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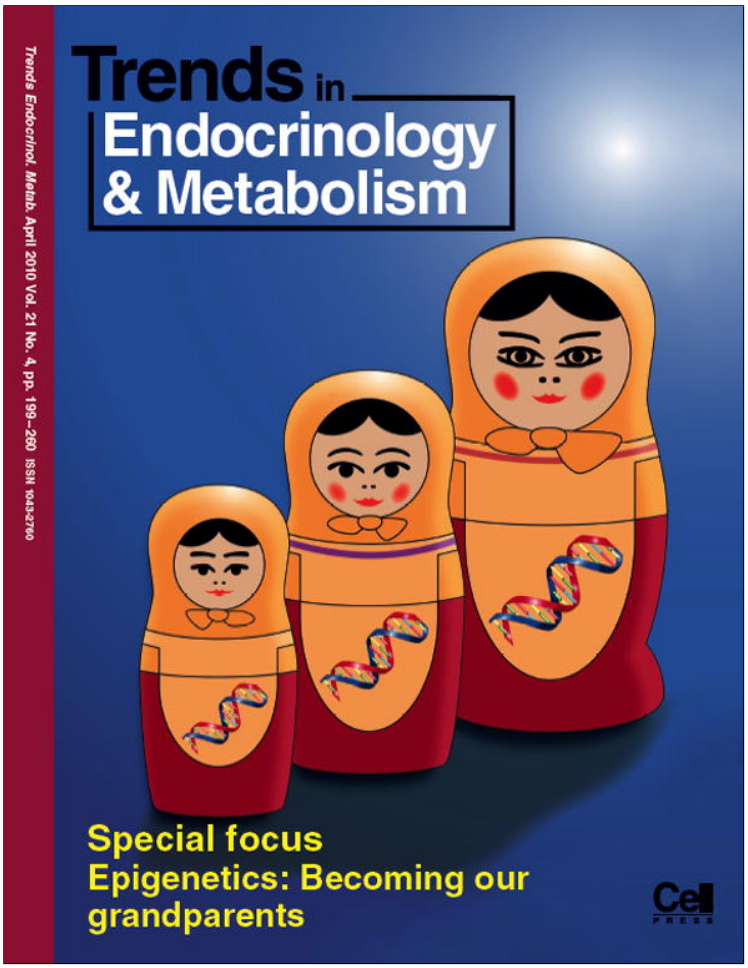

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Review

Epigenetic transgenerational actions of environmental factors in disease etiology

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The ability of environmental factors to promote a phenotype or disease state not only in the individual exposed but also in subsequent progeny for successive generations is termed transgenerational inheritance. The majority of environmental factors such as nutrition or toxicants such as endocrine disruptors do not promote genetic mutations or alterations in DNA sequence. However, these factors do have the capacity to alter the epigenome. Epimutations in the germline that become permanently programmed can allow transmission of epigenetic transgenerational phenotypes. This review provides an overview of the epigenetics and biology of how environmental factors can promote transgenerational phenotypes and disease.

The current paradigm for disease etiology is that the presence of a genetic mutation, polymorphism or chromosomal abnormality promotes disease. Although this is a crucial component of disease, the environment is an equally important consideration in disease etiology (Figure 1). Because the genome is evolutionarily and chemically stable, the ability of the environment to influence or promote disease does not generally involve DNA mutations. Therefore, environmental factors must generally regulate genome activity independent of DNA sequence manipulation (e.g. epigenetics). An additional consideration for environmental influences on disease etiology is the developmental stage of exposure. Exposures during a crucial time of development can alter genome activity associated with the differentiation programming of cells or organ systems. This altered program and gene expression profile can then promote an abnormal physiology and disease at the later adult stage of development.

A large number of epidemiology studies suggest that the environment is a major factor in disease etiology [1,2]. Examples include phenomena such as the regional differences in disease frequency, the low frequency of the genetic component of disease, the increase in the majority of specific disease frequencies, the variability in disease frequency between identical twins, and the large number of environmental exposures that promote disease. This review focuses on how environmental factors promote adult-onset disease transgenerationally.

