) (**Table 1**). Another gene in maize, the *b1* locus, can become stably repressed by epigenetic modifications through paramutation; this repressed state is heritable across generations (**Table 1**). Moreover, the repressed state can silence unmethylated alleles introduced by breeding. Similarly, an epimutation at the *P* locus in maize was stably inherited over five generations, though reversions were noted (37) (**Table 2**). In all these cases, no genetic mutations have been

Epimutations: abnormal epigenetic patterns that can occur in response to a DNA mutation, but the term is generally used in cases without an underlying DNA sequence change

Table 2 Genetic mutation–induced transgenerational epigenetic inheritance

Locus/ epiallele	Organism	Manipulation	Mechanism	Stability	Phenotype	Observed parent of origin effect	Reference
pai2	Maize	Deletion of inverted repeat source of RNA-directed DNA methylation	Upon deletion of the inverted repeat homologous sequences retain methylation in successive generations	Metastable	Metabolic	Maternally inherited, paternal inheritance unknown	(19, 79)
P-pr	Maize	Strain used has increased frequencies of somatic mutation so probably a mutation in cis or in epigenetic modifier caused epimutation	Inherited epimutation (no associated genetic lesion identified)	Stable	Reduced pigmentation	No	(37)
bal	*Arabidopsis thaliana*	DDM1 SWI/ SNF-like chromatin remodeling factor mutant	DDM1 mutant–generated epimutation	Metastable	Dwarfism, elevated disease resistance	No	(119)
sup	*Arabidopsis thaliana*	Chemical mutagenesis and a variety of epigenetic modifier mutants cause the phenotype	Variety of mutants cause epimutation at the SUP gene	Metastable	Abnormal floral organ number	No	(62)
fwa	*Arabidopsis thaliana*	Possible chemical or radiation-induced mutation of epigenetic modifier; phenotype recapitulated in DDM1 mutants	Epimutation (lack of methylation) SINE retrotransposon 5′ of gene causes ectopic expression.	Metastable	Delayed flowering	No	(112)
Genome-wide	Mouse	Pronuclei transfer between different mouse strains	Epimutations in transplanted embryos are paternally transmitted to the next generation; genes in pheromone systems are particularly affected	Metastable	Reduced stature and multiple gene mis-regulation	Possibly male line–specific	(103)

(*Continued*)

Table 2 (*Continued*)

Locus/ epiallele	Organism	Manipulation	Mechanism	Stability	Phenotype	Observed parent of origin effect	Reference
Fab-7 construct	*Drosophila melanogaster*	Fab-7 PRE/TRE construct	Active state of construct is inherited through female line; H4 hyperacetylation transgenerational persistence	Metastable	Larval LacZ and adult eye color markers	No, but phenotype is influenced by sex	(28, 29)
JAK kinase	*Drosophila melanogaster*	JAK kinase overexpression mutant	Maternally-inherited JAK kinase signaling protein overexpression disrupts reprogramming in the early embryo	Metastable	Enhanced offspring tumorigenesis	Yes, parental effects	(136)
Mod(mdg4)	*Drosophila melanogaster*	Mod(mdg4) mutant	Mutation causes abnormal chromatin configuration on the Y chromosome that can be stably inherited	Stable	Enhanced position effect variegation (PEV)	Yes	(41)
Kruppel combined with various others	*Drosophila melanogaster*	Kruppel repetitive element insertional mutant combined with various maternal effect modifier mutations or Hsp90 chemical inhibition	Ectopic overexpression of Kruppel combined with chemical inhibition of Hsp90 causes ectopic bristles in the eye; artificial selection can either fix or remove the phenotype from a population	Metastable	Ectopic large bristle outgrowths from eye	Yes, several maternal effect modifiers	(111)

identified at or near the gene of interest but causative DNA mutations *in cis* remain possible. The recent discovery of previously unnoticed copy number variants (CNVs) in vertebrates is a salient reminder of our need for caution in this regard (17).

Transgenes in plants (and animals, see below) are susceptible to silencing by epigenetic mechanisms. This silencing can be due to integration adjacent to a heterochromatic region or to a poorly understood genome defense system that recognizes the transgene as foreign (76). In many cases the silencing is probabilistic, resulting in mosaic patterns of expression called variegation or position effect variegation (PEV). The silent state is sometimes heritable across generations (76).

Seemingly nonadaptive transgenerational epigenetic effects have been reported following ionizing radiation in plants. The mutagenic

Variegation: mosaic expression of a particular phenotype among cells of the same cell type; for example, mottled coat color

properties of ionizing radiation mean that, after exposure, some inheritance of abnormal phenotype will be the result of inherited genetic lesions. However, growing evidence shows that ionizing radiation also produces nongenetic heritable effects (13). The best example comes from observations in *Arabidopsis thaliana*, where elevated rates of somatic homologous recombination in response to UV-C (UVC) persisted in untreated progeny for up to four generations (82). The phenomena are likely to be epigenetic because the whole population changes its behavior each generation. Mutation would affect only 50% of plants (those that had inherited the mutation). Furthermore, the effect acts *in trans* on a reporter transgene introduced from an untreated parent plant. The effects are independent of the sex of the transmitting parent, suggesting that the memory can be inherited through either gametophyte. The molecular marks that provide this transgenerational persistence of the response to the ionizing radiation are unknown. All the cases of nonadaptive transgenerational effects described in this section are likely to be instances of gametic epigenetic inheritance.

Nonadaptive Transgenerational Epigenetic Effects in Insects

Cases of transgenerational epigenetic effects in *Drosophila* tend to be complex with the involvement of parental effects and genetically compromised backgrounds. All cases appear to involve gametic epigenetic inheritance. Mod(mdg4) is a protein with several roles, including chromatin insulation, apoptosis, and homolog pairing in meiosis. Dorn and coworkers (41) showed that the sons of heterozygous *mod(mdg4)* mutants display enhanced PEV of a reporter locus, even if they do not inherit the mutation. The effect is thought to be caused by an abnormal chromatin configuration on the Y chromosome, which is stably inherited in wild-type males for at least 11 generations. This suggests that certain epigenetic states in flies are not reset each generation and consequently, their perturbation is not rectified.

Transgenerational persistence of polycomb/trithorax–mediated transcriptional regulation has been studied with a reporter construct containing the homeodomain regulator element Fab-7, which contains a polycomb/trithorax response element (PRE/TRE) (28, 29). After embryonic induction of trithorax-mediated expression through the transient binding of the GAL4 transcription factor, the active state is maintained in both the soma and the germline. High levels (though reduced compared with that of the F1) of reporter (lacZ in embryo, red eye color in adult) are still detectable in the F2 and F3 generations. Activated Fab-7 is marked with hyperacetylation of histone H4 and this may be the transgenerationally stable mark. However, it is not known why this reporter construct, unlike endogenous PRE/TRE elements, escapes reprogramming.

Xing and coworkers (136) recently reported a mutant fly in which reprogramming in the early embryo has been disrupted (by overexpression of the JAK kinase signaling protein), and that shows transgenerational inheritance of tumorigenic epimutations. How JAK signaling interferes with reprogramming is unclear, but it inhibits heterochromatin formation (110). The full extent of the epimutations is not understood.

