Spring 2019 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 11 and 12 (March 19 & 26)

Environmental Epigenetics

- Environmental Impacts on Biology
- Environment and Phenotype Variation
- Environmental Factors
- Environmental Epigenetics and Twin Studies
- Early life Exposures and Developmental Effects
- Nutrition and Epigenetics
- Environmental Toxicants and Epigenetics
- Environmental Induced Epigenetic Transgenerational Inheritance

Required Reading

Nilsson E, Sadler-Riggleman I, Skinner MK (2018) Environmentally Induced Epigenetic Transgenerational Inheritance of Disease. Environmental Epigenetics. 4(2):1-13, dvy016.

Books (Reserve in Library)

Scott F. Gilbert and David Epel (2009) Ecological Developmental Biology. Sinauer Associates Inc. Sunderland, Massachusetts

E-Book: Craig and Wong (2011) Epigenetics: A Reference Manual. Caister Academic Press. ISBN-13: 978-1904455882

Literature

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REVIEW ARTICLE

Environmentally induced epigenetic transgenerational inheritance of disease

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Abstract

Ancestral environmental exposures such as toxicants, abnormal nutrition or stress can promote the epigenetic transgenerational inheritance of disease and phenotypic variation. These environmental factors induce the epigenetic reprogramming of the germline (sperm and egg). The germline epimutations can in turn increase disease susceptibility of subsequent generations of the exposed ancestors. A variety of environmental factors, species and exposure specificity of this induced epigenetic transgenerational inheritance of disease is discussed with a consideration of generational toxicology. The molecular mechanisms and processes involved in the ability of these inherited epimutations to increase disease susceptibility are discussed. In addition to altered disease susceptibility, the potential impact of the epigenetic inheritance on phenotypic variation and evolution is considered. Observations suggest environmentally induced epigenetic transgenerational inheritance of disease is a critical aspect of disease etiology, toxicology and evolution that needs to be considered.

Key words: epigenetics; transgenerational; non-genetic inheritance; disease etiology; evolution; review

Introduction

The term epigenetics was originally coined by C.H. Waddington in the 1940s in relation to his studies of gene–environment interactions involving non-Mendelian inherited phenomena [1, 2]. More recent molecular oriented definitions are that epigenetics refers to 'the molecular factors and processes around the DNA that regulate genome activity independent of DNA sequence, and that are mitotically stable' [3] (Table 1). These molecular factors include DNA methylation [4], histone modifications [5], non-coding RNAs [6, 7], chromatin structure [8], and RNA methylation [9] (Fig. 1). The complex integration of epigenetic modifications is referred to as the 'epigenome'. The first whole epigenome analysis was accomplished in 2005, mapping histone acetylation and methylation in yeast [10]. Epigenetic processes are critical for allowing an organism to respond to its environment with changes in gene expression. In addition, epigenetic mechanisms allow a stem cell type to develop into a differentiated cell type [3, 11, 12] (Fig. 2). Therefore, epigenetic processes are an integral part of normal biology.

Molecular Epigenetic Mechanisms

There are a variety of epigenetic factors that act around the DNA in a cell to regulate gene expression and genome activity. DNA methylation is the most extensively studied epigenetic factor. DNA methylation involves a small (methyl) chemical

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Table 1: glossary terms and definitions

Glossary term	Definition
Epigenetics	Molecular factors and processes around DNA that regulate genome activity independent of DNA sequence, and are mitotically stable
Epigenetic transgenerational inheritance	Germline mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences
Multigenerational	Direct exposure of multiple generations
Epimutation	Environmentally induced differential presence of epigenetic alterations that can lead to altered genome activity when compared to organisms not having the exposure

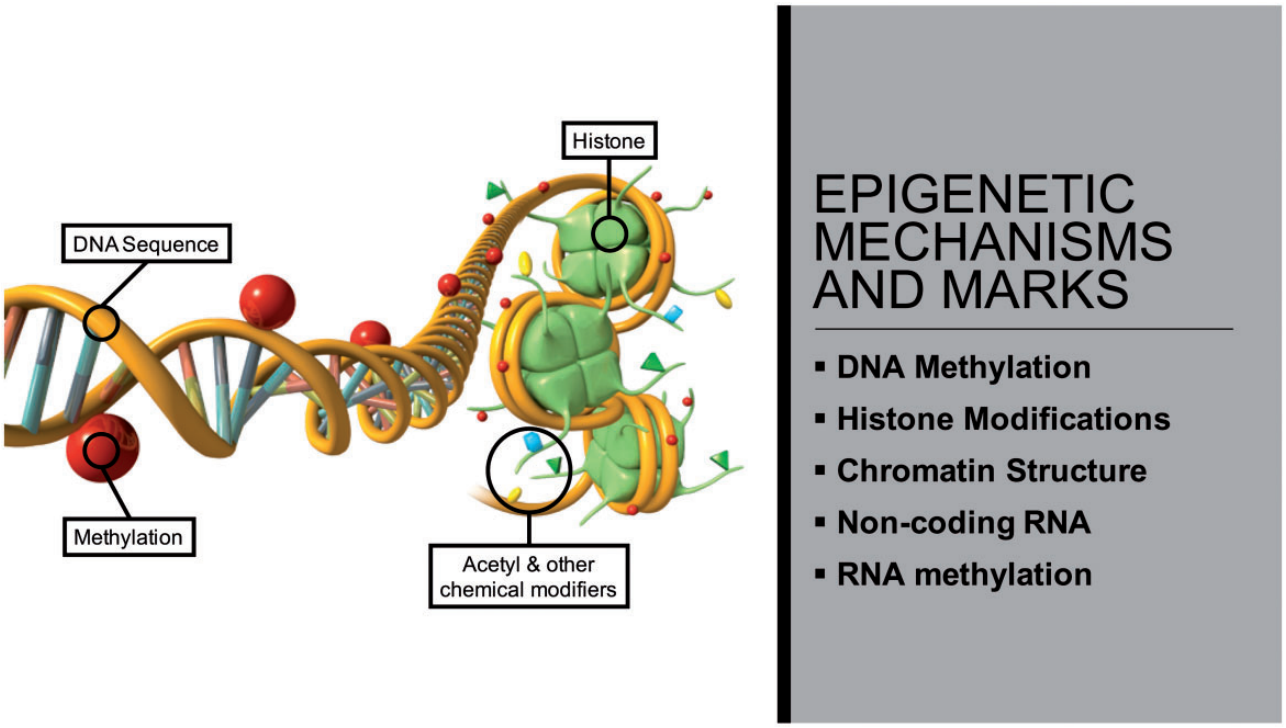

Figure 1: epigenetic mechanisms and processes (marks). Modified from [122]

group being attached to DNA, primarily at the cytosine base when it is adjacent to a guanine residue [4, 13] to produce 5-methylcytosine (5mC). Other chemical modifications of cytosine bases in DNA have also been described. The TET (ten-eleven translocation) family of enzymes can oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) [14]. In broad terms, the presence of 5mC often represses DNA transcription, while 5hmC is permissive to transcription [15, 16]. However, one of the main functions appears in the DNA methylation erasure during early development [17]. *N*(6)-methyladenine is an epigenetic modification to the adenine base of DNA that was once thought to only be present in prokaryotic organisms, but has now been described in mammalian embryonic stem cells [18].

The histone proteins that DNA is wrapped around create the nucleosome and can be chemically modified to alter gene expression. There are many different histone post-translational modifications including lysine acetylation, lysine and arginine methylation, arginine citrullination, lysine ubiquitination, lysine sumoylation, ADP-ribosylation, proline isomerization, and serine/threonine/tyrosine phosphorylation [19]. These modifications can change chromatin structure or recruit transcriptional cofactors to DNA in order to regulate gene expression. Alternatively, they can act as repressive marks to reduce gene expression in major regions of the genome such as heterochromatin. In broad terms, histone acetylation can increase transcription, while methylation can be repressive to transcription.

Non-coding RNA molecules can act as epigenetic factors [20]. These are small and long RNA molecules that do not code for a protein, but rather function as RNA to regulate gene expression. The non-coding RNA molecules that act as epigenetic factors are not DNA sequence dependent, so the majority do not rely on having a nucleotide sequence that is complimentary to a specific DNA or RNA region in order to function. Long non-coding RNAs (lncRNAs) [21] and transfer RNA-derived small RNAs (tsRNAs) [22] are examples of RNA classes that are present in sperm and can act as epigenetic factors that affect subsequent generations [23].