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Environmental factors and disease

Epidemiology research suggests significant environmental effects on disease. Each geographic region around the world generally has a distinct disease frequency. For example, some regions have high rates of prostate disease and low rates of stomach disease (North America), whereas others have low rates of prostate disease and high rates of stomach disease (eastern Asia) [3,4]. If a person is moved early in life from one region to the other, they often develop the new region's disease frequencies. Interestingly, when identical twins develop in different geographic regions, they also have different disease frequencies [5]. Therefore, although the genetics is nearly identical, disease development is different, suggesting an environmental influence [6]. Another example is the dramatic and rapid increase in nearly all disease frequencies over the past several decades that cannot be explained through genetics alone. There are also a large number of environmental compounds and toxicants that have been shown to promote disease, but most do not alter the DNA sequence [7]. Therefore, environmental factors are crucial in the etiology of disease.

Although numerous environmental factors influence and promote adult-onset disease (such as nutrition and stress), this review focuses on endocrine disruptors, as this group of environmental compounds is one of the largest people are exposed to daily in society. Endocrine disruptors are environmental chemicals that affect the function of the endocrine system by mimicking or blocking the actions of hormones, altering hormone signaling or disrupting hormone production [8]. Endocrine disruption can have profound consequences because of the crucial role hormones have in development.

Several disease states are promoted by endocrine disruptors (Table 1). Many endocrine disruptors with reproductive hormone actions (e.g. estrogen or androgen) influence reproduction and fertility including bisphenol-A (BPA), dichlorodiphenyltrichloroethane (the insecticide DDT) and vinclozolin. Activation of the male and female reproductive systems at an inappropriate time during development by endocrine disruptor chemicals can alter normal physiology [9]. For example, prenatal exposure to diethylstilbestrol (DES) produces several developmental abnormalities in the male mouse reproductive tract and increases tumor incidence [10]. Embryonic exposure to the pesticide methoxychlor during the period of sex determination affects the cellular composition of the embryonic testis, and germ cell number and survival [11]. Embryonic

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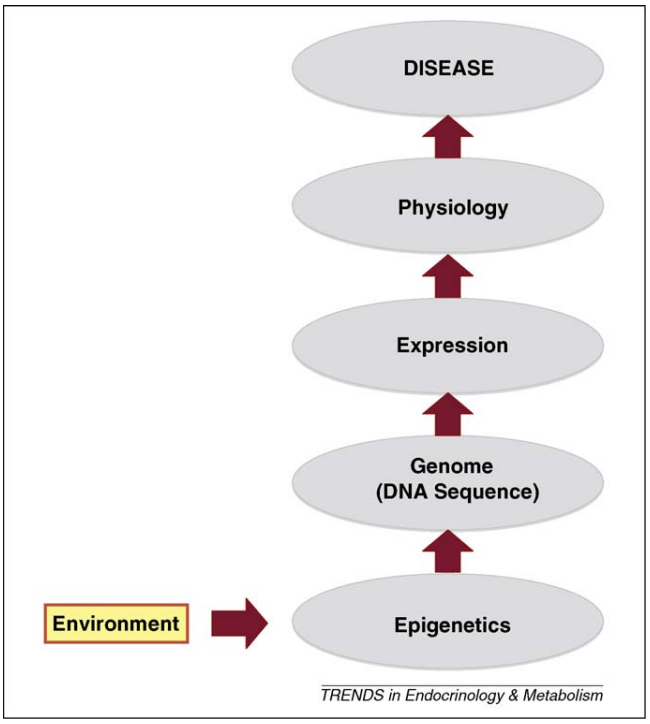

Figure 1. Proposed etiology of how the environment effects disease. The cascade of molecular and physiological processes following an environmental exposure to promote disease is shown.

testicular cord formation is also affected when embryos are exposed *in vitro* to vinclozolin. Transient *in utero* exposure to vinclozolin increases apoptotic germ cell numbers in the testis of pubertal and adult animals, which correlates with reduced sperm motility and number in the adult [12]. *In utero* exposure to the plastic-derived compounds phthalates also disrupts differentiation of androgen-dependent tissues in male rat offspring [13]. A more recent example of an endocrine disruptor is the plastic component BPA, which acts as an estrogenic compound causing numerous pathologies including prostate cancer in low doses [14]. Other examples include the plant-derived estrogenic compounds (phytoestrogens) such as genistein, which influence several reproductive organs [15,16]; aflatoxin-contaminated food, which has been correlated with the incidence of liver cancer in Asia and Africa [17]; tobacco, which contains cadmium, an estrogenic endocrine disruptor (18), and whose use can cause reproductive problems in addition to carcinogen-induced lung cancer. Heterocyclic amines in well-cooked meat products can result in cancer of the colon, breast and stomach in consumers [19]. Abnormalities in mouse testicular Leydig cells are induced by chronic low dose exposure to arsenic [20]. Estrogen receptor-α promoter hypomethylation might play a role in induction of hepatocellular carcinoma by arsenic exposure *in utero* [21]. Therefore, it is apparent that a large number of environmental compounds have endocrine disruptor activity. How an early life exposure to an endocrine disruption can promote an adult-onset disease, long after the compound is removed, is presumed to at least partly involve the epigenetic mechanisms reviewed below.