In *Drosophila* and plants, reduction in the level of the stress response protein Hsp90, by mutation or chemical inhibition, induces unusual morphologies (99, 105). These morphologies are the result of the expression of natural variation that was previously hidden by Hsp90's chaperone function. Selection of the abnormal phenotypes can lead to their fixation in a population. Work by Sollars and colleagues (111) suggests that the phenomenon is, at least in part, epigenetic. Consistent with this hypothesis, mutations in genes encoding trithorax group proteins were commonly found in a screen carried out to identify modifiers of the process. However, transgenerational persistence of an epigenetic mark is yet to be confirmed. These actions of Hsp90 are proposed to be a form of soft inheritance (105, 111). The theory is that environmental stress

Position effect variegation (PEV): variegation caused by the inactivation of a gene in some, but not all, cells of the same cell type through its abnormal juxtaposition with heterochromatin

Metastable epialleles: alleles at which the epigenetic state can switch, creating different phenotypes, and where the establishment is a probabilistic event

Genomic or parental imprinting: the expression of certain genes in a parent-of-origin-specific manner; involves the sex-dependent epigenetic resetting of a germline regulatory element

diverts Hsp90 from its chaperone function of stabilizing aberrant proteins, revealing hidden phenotypes, and that advantageous ones can be selected and fixed. Importantly, an adaptive response would be made without the need to wait for the generation of novel genetic mutations. However, opponents of the theory argue that the effects of Hsp90 reduction are merely nonfunctional consequences and not an evolved evolutionary mechanism (39).

Despite the excellent genetic tractability of flies much remains to be discovered about the mechanisms of gametic epigenetic inheritance in this organism, in particular the nature of the transgenerationally resistant mark.

Nonadaptive Transgenerational Epigenetic Effects in Nonhuman Mammals

There are a number of examples of nonadaptive transgenerational epigenetic effects in mammals. Some cases involve the transgenerational persistence of environmentally induced phenotypes, some display gametic epigenetic inheritance, and a few notable cases involve both.

Transgenes and metastable epialleles. The first molecular evidence for transgenerational epigenetic inheritance (i.e., gametic epigenetic inheritance) in mammals came from studies of metastable epialleles in inbred mouse strains (83, 100) (**Figure 2*a*,*b***; **Table 1**). Inbred mouse strains provide an opportunity to study phenotype differences that occur among genetically identical individuals. Metastable epialleles are loci at which activity is dependent on the epigenetic state. A handful of such alleles have been reported, including the *agouti viable yellow* (A^{vy}) and *axin fused* ($Axin^{Fu}$) alleles, both of which contain intracisternal A particle (IAP) retrotransposons that influence expression of linked genes; this influence is dependent on the methylation status of a cryptic promoter in the IAP long terminal repeat (LTR). The transgenerational memory of these epigenetic states involves gametic epigenetic inheritance and current evidence suggests that DNA methylation is not the mark that is directly inherited (22).

Some transgenes in mice (and in plants, as mentioned previously) show gametic epigenetic inheritance and in many cases this inheritance is multigenerational (5, 52, 67, 123). Interestingly, in most cases the transgenes also show some degree of genomic imprinting (5, 52, 67, 124). In mammals there are approximately one hundred endogenous genes that undergo genomic (parental) imprinting, for which reciprocal DNA methylation patterns are set in male and female germlines. The epigenetic marks associated with imprinting are generally resistant to reprogramming in the early embryo but undergo reprogramming in the germline each generation. Importantly, the finding of long-term transgenerational effects at transgenes implies that in these cases the epigenetic marks also escape reprogramming in the germline. For example, the Tg(13HBV)E36-P transgene, when inherited paternally, is unmethylated, but maternal transmission results in silencing that cannot be reversed, even with subsequent passage through the male germline (52). These studies provided the first models to study the epigenetic transition of a single locus from expressed to permanently transgenerationally silenced. However, what actually makes these sequences resistant to reprogramming remains unclear.

In response to ionizing radiation. Transgenerational epigenetic effects following ionizing radiation, similar to those reported in plants, have been seen in mice. These studies examined germline mutation rates at expanded simple tandem repeat (ESTR) loci following irradiation (12, 43). Exposure of the F0 male with X-rays caused elevated rates of mutation in the F1 and F2 generations. As in plants, the effect can act *in trans*, i.e., ESTR alleles from unexposed mice become unstable in the germlines of progeny of exposed mice. If the effects were caused by mutations in genes that maintain ESTR stability then the effects would lessen through the generations when breeding to wild-type mice as the mutated allele(s)

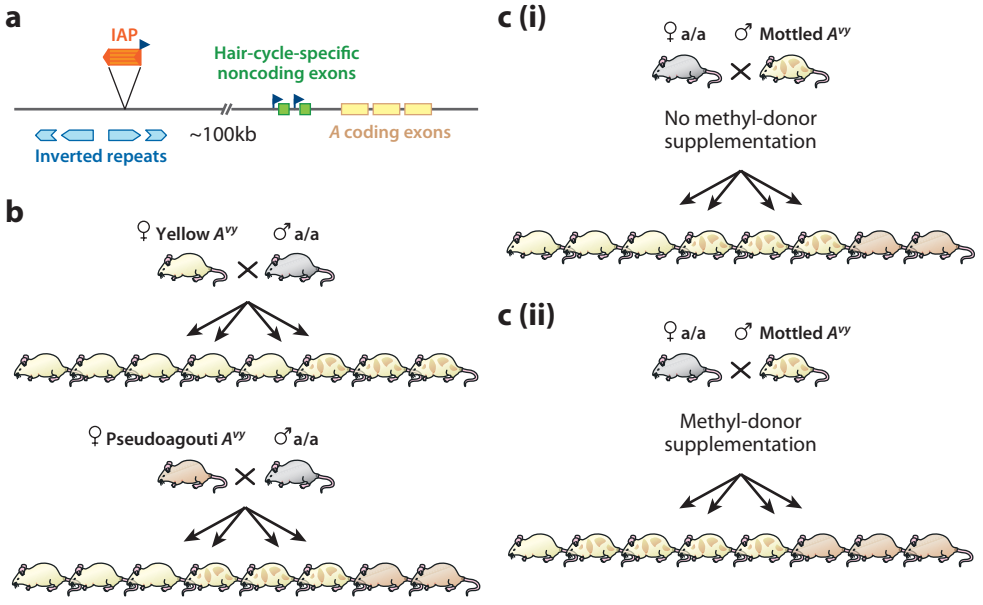

Figure 2

Intracisternal A particle (IAP)-mediated transgenerational epigenetic inheritance at the A^{vy} locus. (*a*) The agouti gene (*A*) is ectopically expressed; a transcript originates from an IAP retrotransposon upstream of the normal promoters. The expression of the cryptic IAP promoter is highly variable among isogenic mice. The agouti protein indirectly results in a yellow coat. The presence or absence of the ectopic transcript correlates with differential DNA methylation at the IAP promoter. The variable expressivity of the IAP creates a range of coat colors from yellow, through mottled, to pseudoagouti. (*b*) A^{vy}/a mice were mated to congenic a/a black mice, and the offspring scored for phenotype at weaning. The phenotype of the A^{vy}/a mother affects the phenotype of the offspring; yellow dams produce a higher proportion of yellow offspring than pseudoagouti dams. There is some memory of the epigenetic state of the maternal A^{vy} locus in the offspring. (*c*) The diets of female a/a mice were supplemented with methyl-donating substances [folic acid, choline, vitamin B_{12}, and betaine (129) or the phytoestrogen genistein (40)] two weeks before mating with male A^{vy}/a mice and throughout pregnancy and lactation. The range of coat colors was shifted toward pseudoagouti in the offspring of mothers with the supplemented diet (*i*) compared with controls (*ii*).

segregate. This is not seen. Mutation rates remain high in the F1 and F2 germline, which points to an epigenetic mechanism. As yet the molecular nature of the transgenerational memory is unknown.