RNA molecules can themselves be epigenetically modified and so affect translation and gene expression [24]. The most prevalent reversible modification to the internal sequence of mRNA is methylation of adenosine to form *N*(6)-methyladenosine (m(6)A). m(6)A mRNA methylation is associated with post-transcriptional regulation [25, 26]. Cytosine methylation (m3C) in both mRNA and tRNA also occurs [27, 28]. Methylation of tRNA inhibits processing of tRNA into tsRNA halves, which

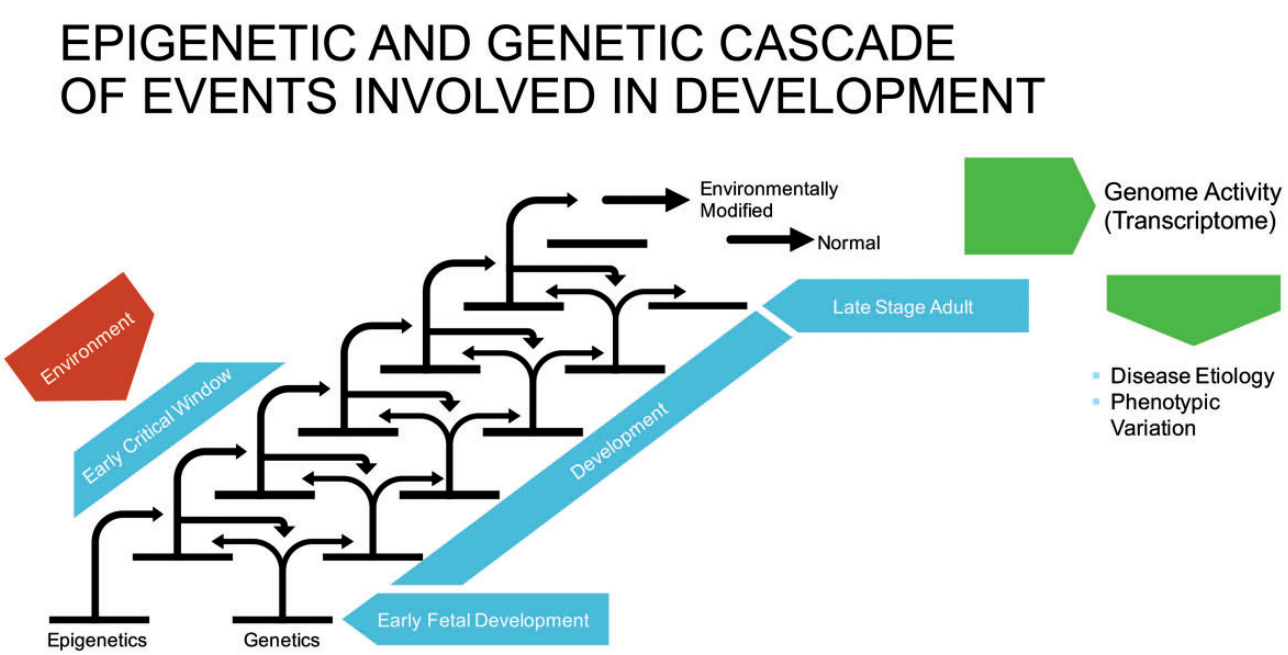

Figure 2: epigenetic and genetic cascade of events involved in development. Cascade of genetic and epigenetic stages interacting to promote differentiated cells. The critical window of exposure allows environmental factors to alter the epigenetic cascade to obtain a modified differentiated site and to cause altered transcriptomes to increase disease susceptibility and phenotypic variation. Modified from [3]

themselves affect transcription [22, 29, 30]. Therefore, RNA methylation is the most recent epigenetic molecular factor identified.

The coiling, looping, and general structure of DNA is termed chromatin structure and is also an epigenetic factor [8]. The three-dimensional structure of DNA can make certain regions of the genome accessible to transcription machinery, such as bring enhancer regions near gene promoters to affect gene expression. Therefore, epigenetic molecular processes include DNA methylation, histone modifications, non-coding RNAs, RNA methylation, and chromatin structure.

Epigenetic Transgenerational Inheritance

The definition of epigenetic transgenerational inheritance is 'germline-mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences that leads to phenotypic variation' [3, 31] (Table 1). Multigenerational exposures, in contrast, refer to observed effects in subsequent generations that are the result of direct exposure [31] (Table 1; Fig. 3). Direct environmental exposure of the parents, considered to be the F0 generation, can also affect the germline (sperm or eggs) of either parent. Therefore, the next generation (F1) derived from this germline is still considered exposed, and so is not truly transgenerational. For preconception parental exposures the F2 generation offspring is considered the first transgenerational unexposed generation (Fig. 3). The situation is different when a gestating female is exposed, because then the fetus and the fetus' germline are directly exposed as well. In that case, the F3 generation is the first unexposed transgenerational offspring [31] (Fig. 3).

The Agouti mouse model is an example of multigenerational inheritance [32–34]. When pregnant Agouti mice are exposed to a methyl donor in their diet, they experience increased methylation on an allele of their Agouti gene, which leads to a coat color change in their offspring. Generally, this change is not passed on to future generations. Instead the normal process of epigenetic reprogramming in the germline and early embryo returns the DNA methylation state to its original setting.

An increasing number of examples of transgenerational inheritance of disease are present in the literature (Table 2). Some of the first experiments to establish the potential for epigenetic transgenerational inheritance were performed by Conrad Waddington, who coined the term 'epigenetic' [1, 35]. In these studies, it was found that a heat shock induced wing structure change in *Drosophila melanogaster* persisted for more than seven generations [35]. An even earlier study in Guinea pigs demonstrated transgenerational inheritance of decreased fertility and increased mortality for four generations after ancestral exposure to ethanol vapor, although this was not attributed to epigenetic inheritance at the time [36]. One of the first studies to associate molecular epigenetic changes with transgenerational inheritance of disease in mammals was an investigation of the effects of treating pregnant rats with the agricultural fungicide vinclozolin [37]. The F3 generation (great-grand offspring) demonstrated reproductive abnormalities such as increased testicular germ cell apoptosis and decreased sperm motility. These transgenerational phenotypes were correlated with changes in DNA methylation in the F3 generation sperm [37].

Several environmental toxicants including vinclozolin, DDT (dichlorodiphenyltrichloroethane), methoxychlor, plastic derived compounds, hydrocarbons, atrazine, tributyltin have been shown to promote the transgenerational inheritance of increased disease susceptibility in rodent models [38, 39] (Table 2). The diseases that were increased transgenerationally included testis, prostate and kidney disease, obesity, polycystic ovaries, reduced oocyte number in the ovaries, and cancer [39]. For the purposes of this review, more recently published investigations of epigenetic transgenerational inheritance of disease will be highlighted, (Table 2). Exposure of mice to the phthalate plastic derived compound DEHP (di(2-ethylhexyl) phthalate) has been shown to result in transgenerational changes to stress

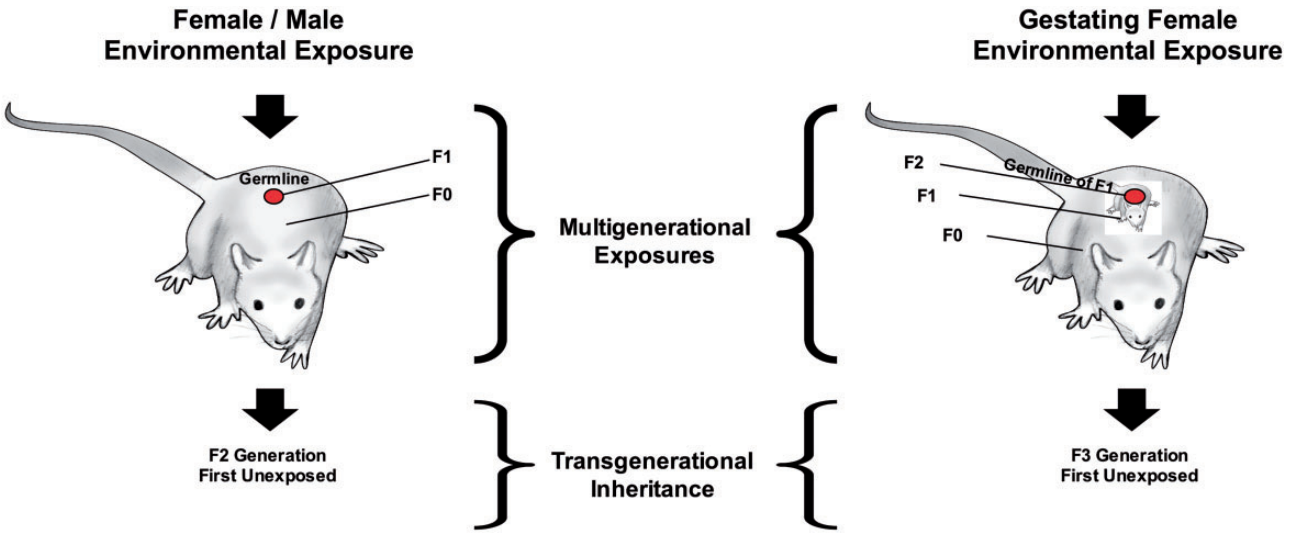

Figure 3: environmentally induced transgenerational epigenetic inheritance. Schematic of multigenerational versus transgenerational environmental exposures. Modified from [31]