Epigenetics

Although the history and definition of epigenetics has evolved (Box 1), the majority of the molecular elements of epigenetic regulatory processes have only been recently elucidated [1]. The first epigenetic molecular factor identified was DNA methylation in the 1970s [22] (Table 2). Significant focus was put on DNA methylation with the analysis of X chromosome inactivation and imprinted genes in the late 1980s and early 1990s [23]. The next epigenetic element identified was histone modifications in the mid 1990s and the appreciation of chromatin structure in the regulation of the genome [24]. This was followed by the identification of non-coding RNA around 2000 and the first whole epigenome analysis in 2005 [25] (Table 2). Epigenetic processes are likely to be expanded in the future. For example, the recent identification of hydroxymethylcytosine residues in the brain is a new epigenetic mark whose function remains to be elucidated [26]. These epigenetic processes are equally important in regulating genome activity (i.e. gene expression) and DNA sequence (i.e. genetics).

A special category of genes called imprinted genes are subject to epigenetic programming and can be influenced by environmental exposures. For example, *in vitro* treatment of preimplantation embryos with the contaminant 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin alters DNA methylation in the *H19* and *IGF-2* imprinted genes [27]. From an epigenetic perspective, imprinted genes are a special class of genes because they have relatively unchanged DNA methylation patterns over generations and are not

Table 1. Common endocrine disruptors and their actions

Endocrine disruptor	Effect	Reference
DDT	Reproductive failure	[110]
Phytoestrogens (e.g. genistein)	Impaired fertility, reproductive effects, breast cancer protection	[15,16]
DES	Vaginal cancer in humans Developmental toxicity in hamsters	[111–113]
Dicofol	Abnormal ovarian follicles, high plasma estrogen levels	[114]
BPA	Prostate cancer	[14,115]
Aflatoxin	Liver cancer	[17]
Cadmium	Lung cancer, reproductive problems	[18]
Heterocyclic amines	Cancer of colon, stomach and breast	[19]
Arsenic	Liver cancer	[21]
Dioxins (TCDD)	Mammary tumor	[116]
Vinclozolin	Impaired male fertility	[33]
Methoxychlor	Impaired male fertility	[117]
Phthalates	Impairs male reproductive tract and testis	[13]

TCDD, 2,3,7,8-Tetrachlorodibenzo-p-dioxin .

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Box 1. Epigenetics

The term 'epigenetics' was coined by Conrad Waddington in the 1940s. Waddington integrated the new knowledge about genes and genetics to embryology. The study of embryological growth and differentiation was commonly known as 'epigenesis', a concept that had been around since Aristotelian times. The integration of the concepts of epigenesis and genetics gave origin to the term 'epigenetics' [101,102]. Waddington's goal with epigenetics was to provide insight into gene–environment interactions that influence development and embryology [101–103]. Pioneering epigenetic experiments from Waddington on *Drosophila* demonstrated that a temperature shock 17–23 hours after puparium formation produced cross veinless wings in flies. Flies with this phenotype were culled from the population and only those showing normal wings were used to carry on the line. After an expected initial reduction of the cross wingless phenotype in the population, it surprisingly recurred after generation 16 [104]. This phenotype was considered a 'genetic assimilation' and dealt with environmental exposures early in development with subsequent consequences on phenotypic inheritance.

The definition of epigenetics has evolved with greater clarity of the molecular mechanisms involved and a better understanding of genetic phenomena. The initial definition of Waddington focused on gene–environment interactions but had no molecular insights to consider [102]. In 1990, Holliday defined epigenetics as 'the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms'. His definition rescues Waddington's original meaning of developmental biology, although it does not differentiate between the action of what we currently know as epigenetic mechanisms and the action of genetic regulators of gene expression such as transcription factors [105]. Another early definition by Riggs and colleagues states that epigenetics is 'the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence' [106]. However, the term heritable is generally used in reference to generational inheritance and is not associated with growth of cells or tissues. Perhaps a more direct term would be 'mitotically stable'. A more recent definition focuses on molecular elements that influence chromatin, independent of DNA sequence. Bird defines epigenetics as the 'structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states' [107]. Because there are several epigenetic elements that do not fit into this definition such as non-coding RNA and minor modifications of histones and DNA methylation of promoters, this definition appears insufficiently global to encompass all of epigenetics. Therefore, we propose a definition that is more global and encompasses all molecular elements and includes the use of the term 'epi' for 'around DNA'. Thus, we define epigenetics as '**molecular factors and processes around DNA that are mitotically stable and regulate genome activity independent of DNA sequence**'.