Maternal exposure to changes in nutrition. Nonadaptive transgenerational epigenetic effects elicited by changes in nutrition have been reported in rodents and lagomorphs. For example, evidence shows that insulin resistance (1, 24), high blood pressure (7, 38), and elevated glucocorticoids (42, 90, 91) can increase the risk of the same condition in the next generation down the female line. Although this constitutes nongenetic perpetuation of phenotype, gametic mechanisms are not necessarily the explanation. For example, low protein diets of F0 females, while pregnant, are associated with a number of abnormalities in the F2 despite normal F1 postnatal nutrition (139). However, because the F1 experienced poor nutrition directly while in utero, the effect in what the authors call F2 is actually only a single generation after the one that experienced dietary restriction (see sidebar, Possible Explanations for Phenotypes Inherited Down the Female Line That Do Not Involve Gametic Epigenetic Inheritance). That is to say, the F2 phenotype could be due to the F1's incapacity to care for the F2 fetus; this

POSSIBLE EXPLANATIONS FOR PHENOTYPES INHERITED DOWN THE FEMALE LINE THAT DO NOT INVOLVE GAMETIC EPIGENETIC INHERITANCE

a) Postfertilization transfer of virus or toxin. This process can occur through the placenta or milk. Examples include the following: a gray mouse phenotype caused by virus in milk, ethanol transfer across the placenta, undernutrition during pregnancy.

b) Poor maternal health compromises pregnancy, which induces a similar phenotype in the next generation. Examples include insulin resistance, high blood pressure, or increased glucocorticoids in females causing complications during pregnancy, which lead indirectly to the same phenotype in the child.

c) Behavioral interactions between mother and child can perpetuate a phenotype. One example is in rats, where reduced maternal care induces a stressed phenotype in offspring and those rats become poor mothers, thus perpetuating the stressed phenotype.

would be a single generation maternal effect. Furthermore, the genome and/or epigenome of the F2 could have been directly affected by the environmental change because the specification of cells in the female germline occurs while the female is still in utero (see sidebar, Possible Explanations for Phenotypes Inherited Down the Female Line That Do Not Involve Gametic Epigenetic Inheritance; **Figure 3**). The problems in interpreting multigenerational effects down the female line also arise in other organisms. For example, in the plant *Plantago major* a collection of juvenile and adult characters in the F2 were influenced by a grandmaternal (F0) nutrient pulse (80). However, the nutrient pulse was administered during the stage of fruit maturation when the seeds that will become the F1 generation are themselves undergoing embryonic development within the F0. Thus, the F1 can be considered to have experienced the nutrient pulse and, similar to the situation in rodents, the effects in the F2 could actually be a maternal effect.

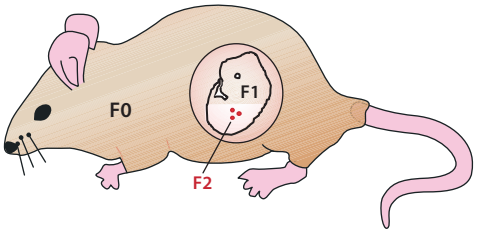

Figure 3

Three generation environmental exposure in pregnant females. In a gestating mother three generations directly experience environmental conditions. The mother (F0), embryo (F1), and the next generation (F2) in the form of the developing germline within the embryo can all be exposed to toxic chemicals, radiation, or dietary fluctuations.

Interestingly, Benyshek and coworkers (20) recently reported effects on glucose metabolism in F3 rats when pregnant (F0) females were fed a protein-restricted diet. Similarly, Stewart and colleagues (118) showed that feeding a low protein or unpalatable diet to rats for ten to twelve generations progressively reduces birth weight, which returns to control levels only three generations after reinstating a balanced diet. In the latter two cases, we can be certain that effects were seen in generations whose germline did not directly experience poor nutrition. Such examples are rare and are not necessarily the result of gametic epigenetic inheritance, because the perpetuation of effects could be mediated by postfertilization fetal-maternal or pup-dam interactions (See sidebar, Possible Explanations for Phenotypes Inherited Down the Female Line That Do Not Involve Gametic Epigenetic Inheritance; **Figure 3**). A good example of the latter is a gray mouse phenotype that is transgenerationally inherited down the female line as a result of transmission of a virus via the mother's milk (84).

Effects on the offspring of mothers fed a protein-restricted diet while pregnant can be reversed by supplementing the pregnant mother with methyl donors (61, 125). Protein restriction of pregnant F0 rats induces DNA hypomethylation and increases the expression of the *GR* and peroxisomal proliferation–activated receptor α (*PPAR*α) genes in the liver of adult F1 offspring (71). So far the molecular studies have been confined to candidate genes.

A follow-up study has found a persistence of GR and PPARα gene promoter hypomethylation in the F2 when the F1s were fed a normal diet (26). Whether this is the result of survival of epigenetic marks through the germline or de novo induction of the state in each generation through maternal-fetal interactions remains unclear.

Other studies reveal methyl donor supplementation of pregnant females with folic acid, vitamin B_{12}, choline, or betaine shifts the spectrum of coat color phenotypes in her offspring toward the repressed state, termed pseudoagouti, by increasing the level of DNA methylation at the A^{vy} allele (33, 135) (**Figure 2*c***). Two studies addressed the question of whether increased DNA methylation at the locus is inherited by the next generation and they came to different conclusions. Waterland and coworkers (130) reported no cumulative effect on DNA methylation when successive generations had the supplemented diet and concluded that the acquired DNA methylation mark was not transgenerationally inherited. Using the same strain of mice but a slightly different approach, Cropley and colleagues (34) came to the opposite conclusion.

Maternal exposure to chemicals. Gestational exposure to carcinogens, endocrine disruptors, and other toxins has been shown to affect more than one generation in some cases; however, most studies do not investigate effects beyond the F2 generation and, as described previously, the F2 may have experienced the exposure directly. Chemicals known to induce phenotypic effects in unexposed generations include alloxan (113), cyclophosphamide (53), orthoaminoasotoluol (98), benzpyrene (35), diethylstilbestrol (DES) (89), and vinclozolin (8). The underlying mechanisms are not known and in most cases inherited genetic lesions cannot be ruled out, especially in cases of mutagens (e.g., cyclophosphamide).