Table 2: examples of transgenerational inheritance from specific exposures and specific effects

Exposure	Effects	Reference
Environmental toxicants		
Vinclozolin	Impaired male fertility; prostate, kidney disease, tumors, immune and reproductive pathologies	[37, 78, 94]
Vinclozolin	Gender-specific changes in anxiety-like behavior	[85]
Methoxychlor	Impaired male fertility; kidney disease, ovary disease, and obesity	[37, 86]
Permethrin/DEET	Prostate, kidney disease	[81]
Dioxin	Prostate, kidney disease, reduced fertility, negative effects on pregnancy outcome	[80, 123]
BPA/phthalates	Prostate, kidney disease; obesity	[43]
Hydrocarbon mixture (jet fuel)	Prostate, kidney disease; obesity; immune and reproductive pathologies	[46]
Vinclozolin, permethrin/DEET, plastics, dioxin, jet fuel	Polycystic ovaries, reduced primordial follicle pool	[82]
DDT	Obesity	[45]
Phthalate	Disruption of testicular germ cell organization and spermatogonial stem cell function, changes in hormones and behavior	[40, 124]
Phthalate	Disrupted ovarian function	[41]
Tributyltin	Increase in fat depot size	[38]
BPA	Cardiac disease; reduced fertility	[48, 72]
BPA	Changes in social behavior and neural gene expression	[42]
Atrazine	Testicular disease, early puberty, lean phenotype	[125]
Benzo[a]pyrene	Behavioral and physiological deficits	[50]
Mercury	Behavior change	[49]
Other exposures		
Caloric restriction	Cardiovascular mortality	[56, 77]
High-fat diet	Increased body size; reduced insulin sensitivity, increased mammary cancer	[57–59]
Folate	Congenital malformations	[126]
Stress	Reduced social interaction; increased stress resilience; disrupted neural connectivity; physiology changes; increased anxiety	[51–55]
Drought	DNA methylation changes	[127]
Heat/salt stress	Accelerated flowering, increased salt tolerance	[128]
Prediabetes/diabetes	Impaired glucose tolerance; reduced insulin sensitivity, male subfertility	[61, 62]
Smoking	Abnormal pulmonary function	[129]
Ethanol	Neurological defects; decreased fertility	[36, 47, 130]
Heat stress	Increased Hsp70 production and tolerance to heat stress; wing structure changes	[131, 132]

hormones, behavior [40], and ovarian function [41]. Earlier studies in mice [42] showed that ancestral exposure to the plastic derived compound bisphenol A (BPA) caused changes in social behavior in juvenile mice and changes in expression of neural genes such as oxytocin and vasopressin. Earlier studies in rats have shown that exposure to a mixture of BPA and phthalates induces transgenerational increases in pubertal abnormalities, testis disease, and ovarian disease [43]. Ancestral exposure of

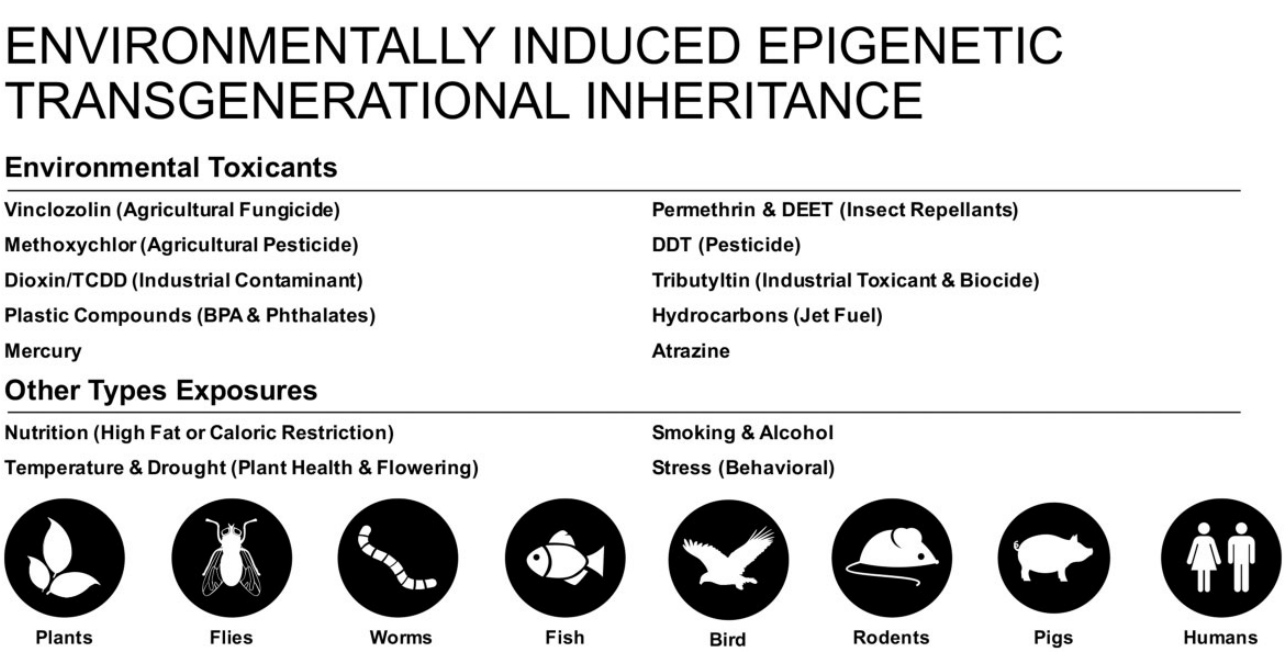

Figure 4: environmentally induced epigenetic transgenerational inheritance. Various exposures and species investigated

mice to the toxicant tributyltin results in a transgenerational increase in obesity [38, 44]. Earlier investigations in pregnant rat exposures to DDT, jet fuel hydrocarbons, or a BPA/phthalates mix will also increase obesity transgenerationally [43, 45, 46]. Other recently published investigations indicate that ethanol exposure of pregnant mice can cause transgenerational neurological changes in descendants that resemble those of Fetal Alcohol Spectrum Disorders [47]. In zebrafish, BPA exposure of males can result in the transgenerational inheritance of heart disorders in the F2 generation [48]. Zebrafish exposure to mercury [49] or to the industrial pollutant benzopyrene [50] induces the transgenerational inheritance of abnormal neurobehaviors that are correlated with epigenetic changes (i.e. epimutations) in sperm (Table 2) [49, 50].

Exposure to environmental factors other than toxicants can also induce transgenerational inheritance (Table 2). The stress of maternal separation in mice transgenerationally disrupts functional connectivity throughout the brain [51], as well as both impairing social interactions and cognition and making the descendant mice more stress resilient [52]. Mice subjected to restraint stress also transmitted reduced anxiety levels to their transgenerational descendants [53]. Conversely, social hierarchy stress in mice was shown to increase anxiety behaviors transgenerationally [54]. This raises the possibility that several psychological stressors can induce different transgenerational effects. In pregnant rats, the stressors of forced swim and restraint induce transgenerational inheritance of physiological changes such as alterations in catecholamine biosynthesis and immune response [55].

Other examples of transgenerational inheritance have been observed with caloric restriction or high fat diets. The Överkalix study by Bygren *et al.* [56] shows how cardiovascular mortality in humans can be influenced by reduced childhood and adolescent food supply. Effects were shown to reach into the second generation. Maternal high fat diet in mice can increase body size and reduce insulin sensitivity in F3 generation female offspring [57], although Masuyama *et al.* [58] demonstrated that a normal diet in utero for three subsequent generations can return glucose and lipid metabolism to normal. In addition, a maternal high fat diet in mice can transgenerationally increase mammary cancer risk [59]. Previous studies with rats demonstrated that exposure of pregnant animals to the environmental toxicant vinclozolin also promoted a transgenerational increase in tumors [60]. Interestingly, diabetes in mice can induce transgenerational inheritance of male subfertility [61]. A paternal prediabetic condition in mice can be inherited transgenerationally as shown by impaired glucose tolerance and decreased insulin sensitivity [62]. Similarly, male rats fed a high fat diet promoted transgenerational inheritance of impaired glucose tolerance in F2 generation offspring [63].