affected by the overall reset in methylation patterns that occur early in development [28]. Imprinted genes carry a molecular memory of their parent of origin allele acquired early in the germline [29]. This molecular memory is associated with differential methylation patterns between the two alleles, which affect monoallelic gene expression [30]. These allelic differences in methylation are defined in the developing embryo during the establishment of germline development [28]. Methylation of imprinted genes initiated during germline development can be completed after fertilization [28,31]. Some imprinted genes remain imprinted throughout the organism's life; however, a group of them are imprinted in specific tissues in a temporally specific manner [32]. Interestingly, if external agents alter DNA methylation in these imprinted genes or induce new methylation sites during crucial periods of their establishment, such changes can persist transgenerationally [33,34] (Figure 2). This heritable transmission of environmentally induced phenotypes is referred to as transgenerational inheritance [1,35].

From a human health perspective, a number of disease states exist that have an epigenetic origin. Several diseases and syndromes have abnormal DNA methylation or imprinted gene sites leading to various pathologies [32]. These include Silver–Russell syndrome [36], Beckwith–Weidemann syndrome [37], and Angelman and Prader–Willi syndromes [38]. Another epigenetic disease caused by abnormal DNA methylation of the X-chromosome is fragile X syndrome [39]. Several brain disorders such as autism, schizophrenia and Rett's syndrome also appear to have major epigenetic components [39–41]. Cancer also has an epigenetic component to regulate genome stability, and is associated with transformation and disease phenotype [42,43]. A growing list of diseases with an epigenetic component suggests that epigenetics will have a crucial role in disease etiology for many disease states (Figure 1).

Table 2. History of epigenetics

Year(s)	Event
1940s	Conrad Waddington defined epigenetics as environment–gene interactions that induce developmental phenotypes
1975	Holliday and Pugh identify DNA methylation
1988	X-chromosome inactivation and DNA methylation
1990s	Imprinted genes, allelic expression and DNA methylation
1995	Histone modifications and chromatin structure
2000s	Small non-coding RNAs
2005	Epigenome mapping

Epigenetics and environmental factors

Initial observations of how the environment can influence epigenetics and phenotype were shown in plants [44]. In animals, many examples associate environmental influences to epigenetic changes. Epigenetic influences have been observed with environmental compounds, nutritional factors [45,46] such as methyl donors (e.g. folate) [47,48], inorganic contaminants such as arsenic [20,21], airborne polycyclic aromatic hydrocarbons [49], drugs such as cocaine [50], endocrine disruptors such as BPA [14,51,52], phytoestrogens [53,54], and chemicals used as fungicides [33] or pesticides [55] (Table 1). Some studies have also demonstrated behavioral effects on DNA methylation including maternal effects on nursing behavior [56] or depression [57]. Therefore, numerous examples of environmental factors have been shown to alter the epigenome.

Holliday initially proposed a link between hormone action and establishment of DNA methylation in mammalian embryos. He proposed that maternal effects of teratogens might disrupt the normal distribution of DNA methylation in a developing embryo, leading to developmental abnormalities or defects that would appear in successive generations [58]. McLachlan and collaborators [59] proposed that exposure to environmental endocrine disrupting chemicals during early development affects adult stages, potentially involving gene imprinting and leading to persistent genetic change at the level of DNA