In particular, the reports in rats of transgenerational epigenetic effects following exposure to the endocrine disruptor vinclozolin have raised considerable public interest. These studies showed that exposure to the fungicide vinclozolin at the time of gonadal sex determination causes a variety of abnormalities in offspring (8, 9). The effects are transmitted down the male line for at least three generations. The high incidence of the defects (approximately 90% of all males in all generations) and the absence of abnormalities when passed down the female line suggest gametic epigenetic inheritance. Importantly, increased DNA methylation was seen in sperm from vinclozolin-exposed males, and these abnormal methylation patterns (epimutations) were inherited. The most important feature of this work is the suggestion that environmentally induced epigenetic marks can survive reprogramming events over multiple generations; this work also highlights potential dangers of current environmental exposures on the health of future generations. However, many unresolved issues remain. How does the induced DNA methylation resist transgenerational reprogramming? How does the passage through the female germline terminate the transmission of the defects? How extensive are the DNA methylation changes? Could genetic changes to the Y chromosome be involved?

Nonadaptive Transgenerational Epigenetic Effects in Humans

For almost 30 years, clinicians prescribed the synthetic estrogen DES to women to prevent miscarriages. Women exposed to DES before birth were later shown to have a greatly increased risk of vaginal adenocarcinoma (55). Animal experiments showed increased tumor risk in the F2 and there is some evidence that this may also be the case in humans (21, 89). Pre- and neonatal DES exposure causes a wide range of gene expression changes and some DNA hypomethylation (70). However, survival of DES-induced epigenetic marks through transgenerational reprogramming is not necessarily the explanation. Estrogenic compounds can induce DNA damage, so the transgenerational incidence of DES-induced tumors could involve DNA mutation (88). Considering

society's increased concern about environmental pollutants, it is likely that this area of research will grow.

Two families with an increased risk of colorectal cancer resulting from heterozygosity for epimutations at tumor suppressor genes provided some evidence that epimutations can be inherited transgenerationally. The best evidence comes from an individual with hereditary nonpolyposis colorectal cancer (HNPCC) (57). The subject had abnormal DNA methylation and silencing of one allele of the DNA mismatch repair/tumor suppressor gene MLH1. The presence of the epimutation in all three germ layers suggests it arose in the parental (in this case maternal) germline or in the zygote and resisted postfertilization reprogramming. No novel DNA mutations were identified in the region and some siblings inherited the same allele [as determined by single nucleotide polymorphism (SNP) analysis] in an unmethylated state. These facts argue against the theory that the epimutation is secondary to a mutation. This is in contrast to an epimutation in another case of HNPCC associated with an epimutation of another tumor suppressor gene (MSH2) (31). In this case a haplotype associated with the epimutation segregated in a Mendelian manner, and no revertants (unmethylated copies) were found. This result suggests that the epimutation was caused by a linked DNA mutation. Unfortunately, in humans it is almost impossible to prove that an epimutation was inherited because of a failure to reprogram in the germline. This is because we are outbred, so even if the DNA in the region of the epimutation has no mutation, the epigenetic status of the epimutation may actually be dependent on the unique genetic background of an individual. Several reports of HNPCC with MLH1 epimutations now exist, suggesting that this may be a hotspot for abnormal DNA methylation (49, 56, 57, 81, 122).

In summary, despite a growing amount of observational and molecular data on induced transgenerational epigenetic effects, incontrovertible evidence for gametic epigenetic inheritance of acquired marks remains scant in vertebrates. However, the recent advances in molecular technologies and high levels of interest in the topic suggest that a resolution is not far away.

GENETIC ELEMENTS NATURALLY RESISTANT TO REPROGRAMMING

Evidence exists that at some regions of the genome, such as centromeres and telomeres, gametic inheritance of epigenetic state is routine; however, these regions do not appear to be prone to acquisition of marks in response to environment and contain few if any genes. Indeed, the inheritance of the epigenetic state at such regions is probably necessary for normal chromosome structure, pairing, and segregation. The study of these genetic elements provides molecular evidence of gametic epigenetic inheritance and guides efforts to unravel events at the biochemical level.

Sequences Naturally Resistant to Reprogramming in Plants

DNA methylation in plants is more complex than in animals and occurs at both CG and CNG sites. Mathieu and colleagues (75) backcrossed mutants with no CG methylation (*met1-3* homozygotes) to wild-type (MET1/MET1) plants and selected MET1/MET1 progeny in the F2 generation. These progeny were then inbred for seven generations. Surprisingly, unmethylated sequences were still present in the F7 generation, showing robust transgenerational inheritance of the unmethylated state. This work built on the results of numerous previous studies (6, 44, 65, 102, 128), suggesting that methylation patterns in plants are resistant to reprogramming. Several studies show that after DNA methylation patterns have been disturbed it can take a number of generations before the patterns revert to normal (**Table 2**). Furthermore, phenotypic consequences of hypomethylation (transposon activation in particular) are known (141). Less is known about the transgenerational

dynamics of histones and PcG proteins in plants (60, 86).

Sequences Naturally Resistant to Reprogramming in Mice

As discussed above, the epigenetic marks that govern transcriptional regulation at imprinted loci are resistant to postfertilization reprogramming and some transgenes are resistant to both postfertilization and gametic reprogramming. Some classes of retrotransposons appear to behave like transgenes and are resistant to reprogramming at both stages, at least with respect to DNA methylation (69). Constitutive heterochromatin at centromeres is resistant to postfertilization demethylation (104). During germline reprogramming, although DNA methylation is erased from centromeric regions, their heterochromatic state is maintained by the continued presence of the repressive histone modification (109).

The removal of histones and replacement with protamines during mammalian spermatogenesis is another point at which epigenetic marks are cleared and replaced. In this case we also know that the clearing is incomplete. In human sperm approximately 15% of DNA remains nuclesome-bound and in the mouse this figure is approximately 2% (18). Nucleosome-bound DNA in sperm localizes to the telomeres and centromeres, consistent with the idea that these regions enter the oocyte already marked for their subsequent heterochromatic structure (93, 127, 138). Modified histones incorporated into the sex chromosomes during spermatogenesis in *Caenorhabditis elegans* and mouse probably persist for several cell divisions postfertilization (16, 87).

Chong and coworkers (32) recently showed that heterozygosity for mutations in epigenetic modifiers can induce phenotypes in the next generation in mice, presumably owing to the retention of abnormal epigenetic states established in the gametes. Roemer and colleagues (103) had previously shown that multiple epimutations caused by pronuclear transplantation can be passed on to the next generation. Therefore it seems likely that in mammals perturbation of global epigenetic patterns established in one generation can be passed on to the next, but this is rarely associated with single copy genes.