Species Diversity of Epigenetic Transgenerational Inheritance

Epigenetic transgenerational inheritance has been identified to occur in a wide variety of organisms (Fig. 4). This review focuses on examples of epigenetic transgenerational inheritance of disease or abnormalities in different animal species. A number of studies have demonstrated the environment (e.g. heat and drought) can induce the epigenetic transgenerational inheritance of phenotypic variation in plants [64]. In the nematode worm *Caenorhabditis elegans* increased longevity that is associated with the histone modification H3K4me3 methylation can be transgenerationally inherited for up to three generations [65]. As mentioned previously, Waddington performed early experiments using the model insect species *D. melanogaster* and demonstrated that a heat shock induced wing structure changes that persisted for more than seven generations [1, 35] and now for hundreds of generations in today's stocks. In more recent examples, it has been found that a high-sugar maternal fly diet can alter the larval body composition for the next two generations [66]. Similarly, a high fat larval diet in fruit flies can cause transgenerational alterations to F2 generation pupal and egg size [67]. Manipulations of the protein levels in the diet of fruit flies can affect longevity and reproduction for three subsequent generations, and this effect is associated with histone

modifications [68, 69]. In another arthropod species, the crustacean *Daphnia magna*, exposure to the toxicant 5-azacytidine results in decreased body length and reduced levels of DNA methylation in non-exposed subsequent generations [70].

Several species of fish have shown epigenetic transgenerational inheritance of disease. Zebrafish exposed to the environmental toxicants benzo(a)pyrene [50], methylmercury [49] or dioxin [71] transmit to their grand-offspring behavioral changes, visual defects, increased body mass, skeletal abnormalities and/or decreased fertility, sometimes associated with changes in DNA methylation. Medaka exposed to the endocrine disruptors BPA or ethinylestradiol produce grand-offspring and great-grand-offspring with reduced fertility [72].

Some bird species have shown evidence of epigenetic transgenerational inheritance. In a study with quail eggs exposed to the environmental estrogen genistein [73] the great-grand offspring age at which the first egg was laid was significantly greater. In ducks, feeding a methionine-deficient diet produces grand-offspring with altered weight gain and changes in metabolic parameters [74].

In mammals most studies of epigenetic transgenerational inheritance have occurred in rodents [75]. Another experimental mammal involves pigs and abnormal nutritional induced epigenetic transgenerational inheritance [76]. Examples of transgenerational inheritance of increased susceptibility to diseases have been outlined above for rats, mice and Guinea pigs [36, 37, 41, 44, 45]. Evidence of epigenetic transgenerational inheritance of disease in humans comes from retrospective studies such as those including the Dutch and Swedish famines [56, 77]. As previously mentioned, the descendants of people exposed to famine conditions as children 9–12 years of age in Sweden were investigated and it was found that men whose grandfathers had been exposed to famine had an increased risk of mortality due to diabetes, and similarly women whose grandmothers were exposed had increased risk [31]. Due to the conservation of environmentally induced epigenetic transgenerational inheritance from plants to humans all organisms will utilize epigenetic inheritance to facilitate environmental adaptation and response.

Phenotypic Diversity of Transgenerationally Inherited Diseases

Studies of the effects of ancestral exposure to an array of toxicants (Table 2) demonstrate epigenetic transgenerational inheritance of a variety of diseases and abnormalities, including testis disease [37], prostate and kidney disease [43, 46, 78–82], mammary tumors [78], immune and reproductive pathologies [46, 78, 83, 84], obesity [45, 46], behavioral effects [85] and many others listed in Table 2. The disease phenotypes observed in these experiments often depend on the specific exposure of the F0 generation. For example, increased obesity risk in rats is inherited transgenerationally after ancestral exposure to DDT, plastic compounds, hydrocarbons and methoxychlor [43, 45, 86], but not dioxins. Jet fuel hydrocarbons induce an elevated rate of luteal ovarian cyst formation in F3 females [46, 82], a phenotype not observed with other exposures. On the other hand, some ovarian disorders such as polycystic ovaries and reduction of the primordial follicle pool size have been shown to be inherited transgenerationally after exposure of the F0 generation to many of the toxicants studied [84, 87]. The explanation for this phenomenon may be that some developmental processes, in this case ovarian follicle development, are more sensitive to epigenetic and gene expression changes in their developmental regulatory networks, and so will be more easily affected than those of other cells and tissues (Fig. 2).

Epigenetic processes are major mechanisms by which organisms respond and adapt to their environment. Therefore, how can environmental epigenetic insults result in transgenerational inheritance of increased disease susceptibility? Since this is a maladaptive response one possible answer may be seen in the predictive adaptive response hypothesis [88]. In this hypothesis an environmental stressor like famine may epigenetically promote an adaptive (thrifty) phenotype in subsequent generations. If the current environment of those descendants has more than adequate nutrients, diseases such as diabetes and obesity are promoted. Another possibility is that an environmental insult, such as exposure to a toxicant, may interfere with the normal molecular epigenetic machinery and result in stochastic and/or directed epigenetic changes that could be considered epimutations. The term epimutation is defined as 'the environmentally induced differential presence of epigenetic alterations that can lead to altered genome activity, when compared to organisms not having exposure' (Table 1). If these epimutations occur in germ cells they can lead to transgenerational inheritance of a wider range of phenotypes in the progeny. Some of those phenotypes may be poorly adapted and develop disease. This would explain an increase in disease susceptibility in organisms whose ancestors were exposed to environmental insults. However, phenotypic variation is the 'raw material' upon which natural selection acts. Therefore, the increased phenotypic variation may also result in some individuals who are better adapted to an altered environment which can facilitate natural selection and evolution [89].

Developmental Etiology of Epigenetic Transgenerational Inheritance

A number of reproductive processes involve DNA methylation changes that normally will be reset by genome-wide DNA methylation reprogramming events. The two main developmental periods are in the early embryo after fertilization and during germ cell specification at gonadal sex determination [90, 91] (Fig. 5). This phenomenon allows embryonic stem cells to develop by removing epigenetic constraints to promote pluripotency. Some parental epigenetic changes, such as imprinted genes, are protected from being reprogrammed during these developmental periods. In contrast, some parent specific imprints are established during this epigenetic reprogramming [92]. Environmentally induced DNA methylation alterations called differential DNA methylation regions (DMRs) [93] present in germ cells behave as imprinted-like genes in the way their methylation patterns persist. By definition, true imprinted genes display 'parent-of-origin allelic transmission with monoallelic gene expression'. DMRs often demonstrate parent-of-origin allelic transmission, but monoallelic gene expression has not been demonstrated. Differentially methylated sites connected with transgenerational inheritance are called 'imprinted-like' [94]. The transmission of epigenetic information to future generations via germ cells can alter the epigenome of the developing embryonic stem cells which would be expected to promote changes to the epigenetic and transcriptomic programming of all derived somatic cells [95]. Those tissues that are sensitive to alterations in their epigenomes and transcriptomes may show increased susceptibility and prevalence of disease development [93, 96] (Fig. 2). Normal biology

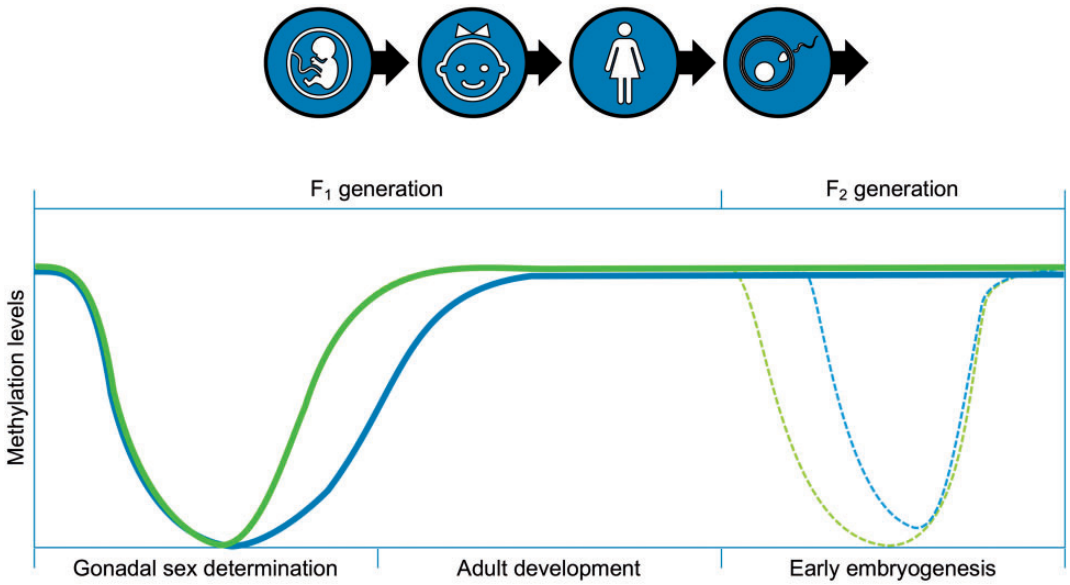

Figure 5: epigenetic reprogramming during primordial germ cell development at gonadal sex determination and following fertilization in the early embryo. Modified from [94]

requires alterations of epigenetics for the development of stem cell populations and subsequent somatic cell differentiation. The epigenetic transgenerational inheritance molecular process is directly linked to these epigenetic reprogramming processes in the germline and the developing embryo.