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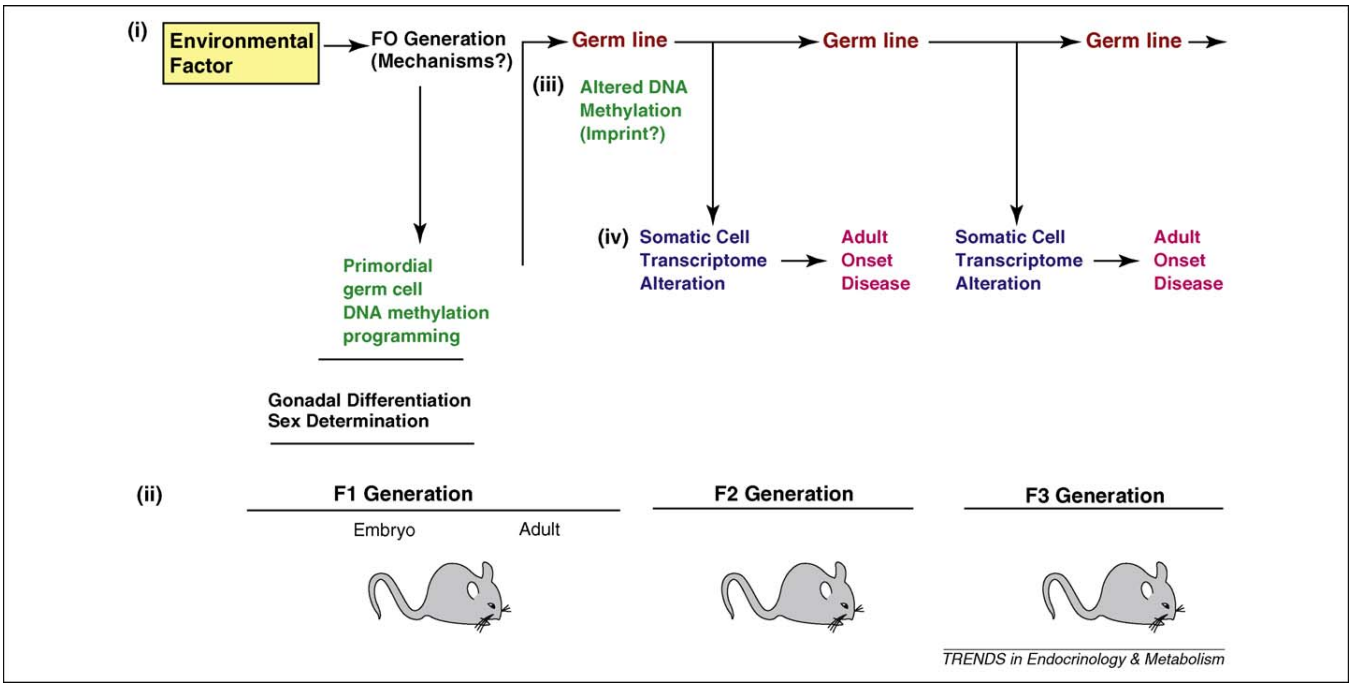

Figure 2. Role of the germline in epigenetic transgenerational inheritance. **(i)** An environmental factor acts on the F0 generation gestating female to influence **(ii)** the developing F1 generation fetus and alter gonadal development to reprogram the primordial germ cell DNA methylation. **(iii)** This altered DNA methylation in the germline becomes permanently programmed, similar to an imprinted-like gene, and is transferred through the germline to subsequent generations. The embryo generated from this germline starts with an altered epigenome that **(iv)** affects developing somatic cells and tissues to have an altered transcriptome. This altered somatic cell transcriptome can then promote adult-onset disease associated with the transgenerational phenotype.

methylation. The first experimental evidence that endocrine-disrupting chemicals produce epigenetic changes came from experiments in which neonatal exposure to DES produced abnormalities in the demethylation of the lactoferrin promoter [60].

A classic model for studying endocrine and nutritional epigenetic effects is the Agouti mouse, which consists of detecting changes in methylation of the A^{vy} allele. Methylation in this meta-stable allele correlates with changes in coat color, which shifts from yellow-agouti to yellow by decreasing DNA methylation in the intracisternal A particle retrotransposon upstream of the *agouti* gene [47]. Maternal methyl donor (i.e. folate) consumption leads to changes in the coat color of offspring, which correlates with alteration in methylation of the A^{vy} allele [47]. Interestingly, transgenerational exposure of $A^{vy/a}$ mice to an *ad libitum* diet produces amplification of obesity, an effect that is suppressed when the diet is methyl-supplemented with extra folate [61]. Maternal BPA treatment also decreases the offspring's CpG DNA methylation in this metastable epiallele, resulting in a change in coat color [51]. Dietary supplementation of BPA or genistein treatments with methyl donors inhibits the hypomethylating effect of BPA or genistein, shifting the coat color of heterozygous yellow–agouti offspring toward pseudo-agouti, which is the same coat color pattern observed in controls [51]. This mouse model has clearly established the ability of environmental factors to influence epigenetics to promote phenotypic changes later in development.