The Molecular Nature of Gametic Epigenetic Inheritance

The normal developmental program of an organism requires that more than simply DNA is transferred to the next generation, because the zygote must have the capacity to initiate transcription. Transcription requires proteins and RNA, which must have originated in the gametes. This requirement creates a molecular memory of the genotype of the parent. In those cases where gametic epigenetic inheritance occurs, the underlying molecular mark could take the form of DNA methylation, chromatin proteins, or RNA. As described above, cytosine methylation has been shown to be involved in gametic epigenetic inheritance in plants; however, little direct evidence exists about the inheritance of chromatin protein. There is increasing interest in the idea that RNA may have a role in this process.

In plants, gametic epigenetic inheritance in the form of RNA is an attractive idea because of the widespread RNA-directed epigenetic pathways that have been uncovered (54). RNA is present in considerable quantities in pollen, where much appears to be dedicated to the growth of the pollen tube (58, 96). It is exciting to speculate that this cache of RNAs could also act as a source of inherited memory to initiate the silencing of relevant transposon classes or genes in the next generation. Consistent with this idea, a genetic screen to identify modifiers of paramutation (which involves gametic epigenetic inheritance) at the aforementioned *b1* locus identified a gene encoding a protein that acts as an RNA-dependent RNA polymerase (4). Alleman and coworkers (4) propose that the polymerase is required to establish and maintain the heritable chromatin state associated with paramutation. Some information is available on RNA transcripts unique to

the female *A. thaliana* gametophyte (137), but the specific functions of these transcripts are unknown.

In animals, the RNA stores in the female gamete are vital for early development. These stores are produced by a set of genes, called maternal effect genes, that are transcribed before the completion of meiosis and originate from alleles present in the mother but not necessarily present in the haploid genetic complement of the oocyte. In insects, RNA stores can also be produced by adjacent diploid nurse cells, which are connected to the oocyte by cytoplasmic bridges (114); the same process may also occur in mammals (95). Similar mechanisms are present during male gametogenesis. Human sperm has 5–10 femtograms of RNA (23), consisting of around 2700 different transcripts (92, 142). There is certainly ample opportunity during male gametogenesis for paternal effects resulting from either RNA or proteins made prior to meiosis or shared between spermatids (25).

In *C. elegans*, microinjection of small RNAs that target genes expressed in the maternal germline can induce phenotypes that last up to three generations (50). Furthermore, in the mouse, a white tail phenotype generally caused by a mutation at the *Kit* gene has been detected in offspring that do not inherit the mutation (101). The phenotype is weaker but still present in F2 offspring from crosses between affected 'wild-type' F1 mice. The authors argue that this phenomenon is the result of the inheritance of abnormal *Kit* RNA from sperm.

SUMMARY

Transgenerational reprogramming is important to ensure that the correct gene expression program is set at the start of embryonic development. The discovery of abnormal phenotypes, including cancer in humans, that are caused by epimutations emphasizes the dangers of abnormal resetting. The evidence at present suggests that for a mark to be resistant requires either a failure of the system (owing to mutation in genes encoding proteins involved in epigenetic reprogramming, abnormal nutritional availability, radiation exposure, or chemical treatment) or the locus to be part of an element involved in maintaining genome integrity (e.g., telomeres or centromeres) or susceptible to genomic imprinting. In the mouse, the IAP retrotransposons are exceptional in their resistance to DNA demethylation. These are the most active transposable elements in the mouse genome, and as such they may attract extra attention from genome defense systems (66). The resistance of some trangenes to reprogramming may occur through a similar mechanism.

Owing to their sessile nature, plants have evolved an enhanced capacity to respond to changes in their environment. Soft inheritance is therefore likely to be of greater use to plants than to animals. Unlike animals, there is no early separation of germline and soma in plants, allowing for epigenetic marks acquired throughout their lifetime to be included in the gametes. Indeed, some epialleles in plants are resistant to reprogramming for many generations, e.g., *Lcyc*. Nevertheless, it seems likely that such examples will be rare. The epimutation at *Lcyc* involves CG methylation, which is known to be resistant to reprogramming. However, most developmentally regulated genes are controlled by non-CG methylation, which requires a continuous remethylation cue and as such is continually reprogrammed (30, 141). Therefore, gain or loss of non-CG methylation at these genes is unlikely to be transferred to the next generation.

In animals, both adaptive and nonadaptive transgenerational epigenetic effects do occur. Many, perhaps most, of these effects are not the result of the direct transfer of information via the gametes. The advantage of epigenetically preadapting offspring to their future environment via the gametes appears to have been mostly outweighed by the desire to prevent inherited epimutations and safeguard the pluripotency of the epigenetic program of early development. The observations of transgenerational effects of nutritional availability, chemical exposure, and inherited epimutations are generally limited to one generation. Indeed,

the theoretical arguments posited for the existence of soft inheritance emphasize the value of flexibility, so multigenerational inheritance of adaptive changes would be counterproductive.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Aerts L, Van Assche FA. 2006. Animal evidence for the transgenerational development of diabetes mellitus. *Int. J. Biochem. Cell Biol.* 38:894–903
2. Agrawal AA, Laforsch C, Tollrian R. 1999. Transgenerational induction of defences in animals and plants. *Nature* 401:60–63
3. Alekseev V, Lampert W. 2001. Maternal control of resting-egg production in *Daphnia*. *Nature* 414:899–901
4. Alleman M, Sidorenko L, McGinnis K, Seshadri V, Dorweiler JE, et al. 2006. An RNA-dependent RNA polymerase is required for paramutation in maize. *Nature* 442:295–8
5. Allen ND, Norris ML, Surani MA. 1990. Epigenetic control of transgene expression and imprinting by genotype-specific modifiers. *Cell* 61:853–61
6. Amado L, Abranches R, Neves N, Viegas W. 1997. Development-dependent inheritance of 5-azacytidine-induced epimutations in triticale: analysis of rDNA expression patterns. *Chromosome Res.* 5:445–50
7. Anderson CM, Lopez F, Zimmer A, Benoit JN. 2006. Placental insufficiency leads to developmental hypertension and mesenteric artery dysfunction in two generations of Sprague-Dawley rat offspring. *Biol. Reprod.* 74:538–44
8. Anway MD, Cupp AS, Uzumcu M, Skinner MK. 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308:1466–69
9. Anway MD, Skinner MK. 2006. Epigenetic transgenerational actions of endocrine disruptors. *Endocrinology* 147:S43–49
10. Armitage JA, Taylor PD, Poston L. 2005. Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. *J. Physiol.* 565:3–8
11. Banks JA, Masson P, Fedoroff N. 1988. Molecular mechanisms in the developmental regulation of the maize *Suppressor-mutator* transposable element. *Genes Dev.* 2:1364–80
12. Barber R, Plumb MA, Boulton E, Roux I, Dubrova YE. 2002. Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. *Proc. Natl. Acad. Sci. USA* 99:6877–82
13. Barber RC, Dubrova YE. 2006. The offspring of irradiated parents, are they stable? *Mutat. Res.* 598:50–60
14. Barker DJP. 1998. *Mothers, Babies and Health in Later Life*. Edinburgh: Churchill Livingstone. ix, 217 pp. 2nd ed.
15. Bateson P. 2001. Fetal experience and good adult design. *Int. J. Epidemiol.* 30:928–34
16. Bean CJ, Schaner CE, Kelly WG. 2004. Meiotic pairing and imprinted X chromatin assembly in *Caenorhabditis elegans*. *Nat. Genet.* 36:100–5
17. Beckmann JS, Estivill X, Antonarakis SE. 2007. Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability. *Nat. Rev. Genet.* 8:639–46
18. Bench GS, Friz AM, Corzett MH, Morse DH, Balhorn R. 1996. DNA and total protamine masses in individual sperm from fertile mammalian subjects. *Cytometry* 23:263–71
19. Bender J, Fink GR. 1995. Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of *Arabidopsis*. *Cell* 83:725–34