Germline Epimutations

It is a prerequisite for environmentally induced epigenetic transgenerational inheritance that there be epigenetic changes (i.e. epimutations) in the germline, because the germ cells (sperm and egg) are the only cells that can transmit molecular information between generations from the parents to their offspring. Early studies investigating transgenerational epimutations in germ cells used pregnant rats exposed to vinclozolin during the period of gonadal sex determination when epigenetic reprogramming of the fetal germ cells occurs. A genome-wide promoter analysis was applied to look for epigenetic changes in the sperm DNA and approximately 50 differential DMRs were identified in gene promoters in vinclozolin lineage F3 generation sperm DNA versus control lineage [97]. Similar experiments have been performed in rats using a number of additional toxicants including dioxin [80], a mixture of permethrin and DEET (N,N-diethyl-meta-toluamide) [81], BPA and phthalates [43] and jet fuel (hydrocarbons) [46]. All these toxicants were found to promote transgenerational inheritance of both disease and sperm DMRs. Interestingly, it was observed that each toxicant produced an exposure-specific set of DNA methylation changes in the sperm, and comparisons between the different toxicant exposures demonstrated negligible overlap between them [82]. This raises the possibility that these 'epigenetic signatures' may be used in the future as a diagnostic tool to determine if an individual has had a particular environmental toxicant exposure in their ancestry. The examination of the genomic features of all these DMRs identified a low CpG density termed CpG deserts [98] and a DNA sequence motif called Environmental Induced Differential Methylation Consensus Sequence 1 (EDM1). Nearly all the DMRs identified with numerous exposures had these genomic features [39]. A machine learning analysis used this data to identify approximately 40 000 potential genome-wide DMR sites susceptible to environmental alterations [99]. Further studies are needed to determine the utility of these potential epimutation sites as biomarkers for exposure and disease.

Comparisons have been made of the DMRs induced in the direct exposure F1 generation and transgenerational F3 generation vinclozolin lineage male sperm [100]. As described above, when the gestating female is directly exposed to a toxicant the F1 generation fetus is also directly exposed, as are the developing germ cells within the F1 generation fetus that will generate the F2 generation. The F3 generation animals are the first non-exposed transgenerational descendants (Fig. 3). Therefore, the molecular mechanisms of inducing epigenetic changes is different in the direct exposure F1 generation, and in the F1 generation germ cells (sperm) that will produce the F2 generation, when compared with mechanisms by which epimutations are induced in the transgenerational F3 generation. In a study involving vinclozolin exposure of gestating female rats there was a distinct set of DNA methylation changes in the F1 generation sperm that was different from the set of methylation changes in the transgenerational F3 generation sperm [100]. This suggests that the direct exposure induced F1 generation sperm epimutations promote epigenetic alterations during germ cell development in subsequent generations that lead to the different DMRs in the F3 generation. This mechanism appears to be associated with altered early embryonic development of the stem cells.

In addition to DNA methylation, other epigenetic factors such as non-coding RNA (ncRNA) can also contribute to epigenetic transgenerational inheritance. Small ncRNAs of the microRNA class are altered in the sperm of stressed vs. unstressed mice and have been shown experimentally to promote a change in the hypothalamic–pituitary–adrenal stress axis reactivity of offspring [101]. Another class of small non-coding RNAs associated with transgenerational sperm are 5′ halves of tRNAs [102]. These stRNA 5′ halves and microRNAs are transgenerationally altered in the F3 generation sperm of rats ancestrally exposed to vinclozolin during pregnancy [102]. A number

of studies have demonstrated the potential role of ncRNA in epigenetic transgenerational inheritance [103].

Another epigenetic factor present in sperm and associated with transgenerational inheritance is the retention of histone proteins [104]. During spermatogenesis in vertebrates the histone cores that DNA is wrapped around in most somatic cell types are replaced by protamines, allowing for more tightly compacted DNA in sperm heads [105]. However, 1–10% of histones are retained in mammals, depending on species [106]. These retained sperm histones have been implicated in regulating gene expression in the resulting offspring [107]. In a recent transgenerational study using rats, Ben Mammar *et al.* [108] demonstrated that a specific set of histones are retained in F3 generation control lineage sperm. This same set of histones is retained in F3 generation rats ancestrally exposed to vinclozolin or DDT, but additional sites of histone retention are induced in the vinclozolin and DDT lineage sperm [108]. Therefore, histone retention also appears to be associated with sperm mediated transgenerational inheritance of disease following ancestral DDT or vinclozolin exposure [104, 108].

Since post-translational modifications of histones are known to be an epigenetic factor that regulates gene expression studies have investigated histone modifications present in sperm. Histone H3 methylation changes in retained sperm histones have been correlated with fertility in humans [109] and with survival of offspring in mice [110]. Histone modifications have been correlated with epigenetic transgenerational inheritance of altered phenotypes in *C. elegans* [111], *Drosophila* [112], and recently in mammals [104].

Previous transgenerational studies have focused on epigenetic factors and epimutations in sperm due to the relative ease of obtaining large numbers of sperm cells. Several studies have shown that epigenetic transgenerational inheritance is mediated through the female germline [45, 86]. Eggs cannot be obtained in large enough quantity to allow traditional molecular analysis. Future studies with single cell analyses may be needed to document the role of epimutations in eggs. Epigenetic factors in eggs appear to play an equally important role in epigenetic inheritance, but remain to be investigated. The epigenetic transgenerational inheritance of disease following environmental exposures will likely be mediated by the integrated actions and combination of different epigenetic factors present in gametes. A recent study in rats demonstrated that after treatment of gestating females with DDT or vinclozolin there were concurrent transgenerational alterations in F3 generation sperm in DNA methylation, histone retention, and non-coding RNAs [108, 113]. Therefore, transgenerational alterations in all the different epigenetic processes appear to be involved in the epigenetic transgenerational inheritance phenomenon.

Transgenerational Gene Expression Changes

Transgenerational inheritance of environmentally induced epigenetic changes requires transmission through the germ line from parents to future generations. However, epigenetic changes themselves would not cause disease, rather they must manifest as changes in gene expression. Ensuing disease such as cancer, prostate or kidney abnormalities, and obesity are brought on by disturbances in gene expression in the pertinent somatic cells. The hypothesis is that the epimutations in the germline alter the epigenome of the embryonic stem cells that then affect all subsequent somatic cell epigenomes and transcriptomes [87, 95] (Fig. 2). These cell and tissue specific epimutations promote tissue specific alterations in transcriptomes [96]. These aberrant transcriptomes could then lead to a susceptibility for physiological abnormalities and disease (Fig. 2).

Exposure of F0 generation animals to environmental toxicants will affect and change the transcriptomes of potentially all tissues in future generations [96]. In a study of rats ancestrally exposed to vinclozolin the transcriptomes of 11 different tissue types from adult male and female animals were examined [96]. It was found that there were gene expression differences between control and vinclozolin lineage animals in the different tissues with minimal overlap in the differentially expressed genes between tissues. However, there was significant overlap in the physiological pathways and cellular processes that were affected by gene expression changes in different tissues. For example, both prostate and liver tissues were enriched for genes in transcription and focal adhesion processes, but the specific genes altered were not the same in each tissue [96]. These observations warranted a closer look at the genomic locations of epimutations and differentially expressed genes. Looking across the different tissue types it was found that there were regions of the genome that had statistically over-represented clusters of gene expression changes [96]. These regions in the genome were called epigenetic control regions (ECR). These ECR are 2–5 megabase in size and have clusters of genes. Within these ECRs are DNA methylation epimutations and long non-coding RNA (ncRNA) expression sites [114]. The long ncRNAs play a role in regulation of distal gene transcription and epigenetic regulation [115, 116]. Observations suggest that within an ECR many of the genes are epigenetically regulated up or down as a block. Therefore, in one cell type those genes within the ECR normally expressed would be regulated while in another cell type a different set of genes within the ECR normally expressed would be affected. Epigenetic alterations within the ECR can influence gene expression in a variety of cell types differently [96]. Interestingly, the location of ECRs has been shown to co-localize with clusters of transgenerational epimutations (e.g. DMRs) found after ancestral toxicant exposures [117].