Endocrine disruptors have the ability to alter the DNA methylation patterns of key genes that produce related transcriptional changes [1,7,62,63]. Administration of the plant-derived endocrine-disrupting phytoestrogens, coumestrol and equol, to newborn mice enhances DNA methylation to inactivate the proto-oncogene *H-ras* [64]. DNA methylation patterns were altered in 8-week-old mice that consumed high doses of the phytoestrogen genistein [65]. Recently, gender-specific changes in *Acta1* methylation have been shown to occur as a response to dietary isoflavones in mice [54]. Environmental compounds with endocrine disruptor activity tested for epigenetic effects include the fungicide vinclozolin, the plastic residue BPA, and the pharmacological compound DES (Table 1). Exposure to environmentally relevant doses of BPA during the neonatal developmental period in rats produces DNA methylation changes associated with carcinogenic processes [14]. Maternal exposure to BPA has also been shown to alter methylation in the fetal mouse forebrain [52] and to produce changes in behavior responses in the offspring [66]. These findings correlate with other studies showing epigenetic changes resulting from endocrine disruptor exposure, which affected aspects of neuroendocrine systems [67] and behavioral neuroendocrinology [68–70]. Changes in methylation also explain the reappearance of increased susceptibility for tumor formation in F2 generation mice after developmental exposure to DES [71,72]. Therefore, the actions of a number of endocrine disruptors involve alterations in epigenetic processes.

The implication of these environmentally induced epigenetic effects in evolutionary biology is also a topic of interest. An assumption of new-Darwinian theory is that evolution proceeds based on random DNA sequence mutations and that the environment is not able to alter the occurrence or frequency of these mutations [73]. Epigenetics offers an alternative view regarding the molecular mechanism involved. For example, DNA methylation of

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CpG sites increases the rate of mutations of methylated cytosines by an order of magnitude [74]. Therefore, in the event of DNA methylation patterns being altered by an environmental stimulus, these CpG sites will be more prone to undergo mutations than will sites that are not methylated [34]. If this is transgenerationally maintained in a population, this is an epigenetically controlled mutation frequency. This bias in the mutation rates over generations is environmentally induced. Simulations in the evolution of the *BRCA1* gene show that methylation-biased derived mutations are a feasible process [75]. Recent studies highlight the role of environmental compounds on epigenetic mechanisms from an evolutionary and ecological perspective [34,54,69,76].

Epigenetic transgenerational phenomena

Because the germline is required for transmitting genetic information between generations, a permanent epigenetic modification in it can result in transgenerational phenomena (Box 2). Epigenetic programming of the germline occurs during the migration of the primordial germ cells in the embryo. The migrating primordial germ cells in the genital ridge undergo an erasure of methylation of the DNA during migration and colonize the early bipotential gonad before gonadal sex determination [77,78]. Once gonadal sex determination is initiated, the primordial germ cells develop female or male germ cell lineage and remethylate the DNA in a male- or female-specific manner. Therefore, the germ cell epigenetic programming during gonadal sex determination is a period sensitive to environmental factors [77] (Box 3).

Although there are alterations in the male and female germline epigenomes (i.e. DNA methylation) during gametogenesis in the adult gonads [79], the embryonic period of gonadal sex determination is the most sensitive to environmental insults. During spermatogenesis, the male germ cell replaces the majority of histones with protamines, DNA condensation occurs to eliminate chromatin structure, and the genome is silenced for reduced expression of non-coding RNAs [80]. Although a small percentage of histones are maintained in developmentally important loci [81], the role of histones in sperm remains to be established. Therefore, the primary epigenetic process that is transmitted through the male germline is DNA methylation.

Box 2. Definition of transgenerational phenotype

Most of the actions of environmental factors or toxicants involve direct exposure of somatic tissues that are important for the exposed individual's disease, but will not be transmitted to the next generation. By contrast, transgenerational phenotypes and toxicology by definition excludes direct exposure and must be transmitted through several generations [1,108]. For example, exposure of a gestating female provides direct exposure of the F0 generation female, the F1 generation embryo, and the germline that will generate the F2 generation [108]. Therefore, a phenotype in the F3 generation is required to have a transgenerational phenomenon or phenotype. The effects observed in the F0 and F1 generations are caused by direct exposure, as is that in the F2 generation germline [1,108]. The ability of a direct exposure to influence several generations is defined as a multiple generation phenotype and not a transgenerational phenotype. By contrast, a transgenerational phenotype requires the absence of a direct exposure and transmission of the phenotype to at least the F3 generation [108].