20. Benyshek DC, Johnston CS, Martin JF. 2006. Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life. *Diabetologia* 49:1117–19
21. Blatt J, Van Le L, Weiner T, Sailer S. 2003. Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. *J. Pediatr. Hematol. Oncol.* 25:635–36
22. Blewitt ME, Vickaryous NK, Paldi A, Koseki H, Whitelaw E. 2006. Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. *PLoS Genet.* 2:e49
23. Boerke A, Dieleman SJ, Gadella BM. 2007. A possible role for sperm RNA in early embryo development. *Theriogenology* 68(Suppl 1):S147–55
24. Boloker J, Gertz SJ, Simmons RA. 2002. Gestational diabetes leads to the development of diabetes in adulthood in the rat. *Diabetes* 51:1499–506
25. Braun RE, Behringer RR, Peschon JJ, Brinster RL, Palmiter RD. 1989. Genetically haploid spermatids are phenotypically diploid. *Nature* 337:373–76
26. Burge GC, Slater-Jeffries J, Torrrens C, Phillips ES, Hanson MA, Lilliycrop KA. 2007. Dietary protein restriction of pregnant rats in the F_0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F_1 and F_2 generations. *Br. J. Nutr.* 97:435–9
27. Bygren LO, Kaati G, Edvinsson S. 2001. Longevity determined by paternal ancestors' nutrition during their slow growth period. *Acta Biotheor.* 49:53–59
28. Cavalli G, Paro R. 1998. The *Drosophila Fab-7* chromosomal element conveys epigenetic inheritance during mitosis and meiosis. *Cell* 93:505–18
29. Cavalli G, Paro R. 1999. Epigenetic inheritance of active chromatin after removal of the main transactivator. *Science* 286:955–58
30. Chan SW, Henderson IR, Zhang X, Shah G, Chien JS, Jacobsen SE. 2006. RNAi, DRD1, and histone methylation actively target developmentally important non-CG DNA methylation in Arabidopsis. *PLoS Genet.* 2:e83
31. Chan TL, Yuen ST, Kong CK, Chan YW, Chan AS, et al. 2006. Heritable germline epimutation of *MSH2* in a family with hereditary nonpolyposis colorectal cancer. *Nat. Genet.* 38:1178–83
32. Chong S, Vickaryous N, Ashe A, Zamudio N, Youngson N, et al. 2007. Modifiers of epigenetic reprogramming show paternal effects in the mouse. *Nat. Genet.* 39:614–22
33. Cooney CA, Dave AA, Wolff GL. 2002. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J. Nutr.* 132:2393–400
34. Cropley JE, Suter CM, Beckman KB, Martin DI. 2006. Germ-line epigenetic modification of the murine A^{vy} allele by nutritional supplementation. *Proc. Natl. Acad. Sci. USA* 103:17308–12
35. Csaba G, Inczefi-Gonda A. 1998. Transgenerational effect of a single neonatal benzpyrene treatment on the glucocorticoid receptor of the rat thymus. *Hum. Exp. Toxicol.* 17:88–92
36. Cubas P, Vincent C, Coen E. 1999. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401:157–61
37. Das OP, Messing J. 1994. Variegated phenotype and developmental methylation changes of a maize allele originating from epimutation. *Genetics* 136:1121–41
38. Denton KM, Flower RL, Stevenson KM, Anderson WP. 2003. Adult rabbit offspring of mothers with secondary hypertension have increased blood pressure. *Hypertension* 41:634–39
39. Dickinson WJ, Seger J. 1999. Cause and effect in evolution. *Nature* 399:30
40. Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL. 2006. Maternal genistein alters coat color and protects A^{vy} mouse offspring from obesity by modifying the fetal epigenome. *Environ. Health Perspect.* 114:567–72
41. Dorn R, Krauss V, Reuter G, Saumweber H. 1993. The enhancer of position-effect variegation of *Drosophila*, *E(var)3-93D*, codes for a chromatin protein containing a conserved domain common to several transcriptional regulators. *Proc. Natl. Acad. Sci. USA* 90:11376–80
42. Drake AJ, Walker BR, Seckl JR. 2005. Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *Am J. Physiol. Regul. Integr. Comp. Physiol.* 288:R34–38
43. Dubrova YE, Plumb M, Gutierrez B, Boulton E, Jeffreys AJ. 2000. Transgenerational mutation by radiation. *Nature* 405:37

44. Finnegan EJ, Peacock WJ, Dennis ES. 1996. Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proc. Natl. Acad. Sci. USA* 93:8449–54
45. Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D. 2000. The fetal and childhood growth of persons who develop type 2 diabetes. *Ann. Intern. Med.* 133:176–82
46. Forsen T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJ. 1999. Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. *BMJ* 319:1403–7
47. Francis D, Diorio J, Liu D, Meaney MJ. 1999. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* 286:1155–58
48. Galloway LF. 2005. Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytol.* 166:93–99
49. Gazzoli I, Loda M, Garber J, Syngal S, Kolodner RD. 2002. A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the *MLH1* gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor. *Cancer Res.* 62:3925–28
50. Grishok A, Tabara H, Mello CC. 2000. Genetic requirements for inheritance of RNAi in *C. elegans*. *Science* 287:2494–97
51. Groothuis TG, Muller W, von Engelhardt N, Carere C, Eising C. 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neurosci. Biobehav. Rev.* 29:329–52
52. Hadchouel M, Farza H, Simon D, Tiollais P, Pourcel C. 1987. Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with de novo methylation. *Nature* 329:454–56
53. Hales BF, Crosman K, Robaire B. 1992. Increased postimplantation loss and malformations among the F2 progeny of male rats chronically treated with cyclophosphamide. *Teratology* 45:671–78
54. Henderson IR, Jacobsen SE. 2007. Epigenetic inheritance in plants. *Nature* 447:418–24
55. Herbst AL, Ulfelder H, Poskanzer DC. 1971. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N. Engl. J. Med.* 284:878–81
56. Hitchins M, Williams R, Cheong K, Halani N, Lin VA, et al. 2005. *MLH1* germline epimutations as a factor in hereditary nonpolyposis colorectal cancer. *Gastroenterology* 129:1392–99
57. Hitchins MP, Wong JJ, Suthers G, Suter CM, Martin DI, et al. 2007. Inheritance of a cancer-associated *MLH1* germ-line epimutation. *N. Engl. J. Med.* 356:697–705
58. Honys D, Twell D. 2004. Transcriptome analysis of haploid male gametophyte development in *Arabidopsis*. *Genome Biol.* 5:R85
59. Ims RA. 1990. Determinants of natal dispersal and space use in gray-sided voles, *Clethrionomys rufocanus*: A combined field and laboratory experiment. *Oikos* 57:106–13
60. Ingouff M, Hamamura Y, Gourgues M, Higashiyama T, Berger F. 2007. Distinct dynamics of HISTONE3 variants between the two fertilization products in plants. *Curr. Biol.* 17:1032–37
61. Jackson AA, Dunn RL, Marchand MC, Langley-Evans SC. 2002. Increased systolic blood pressure in rats induced by a maternal low-protein diet is reversed by dietary supplementation with glycine. *Clin. Sci.* 103:633–39
62. Jacobsen SE, Meyerowitz EM. 1997. Hypermethylated *SUPERMAN* epigenetic alleles in *Arabidopsis*. *Science* 277:1100–3
63. Kaati G, Bygren LO, Edvinsson S. 2002. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *Eur. J. Hum. Genet.* 10:682–88
64. Kaati G, Bygren LO, Pembrey M, Sjostrom M. 2007. Transgenerational response to nutrition, early life circumstances and longevity. *Eur. J. Hum. Genet.* 15:784–90
65. Kakutani T, Munakata K, Richards EJ, Hirochika H. 1999. Meiotically and mitotically stable inheritance of DNA hypomethylation induced by *ddm1* mutation of *Arabidopsis thaliana*. *Genetics* 151:831–38
66. Kantheti P, Diaz ME, Peden AE, Seong EE, Dolan DF, et al. 2003. Genetic and phenotypic analysis of the mouse mutant mh2J, an Ap3d allele caused by IAP element insertion. *Mamm. Genome* 14:157–67
67. Kearns M, Preis J, McDonald M, Morris C, Whitelaw E. 2000. Complex patterns of inheritance of an imprinted murine transgene suggest incomplete germline erasure. *Nucleic Acids Res.* 28:3301–9
68. Lacey EP. 1996. Parental effects in *Plantago lanceolata* L. I. A growth chamber experiment to examine pre- and postzygotic temperature effects. *Evolution* 50:865–78
69. Lane N, Dean W, Erhardt S, Hajkova P, Surani A, et al. 2003. Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* 35:88–93