Several studies have suggested how the molecular mechanisms of environmentally induced transgenerational inheritance may lead to tissue specific disease occurrence. As mentioned earlier, two ovarian disorders, polycystic ovarian syndrome, and primary ovarian insufficiency (premature reduction of the primary follicle pool) were both induced transgenerationally by a number of environmental toxicants [83]. Analysis of this phenomenon involved the isolation of a specific cell type from the tissue that is associated with the disease in the vinclozolin lineage animals. The granulosa cells were isolated from the ovarian follicles of young female rats prior to disease onset. The epigenomes and transcriptomes of these granulosa cells from control and vinclozolin lineages were analyzed and compared [87]. Granulosa cells from F3 generation vinclozolin lineage rats had differences in both the epigenome and the transcriptome compared with the control lineage. Interestingly, some of the affected genes had been previously shown to be associated with polycystic ovarian syndrome and primary ovarian insufficiency [87]. Similar results were obtained when the molecular basis of transgenerational male infertility in rats was examined. As above, changes in the epigenome and transcriptome were found in testicular Sertoli cells of F3 generation rats after ancestral vinclozolin exposure [95]. Several of the differentially regulated genes identified were known to be associated with male infertility, such as HDAC1 and HSP90AA1 [118, 119]. In addition, a number of Sertoli cell genes associated with pyruvate production were down-regulated and this is

known to impact spermatogenic cell survival and promote germ cell apoptosis, which is one of the testis pathology phenotypes observed [95]. Therefore, the environmentally induced transgenerational changes in the somatic cell epigenomes are associated with transgenerational changes in gene expression, which are related to the increases in disease development observed.

Experimental and Technical Approach Limitations

One of the main experimental design issues and limitations is a consideration of what constitutes a multigenerational or intergenerational direct exposure versus a true non-exposed transgenerational generation. A number of past studies have referred to F1 generation fetal exposure or F1 generation germline that will generate the F2 generation as transgenerational experiments (Fig. 3). Many previous reports have not carefully considered this issue and misinterpreted the results as transgenerational. A multigenerational or intergenerational exposure experiment is important and helps elucidate risk of exposure on multiple generations physiology and pathology. However, the mechanisms involved are distinct and impacts different than transgenerational generations [31]. This non-genetic form of inheritance needs to be distinguished from multiple generation exposure that is due to direct exposures and toxicities.

Another experimental design issue is the use of mixed cell populations for an epigenetic analysis [120]. Every cell type in the body has the same DNA sequence, so for genetic analysis a mixed cell population does not affect the data or observations. In contrast, each cell type in the organism has a very distinct epigenome to allow the cell type to have its unique cell biology and physiology. The reason a neuron is distinct from a hepatocyte is not the genetic sequence, but the epigenetic differences between the cell types that regulate the unique gene expression. Therefore, an epigenetic analysis of mixed cell populations are influenced by small changes in specific cell population numbers which will alter the epigenetic data experimentally observed without an actual change in molecular epigenetics [120]. A number of epigenetic studies have used whole blood which contains over 20 different cell populations to do epigenetics. Twin studies using this approach have not been revealing due to the variation in cell populations in the blood and inability to dissect out specific epigenetic changes. Purifying a specific cell type such as monocytes from the blood will be far more useful for epigenetic analyses than use of the mixed cell population. Therefore, epigenetic analysis optimally requires purified cell populations [120].

Epigenetic molecular procedures have dramatically developed over the past decade to provide greater accuracy and precision. The technology of next generation sequencing is superior to array technology and previous biochemical procedures. The current procedures for DNA methylation, ncRNA and histone modifications use next generation sequencing which should be considered the optimal approach for any genome-wide analysis. If a few selected sites are examined then the array technology or biochemical approach can be used and are less costly. For the genome-wide approaches, the different DNA methylation approaches are methylated DNA immunoprecipitation (MeDIP) sequencing (MeDIP-Seq) and bisulfite sequencing (BS-Seq). The MeDIP-Seq is biased to low density CpG $<$20% while the BS-Seq is biased to high density CpG. All these procedures are efficient, but the limitation in CpG bias needs to be considered in the interpretation of the data obtained. The RNA-Seq and chromatin immunoprecipitation ChIP-Seq approaches are the optimal procedures currently available with few alterations. Third generation sequencing that may be able to assess epigenetic modifications during the sequencing will be a future technology to elucidate the DNA methylation CpG density bias, but remains to be optimized. The rate at which epigenetic technology is developing suggests within the next five years we will likely be using new technologies. The research in this area needs to consider the limitations of some of the technology currently used.

Conclusions

Research in the area of environmentally induced epigenetic transgenerational inheritance of disease and phenotypic variation has provided evidence of transgenerational inheritance of epimutations in plants, worms, flies, fish, birds, pigs, mice, rats, and humans [121] (Fig. 4). Ancestral exposure to environmental influences such as toxicants, abnormal nutrition, or stress can induce changes in the germline epigenome that are transmitted to descendants. These epimutations caused by individual exposures must occur in the germline in order to be transmitted. When these germline epigenetic changes become imprinted-like and escape the normal processes of epigenetic reprogramming, then epigenetic transgenerational inheritance can occur. Since the embryonic stem cells develop an altered epigenome, these epimutations subsequently induce somatic cell alterations in the epigenome and transcriptome, which will increase disease susceptibility in the offspring. Therefore, these ancestral exposures to environmental toxicants can lead to transgenerational changes in the epigenome and transcriptome of future generations and lead to an increased incidence of disease. Although DNA methylation is the most thoroughly studied epigenetic mechanism, other epigenetic processes are equally important. Future research will need to investigate the multiple epigenetic mechanisms and how they integrate. The developmental aspects of how the epigenetic transgenerational inheritance of disease develops are still unclear. How epimutations in sperm result in epigenetic changes in the resultant embryo needs to be investigated. How the derived embryonic stem cell changes can lead to epigenetic and transcriptome changes in the function of an adult organ associated with disease also remain to be elucidated on a molecular level. The potential role these ancestral exposures and epigenetic transgenerational inheritance have on disease etiology needs to be seriously considered. In addition, it may be clinically useful to determine what epimutation patterns or signatures are associated with specific disease and/or ancestral exposures in humans. Epigenetic biomarker signatures may be used in the future as a diagnostic tool to assess if an individual has a specific disease susceptibility or environmental toxicant exposures. This will facilitate preventative medicine and therapeutic approaches to mitigate associated disease risks.

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Spring 2019 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 11 and 12 (March 19 & 26)

Environmental Epigenetics

- Environmental Impacts on Biology
- Environment and Phenotype Variation
- Environmental Factors
- Environmental Epigenetics and Twin Studies
- Early life Exposures and Developmental Effects
- Nutrition and Epigenetics
- Environmental Toxicants and Epigenetics
- Environmental Induced Epigenetic Transgenerational Inheritance

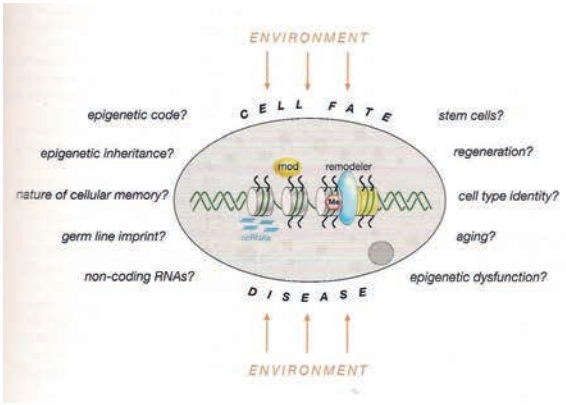
Required Reading

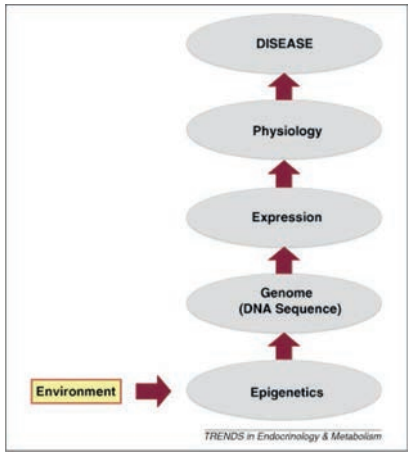
Nilsson E, Sadler-Riggleman I, Skinner MK (2018) Environmentally Induced Epigenetic Transgenerational Inheritance of Disease. Environmental Epigenetics. 4(2):1-13, dvy016.