Box 3. Germ cell developmental epigenetics

An important factor to consider with a transgenerational phenotype is the action of environmental factors on the germline and gonadal development. During embryonic development in mammalian species, the primordial germ cells migrate down the genital ridge towards the developing gonad before sex determination occurs [77,78,109]. At the time of gonadal sex determination, the germ cell develops into a male or female germ cell lineage at the initial stages of gonadal sex determination. The female germline then enters meiosis in the developing embryonic ovary, whereas male germ cells continue to proliferate until immediately before birth and then resume proliferation after birth until puberty [77,78,109]. In the adult, the female germline undergoes oogenesis during follicle development to generate oocytes. The male germline, in turn, develops from spermatogonial stem cells and undergoes spermatogenesis for the production of spermatozoa in the testis. The crucial period for epigenetic regulation and modification of the germline is during the period of primordial germ cell migration and gonadal sex determination. The permanent alteration in the epigenetic programming of the germline appears to be the mechanism involved in the transgenerational phenotype [1,33].

One of the first studies to demonstrate the ability of an environmental factor to modify the epigenetic programming of the male germline used the endocrine disruptor vinclozolin. When embryonic rats were exposed through maternal administration to vinclozolin, an anti-androgenic environmental endocrine disruptor, during gonadal sex determination, adult-onset disease occurred in the first generation and persisted for four subsequent generations [33] (Figure 2). This phenomenon was found to be caused by male germline changes in DNA methylation, which resulted in heritable changes in transcription in several tissues, such as the testis [82], brain [70] and prostate [83]. The pathology of adult-onset disease from vinclozolin exposure during embryonic life included testicular, prostate and renal abnormalities, and increased the incidence of tumors [33,84,85]. A modification of the sperm epigenome appears to have occurred following vinclozolin exposure at the time of gonadal sex determination, which enabled transgenerational transmission to subsequent generations to promote adult-onset disease [1] (Figure 2). This was one of the first reports of an environmental factor promoting epigenetic transgenerational inheritance.

A follow-up study by a company that produces vinclozolin (BASF, Ludwigshafen, Germany) found that oral administration of the same dose used intraperitoneally (IP) [33] did not have transgenerational effects nor major effects in the F1 generation [86]. Previously, we found that a fourfold decrease in the dose eliminated the vinclozolin effect [84]. For most compounds, oral gavage treatment generally has a circulating dose an order of magnitude lower than an intraperitoneal injection, thus the lack of effect was probably a result of insufficient dosing [33]. Regarding toxicology, this study suggests that vinclozolin might not be a significant risk factor at the dose used [86]. However, in our studies, we used vinclozolin as a pharmacologic agent to promote the transgenerational phenotype and to study its mechanism [33], and did not perform risk assessment or classic toxicology experiments. A second

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Table 3. Epigenetic transgenerational events

Epigenetic transgenerational event, environmental factor and generation	Reference
Paramutation in maize Modification of plant color (F1–F2)	[118]
Paramutation in *Arabidopsis* (F1–F4)	[91]
Epigenetic (paramutation) non-mendelian change in mouse (F1–F6)	[92,93]
Vinclozolin-induced epigenetic transgenerational adult-onset disease in rat (F1–F4)	[33,86]
BPA-induced transgenerational testicular abnormality (F1–F3)	[89]
Transgenerational promotion of long term potentiation (F1–F2) by altered environment	[95]
Stress-induced behavior alterations (F0–F2)	[96]
Nutrition-induced transgenerational obesity in mice (F1–F3)	[61]
Transgenerational response in longevity to nutrition (F0–F2)	[94]
Gender bias in multiple sclerosis following epigenetic changes in HLA class III risk haplotypes (F1–F2)	[98]
Tumor susceptibility in *Drosophila* (F1–F3)	[119]
Stem cell culture-induced adult-onset disease (F0–F4)	[99]

Box 4. Future questions and considerations

- The epigenetic and genetic mechanisms of how the germline epigenome becomes permanently programmed to transmit a transgenerational phenotype need to be determined.
- A correlation of epigenetic biomarkers with disease needs to be assessed for the potential future development of early stage diagnosis of disease.
- A correlation of epigenetic biomarkers with environmental exposures is needed to develop advanced risk and toxicology assessments.
- The paradigm that genetics is the primary molecular mechanism involved in biology and medicine needs to be modified to incorporate epigenetics as a crucial regulatory factor as well.