70. Li S, Washburn KA, Moore R, Uno T, Teng C, et al. 1997. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res.* 57:4356–59
71. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. 2005. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J. Nutr.* 135:1382–86
72. Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ. 2000. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. *J. Neuroendocrinol.* 12:5–12
73. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, et al. 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 277:1659–62
74. Lumey LH. 1992. Decreased birthweights in infants after maternal in utero exposure to the Dutch famine of 1944–1945. *Paediatr. Perinat. Epidemiol.* 6:240–53
75. Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszkowski J. 2007. Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell* 130:851–62
76. Matzke AJ, Matzke MA. 1998. Position effects and epigenetic silencing of plant transgenes. *Curr. Opin. Plant Biol.* 1:142–48
77. Mayr E. 1982. *The Growth of Biological Thought: Diversity, Evolution, and Inheritance*. Cambridge, MA/London: Belknap Press of Harvard Univ. Press. 974 pp.
78. Mayr E, Provine WB. 1980. *The Evolutionary Synthesis: Perspectives on the Unification of Biology*. Cambridge, MA/ London: Harvard Univ. Press. 487 pp.
79. Melquist S, Bender J. 2003. Transcription from an upstream promoter controls methylation signaling from an inverted repeat of endogenous genes in *Arabidopsis*. *Genes Dev.* 17:2036–47
80. Miao SL, Bazzaz FA, Primack RB. 1991. Persistence of maternal nutrient effects in *Plantago major*: The third generation. *Ecology* 72:1634–42
81. Miyakura Y, Sugano K, Konishi F, Fukayama N, Igarashi S, et al. 2003. Methylation profile of the *MLH1* promoter region and their relationship to colorectal carcinogenesis. *Genes Chromosomes Cancer* 36:17–25
82. Molinier J, Ries G, Zipfel C, Hohn B. 2006. Transgeneration memory of stress in plants. *Nature* 442:1046–49
83. Morgan HD, Sutherland HG, Martin DI, Whitelaw E. 1999. Epigenetic inheritance at the agouti locus in the mouse. *Nat. Genet.* 23:314–18
84. Morse HC 3rd, Yetter RA, Stimpfling JH, Pitts OM, Fredrickson TN, Hartley JW. 1985. Greying with age in mice: Relation to expression of murine leukemia viruses. *Cell* 41:439–48
85. Mousseau TA, Fox CW, eds. 1998. *Maternal Effects as Adaptations*. New York/Oxford: Oxford Univ. Press. 375 pp.
86. Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, et al. 2006. LHP1, the *Arabidopsis* homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of *FLC*. *Proc. Natl. Acad. Sci. USA* 103:5012–17
87. Namekawa SH, Park PJ, Zhang LF, Shima JE, McCarrey JR, et al. 2006. Postmeiotic sex chromatin in the male germline of mice. *Curr. Biol.* 16:660–67
88. Newbold RR, Liehr JG. 2000. Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. *Cancer Res.* 60:235–37
89. Newbold RR, Padilla-Banks E, Jefferson WN. 2006. Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinology* 147:S11–17
90. Newnham JP, Evans SF, Godfrey M, Huang W, Ikegami M, Jobe A. 1999. Maternal, but not fetal, administration of corticosteroids restricts fetal growth. *J. Matern. Fetal Med.* 8:81–87
91. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. 1998. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J. Clin. Invest.* 101:2174–81
92. Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz SA. 2002. Spermatozoal RNA profiles of normal fertile men. *Lancet* 360:772–77