Books (Reserve in Library)

Scott F. Gilbert and David Epel (2009) Ecological Developmental Biology. Sinauer Associates Inc. Sunderland, Massachusetts

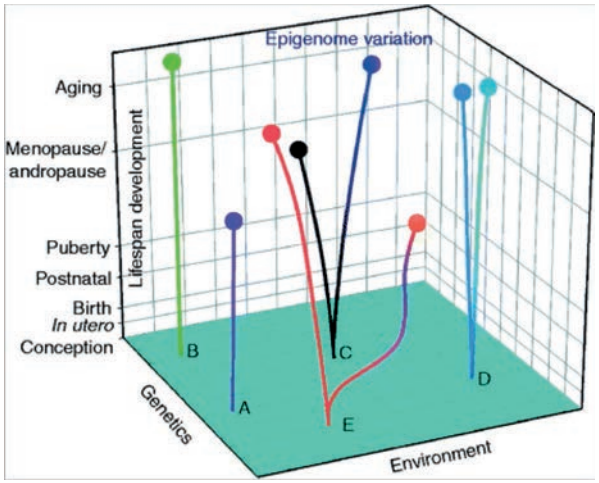
E-Book: Craig and Wong (2011) Epigenetics: A Reference Manual. Caister Academic Press. ISBN-13: 978-1904455882





Agents of developmental plasticity

- Temperature
- Nutrition
- Pressure and gravity
- Light
- The presence of dangerous conditions (predators or stress)
- The presence or absence of conspecifics (other members of the same species)



Environmental Epigenetics
(Phenotypic Variation)

FIGURE 1.5 Polyphenic variation in *Pontia* (Pieridae) butterflies. The top row shows summer morphs: *P. protodice* female (left) and male (center), *P. occidentalis* male (right). The bottom row shows spring morphs, which have a more highly pigmented ventral hindwing: *P. protodice* female (left) and male (center), *P. occidentalis* male (right). (Photograph courtesy of T. Valente.)

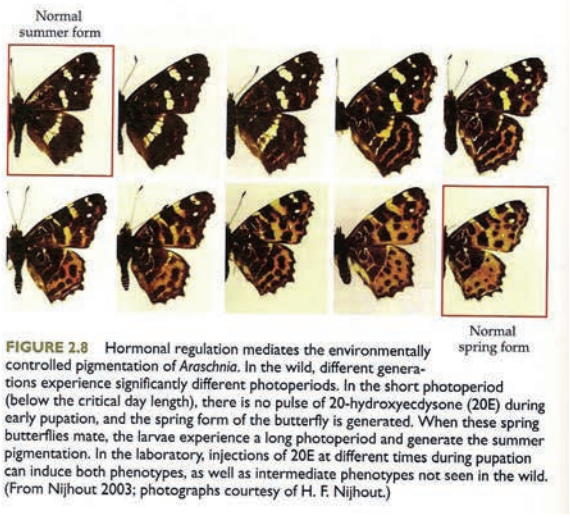


FIGURE 2.8 Hormonal regulation mediates the environmentally controlled pigmentation of *Araschnia*. In the wild, different generations experience significantly different photoperiods. In the short photoperiod (below the critical day length), there is no pulse of 20-hydroxyecdysone (20E) during early pupation, and the spring form of the butterfly is generated. When these spring butterflies mate, the larvae experience a long photoperiod and generate the summer pigmentation. In the laboratory, injections of 20E at different times during pupation can induce both phenotypes, as well as intermediate phenotypes not seen in the wild. (From Nijhout 2003; photographs courtesy of H. F. Nijhout.)

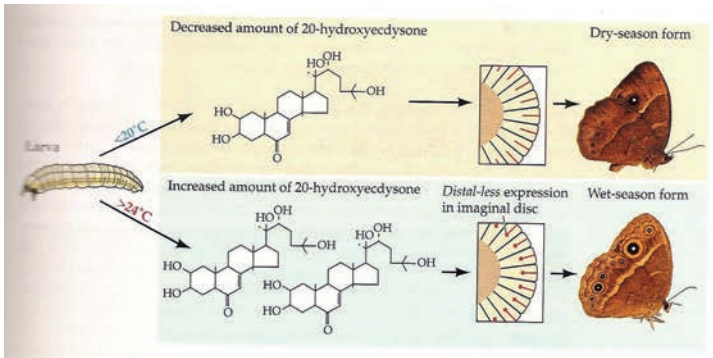


FIGURE 2.9 Phenotypic plasticity in *Bicyclus anynana* is regulated by temperature. High temperature (either in the wild or in controlled laboratory conditions) allows the accumulation of 20-hydroxyecdysone (20E), a hormone that is able to sustain *Distal-less* expression in the pupal imaginal disc. The region of *Distal-less* expression becomes the focus of each eyespot. In cooler weather, 20E is not formed, *Distal-less* expression in the imaginal disc begins but is not sustained, and eyespots fail to form. (Photographs courtesy of S. Carroll and P. Brakefield.)

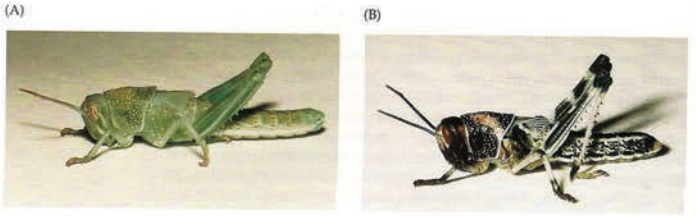


FIGURE 1.3 Density-induced polyphenism in the desert (or "plague") locust *Schistocerca gregaria*. (A) The low-density morph has green pigmentation and miniature wings. (B) Triggered by crowding, the high-density morph develops with deep pigmentation and wing and leg development suitable for migration. (From Tawfik et al. 1999, courtesy of S. Tanaka.)

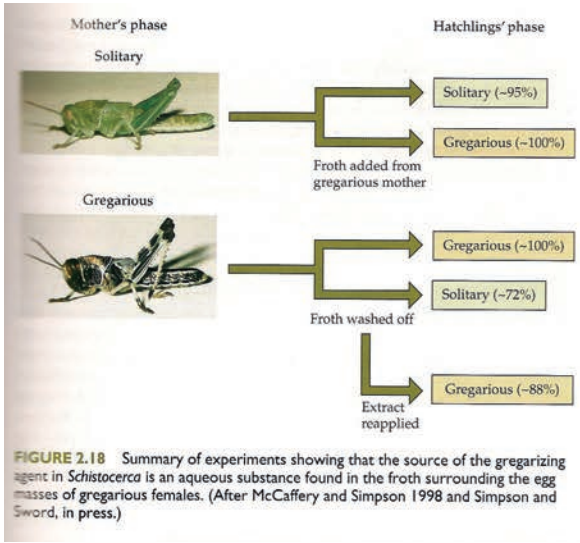


FIGURE 2.18 Summary of experiments showing that the source of the gregarizing agent in *Schistocerca* is an aqueous substance found in the froth surrounding the egg masses of gregarious females. (After McCaffery and Simpson 1998 and Simpson and Sword, in press.)

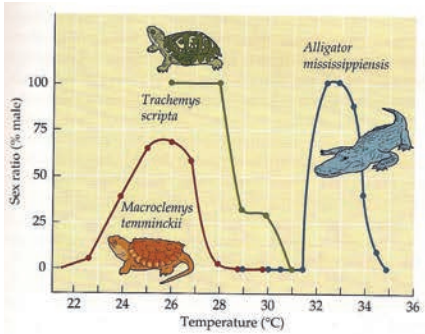
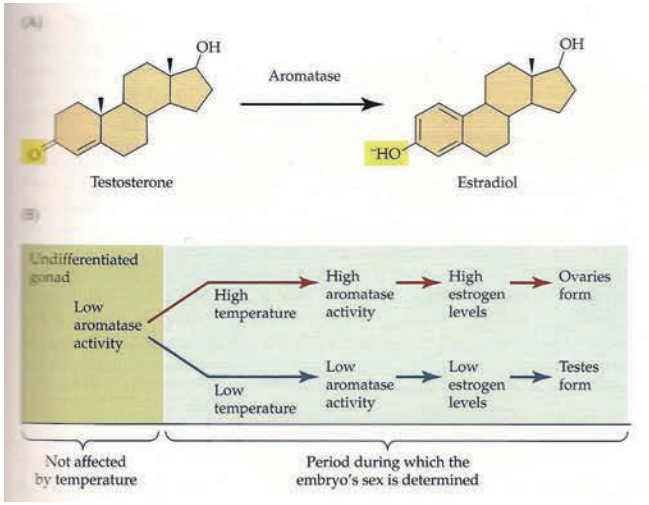


FIGURE 1.6 Temperature-dependent sex determination in three different reptilian species: the American alligator (*Alligator mississippiensis*), the red-eared slider turtle (*Trachemys scripta*), and the alligator snapping turtle (*Macroclemys temminckii*). (After Crain and Guillette 1998.)