study repeated the vinclozolin experiment [87] using a more inbred CD–Sprague Dawley (Charles River) rat line versus the outbred Harlan Sprague Dawley line [33]. This study did not obtain a dramatic transgenerational phenotype [87]. Previously, we reported that the inbred Fisher rat line did not respond as well as the outbred Harlan Sprague Dawley line [33,84], and we have recently found the CD–Sprague Dawley response is also not as robust. The hypothesis is proposed that the inbred status of the line might be a factor in the efficiency of promoting the phenotype. We recently repeated the original observation [33] with the outbred Harlan Sprague Dawley line [88]. In addition to the outbred status of the line, we found the exposure timing and duration to be crucial. The parameters required to obtain the transgenerational phenotype should help to reveal aspects of the mechanisms involved. Several other recent studies confirm the ability of environmental agents to promote transgenerational phenotypes [89], and a recent independent study confirmed the epigenetic transgenerational actions of vinclozolin [90].

Several epigenetic transgenerational phenomena and phenotypes have since been observed in various species and with various environmental factors involved (Table 3). The first non-mendelian hereditary phenomenon reported in plants was called paramutation [44] and later this transgenerational phenomenon was found to be epigenetic in nature and controlled by DNA methylation [91]. This event was also observed in mammals, with a similar mode of inheritance found in mice [92,93]. Nutrition also promotes a transgenerational adult-onset obesity phenotype, as described in the Agouti mouse model [61], and there is also documentation of transgenerational responses to nutrition in humans [94]. A transgenerational mechanism exists that appears to capture an alteration in nutrition in a sensitive period of perinatal development from the previous generation(s). This requires a mechanism for transmitting the change in environmental exposure (epigenetic) that then alters gene expression and phenotype in the next generation (Figure 2). A nutritionally induced transgenerational response has been observed down the male line, and implies that the sperm carries the ancestral exposure information. A study by Arai *et al*. [95] demonstrated the ability of an animal's environment to modulate the signaling network that promotes long-term potentiation (LTP) in the hippocampus and to improve contextual fear memory formation across generations. In addition, environment also enhances LTP in their future offspring through adolescence, even if the offspring are not exposed. Stress-induced maternal programming also promotes behavioral changes transgenerationally [96,97].

Heritable disease states such as multiple sclerosis (MS) also appear to have an epigenetic origin [98]. Epigenetic modifications differentiate among human leukocyte antigen class II risk haplotypes and are involved in the gender bias in MS [98]. Processes such as embryonic stem cell culture to generate spermatogonial stem cells have been shown to epigenetically alter the germline and promote abnormalities transgenerationally (F0–F4) in mice [99]. As discussed, environmental toxicants such as vinclozolin [33] and the plastic component BPA promote transgenerational disease. The plasticizer BPA also promotes testicular disease from F1 to F3 generations in rats [89]. Further studies (Box 4) are required to determine the crucial time of exposure of environmental toxicants, and to identify factors that result in germline-transmitted adult-onset diseases and those that have an epigenetic basis.

Concluding remarks

Epigenetic transgenerational phenomena generally require the involvement of the germline to allow the transmission of an epigenetic abnormality down several generations. The ability of environmental factors or toxicants to alter the epigenome will be common in somatic tissues, but is less common for the germline because of the limited developmental period it is sensitive to reprogramming. In the event of an altered germline epigenome becoming permanently programmed, an epigenetic transgenerational phenotype is possible (Figure 2).

The phenomenon of the fetal basis of adult-onset disease has been established [1,100], and epigenetics probably plays a crucial role in this process. Transient early life exposures in the exposed individual, or transgenerational exposures if the germline is involved, are now included as causal factors for adult-onset disease. Further investigation into the role of epigenetics in disease etiology is

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needed to determine how important early life toxicology is to disease. Elucidating the epigenetic mechanisms involved in transgenerational toxicology will provide insights into the diagnosis of environmental exposures and provide potential therapeutic targets for disease. Although the prevalence of epigenetic transgenerational inheritance needs to be assessed in various disease states, the role of epigenetics is likely to be a major factor to consider in toxicology and medicine in the future.

Endocrine disruptors are one of the most prevalent groups of environmental compounds we are exposed to daily. Although these compounds disrupt the endocrine system, it is the long-term response of molecular processes such as epigenetics that will promote downstream developmental events and adult-onset disease (Figure 1). Elucidation of the role of epigenetics in endocrine disruptor actions and in the etiology of disease will undoubtedly provide insights into diagnostics and therapeutics for environmental exposures, risk assessment and adult-onset disease (Box 4). In addition to these abnormal endocrine disrupting agents, it is likely that epigenetics will also be essential to consider in normal endocrinology and metabolic events.

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