93. Palmer DK, O'Day K, Margolis RL. 1990. The centromere specific histone CENP-A is selectively retained in discrete foci in mammalian sperm nuclei. *Chromosoma* 100:32–36
94. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, et al. 2006. Sex-specific, male-line transgenerational responses in humans. *Eur. J. Hum. Genet.* 14:159–66
95. Pepling ME, Spradling AC. 1998. Female mouse germ cells form synchronously dividing cysts. *Development* 125:3323–28
96. Pina C, Pinto F, Feijo JA, Becker JD. 2005. Gene family analysis of the *Arabidopsis* pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. *Plant Physiol.* 138:744–56
97. Platenkamp GJ, Shaw RG. 1993. Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. *Evolution* 47:540–55
98. Popova NV. 1989. Transgenerational effect of orthoaminoasotoluol in mice. *Cancer Lett.* 46:203–6
99. Queitsch C, Sangster TA, Lindquist S. 2002. Hsp90 as a capacitor of phenotypic variation. *Nature* 417:618–24
100. Rakyan VK, Chong S, Champ ME, Cuthbert PC, Morgan HD, et al. 2003. Transgenerational inheritance of epigenetic states at the murine $Axin^{Fu}$ allele occurs after maternal and paternal transmission. *Proc. Natl. Acad. Sci. USA* 100:2538–43
101. Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F. 2006. RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. *Nature* 441:469–74
102. Riddle NC, Richards EJ. 2005. Genetic variation in epigenetic inheritance of ribosomal RNA gene methylation in *Arabidopsis*. *Plant J.* 41:524–32
103. Roemer I, Reik W, Dean W, Klose J. 1997. Epigenetic inheritance in the mouse. *Curr. Biol.* 7:277–80
104. Rougier N, Bourc'his D, Gomes DM, Niveleau A, Plachot M, et al. 1998. Chromosome methylation patterns during mammalian preimplantation development. *Genes. Dev.* 12:2108–13
105. Rutherford SL, Lindquist S. 1998. Hsp90 as a capacitor for morphological evolution. *Nature* 396:336–42
106. Schmid B, Dolt C. 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima*. *Evolution* 48:1525–49
107. Schmitt J, Gamble SE. 1990. The effect of distance from the parental site on offspring performance and inbreeding depression in *Impatiens capensis*: A test of the local adaptation hypothesis. *Evolution* 44:2022–30
108. Schmitt J, Niles J, Wulff R. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. *Am. Nat.* 139:451–66
109. Seki Y, Yamaji M, Yabuta Y, Sano M, Shigeta M, et al. 2007. Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. *Development* 134:2627–38
110. Shi S, Calhoun HC, Xia F, Li J, Le L, Li WX. 2006. JAK signaling globally counteracts heterochromatic gene silencing. *Nat. Genet.* 38:1071–76
111. Sollars V, Lu X, Xiao L, Wang X, Garfinkel MD, Ruden DM. 2003. Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nat. Genet.* 33:70–74
112. Soppe WJ, Jacobsen SE, Alonso-Blanco C, Jackson JP, Kakutani T, et al. 2000. The late flowering phenotype of *fwa* mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. *Mol. Cell* 6:791–802
113. Spergel G, Levy LJ, Goldner MG. 1971. Glucose intolerance in the progeny of rats treated with single subdiabetogenic dose of alloxan. *Metabolism* 20:401–13
114. Spradling AC. 1993. Developmental genetics of oogenesis. In *The Development of Drosophila melanogaster*, ed. M Bates, A Martinez-Arias, pp. 1–70. New York: Cold Spring Harbor Lab. Press
115. Stam M, Belele C, Dorweiler JE, Chandler VL. 2002. Differential chromatin structure within a tandem array 100 kb upstream of the maize *b1* locus is associated with paramutation. *Genes Dev.* 16:1906–18
116. Stanner SA, Bulmer K, Andres C, Lantseva OE, Borodina V, et al. 1997. Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. *Br. Med. J.* 315:1342–48

117. Stein AD, Lumey LH. 2000. The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: The Dutch Famine Birth Cohort Study. *Hum. Biol.* 72:641–54
118. Stewart RJ, Sheppard H, Preece R, Waterlow JC. 1980. The effect of rehabilitation at different stages of development of rats marginally malnourished for ten to twelve generations. *Br. J. Nutr.* 43:403–12
119. Stokes TL, Kunkel BN, Richards EJ. 2002. Epigenetic variation in *Arabidopsis* disease resistance. *Genes Dev.* 16:171–82
120. Stratton DA. 1989. Competition prolongs expression of maternal effects in seedlings of *Erigeron annuus* (Asteraceae). *Am. J. Bot.* 76:1646–53
121. Sultan SE. 1996. Phenotypic plasticity for offspring traits in *Polygonum persicaria*. *Ecology* 77:1791–807
122. Suter CM, Martin DI, Ward RL. 2004. Germline epimutation of *MLH1* in individuals with multiple cancers. *Nat. Genet.* 36:497–501
123. Sutherland HG, Kearns M, Morgan HD, Headley AP, Morris C, et al. 2000. Reactivation of heritably silenced gene expression in mice. *Mamm. Genome* 11:347–55
124. Swain JL, Stewart TA, Leder P. 1987. Parental legacy determines methylation and expression of an autosomal transgene: a molecular mechanism for parental imprinting. *Cell* 50:719–27
125. Torrens C, Brawley L, Anthony FW, Dance CS, Dunn R, et al. 2006. Folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction. *Hypertension* 47:982–87
126. Tschirren B, Fitze PS, Richner H. 2007. Maternal modulation of natal dispersal in a passerine bird: An adaptive strategy to cope with parasitism? *Am. Nat.* 169:87–93
127. van der Heijden GW, Derijck AA, Ramos L, Giele M, van der Vlag J, de Boer P. 2006. Transmission of modified nucleosomes from the mouse male germline to the zygote and subsequent remodeling of paternal chromatin. *Dev. Biol.* 298:458–69
128. Vongs A, Kakutani T, Martienssen RA, Richards EJ. 1993. *Arabidopsis thaliana* DNA methylation mutants. *Science* 260:1926–28
129. Waterland RA, Jirtle RL. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Cell. Biol.* 23:5293–300
130. Waterland RA, Travisano M, Tahiliani KG. 2007. Diet-induced hypermethylation at *agouti viable yellow* is not inherited transgenerationally through the female. *FASEB J.* 21:3380–5
131. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, et al. 2004. Epigenetic programming by maternal behavior. *Nat. Neurosci.* 7:847–54
132. Weaver IC, D'Alessio AC, Brown SE, Hellstrom IC, Dymov S, et al. 2007. The transcription factor nerve growth factor-inducible protein a mediates epigenetic programming: Altering epigenetic marks by immediate-early genes. *J. Neurosci.* 27:1756–68
133. Weaver IC, Grant RJ, Meaney MJ. 2002. Maternal behavior regulates long-term hippocampal expression of BAX and apoptosis in the offspring. *J. Neurochem.* 82:998–1002
134. Weaver IC, Meaney MJ, Szyf M. 2006. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc. Natl. Acad. Sci. USA* 103:3480–85
135. Wolff GL, Kodell RL, Moore SR, Cooney CA. 1998. Maternal epigenetics and methyl supplements affect *agouti* gene expression in A^{vy}/a mice. *FASEB J.* 12:949–57
136. Xing Y, Shi S, Le L, Lee CA, Silver-Morse L, Li WX. 2007. Evidence for transgenerational transmission of epigenetic tumor susceptibility in *Drosophila*. *PLoS Genet.* 3:1598–606
137. Yu HJ, Hogan P, Sundaresan V. 2005. Analysis of the female gametophyte transcriptome of *Arabidopsis* by comparative expression profiling. *Plant Physiol.* 139:1853–69
138. Zalenskaya IA, Bradbury EM, Zalensky AO. 2000. Chromatin structure of telomere domain in human sperm. *Biochem. Biophys. Res. Commun.* 279:213–18
139. Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, et al. 2005. Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. *J. Physiol.* 566:225–36

140. Zhang TY, Bagot R, Parent C, Nesbitt C, Bredy TW, et al. 2006. Maternal programming of defensive responses through sustained effects on gene expression. *Biol. Psychol.* 73:72–89
141. Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, et al. 2006. Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell* 126:1189–201
142. Zhao Y, Li Q, Yao C, Wang Z, Zhou Y, et al. 2006. Characterization and quantification of mRNA transcripts in ejaculated spermatozoa of fertile men by serial analysis of gene expression. *Hum. Reprod.* 21:1583–90

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