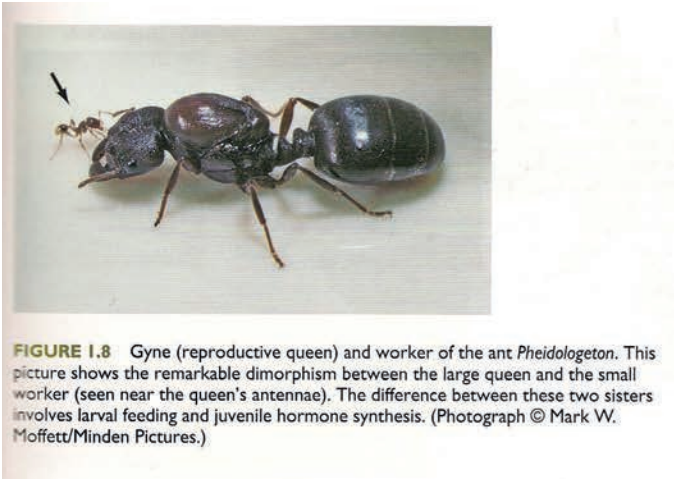
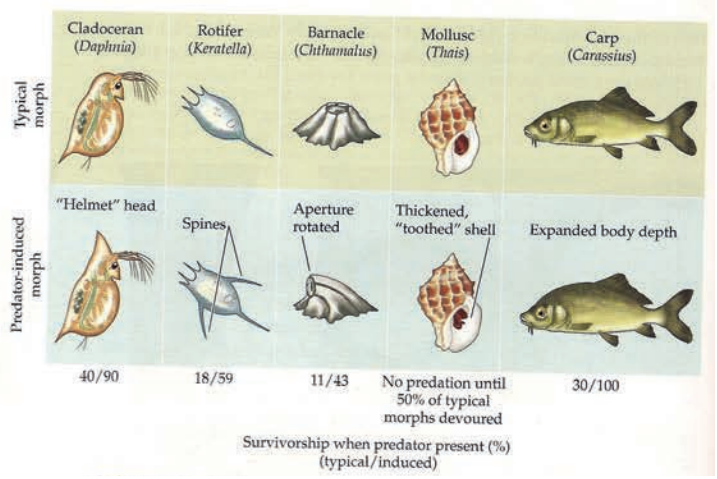


FIGURE 1.8 Gyne (reproductive queen) and worker of the ant *Pheidologeton*. This picture shows the remarkable dimorphism between the large queen and the small worker (seen near the queen's antennae). The difference between these two sisters involves larval feeding and juvenile hormone synthesis. (Photograph © Mark W. Moffett/Minden Pictures.)



Environmental Epigenetics
(Historic Observation)

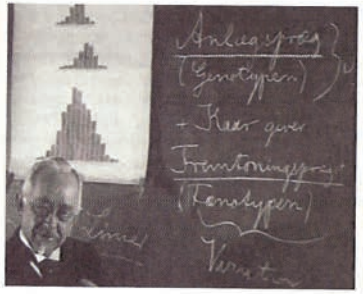
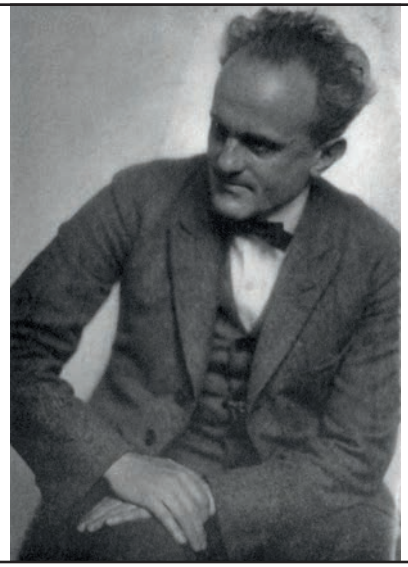
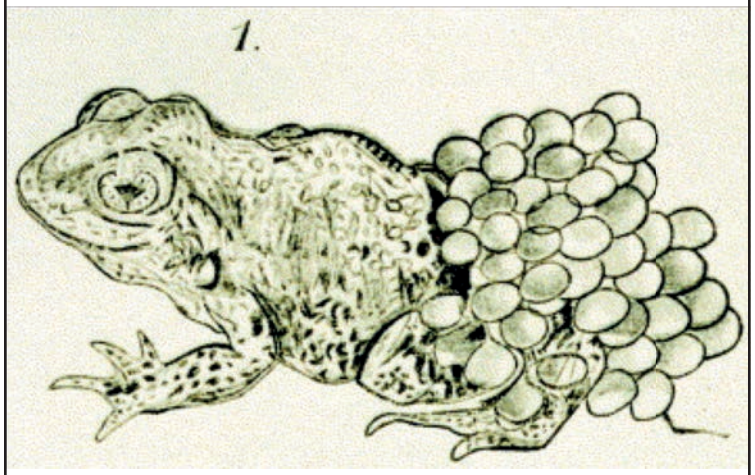


FIGURE 1.2 One hundred years ago, Wilhelm Johannsen noted that the phenotype is the product of both the genome and environmental circumstances. Here he writes on the board that *Anlaegspraeg* (genotype) + *Kaar* (Danish for "conditions" or "circumstances") gives *Fremtonigspraeg* (phenotype). (Photograph from a movie of Professor Johannsen at http://www.wjc.ku.dk/library/video/original.avi.)

Did Paul Kammerer Discover Epigenetic Inheritance? A Modern Look at the Controversial Midwife Toad Experiments





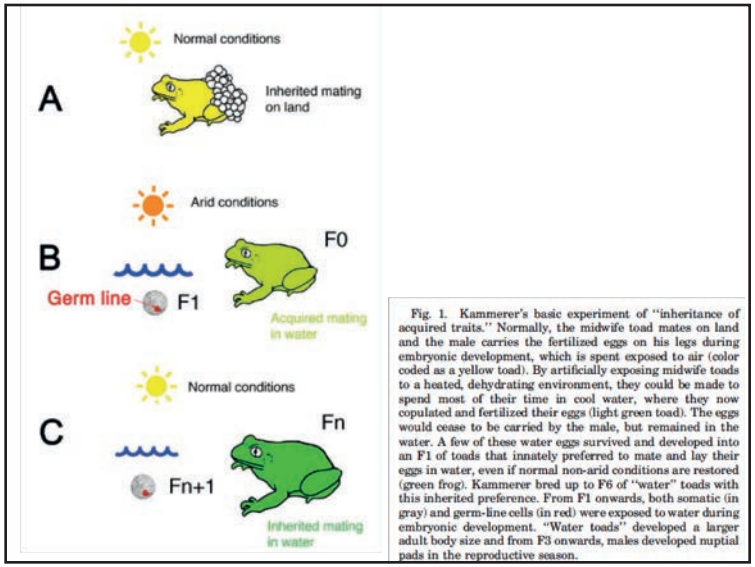


Fig. 1. Kammerer's basic experiment of "inheritance of acquired traits." Normally, the midwife toad mates on land and the male carries the fertilized eggs on his legs during embryonic development, which is spent exposed to air (color coded as a yellow toad). By artificially exposing midwife toads to a heated, dehydrating environment, they could be made to spend most of their time in cool water, where they now copulated and fertilized their eggs (light green toad). The eggs would cease to be carried by the male, but remained in the water. A few of these water eggs survived and developed into an F1 of toads that innately preferred to mate and lay their eggs in water, even if normal non-arid conditions are restored (green frog). Kammerer bred up to F6 of "water" toads with this inherited preference. From F1 onwards, both somatic (in gray) and germ-line cells (in red) were exposed to water during embryonic development. "Water toads" developed a larger adult body size and from F3 onwards, males developed nuptial pads in the reproductive season.

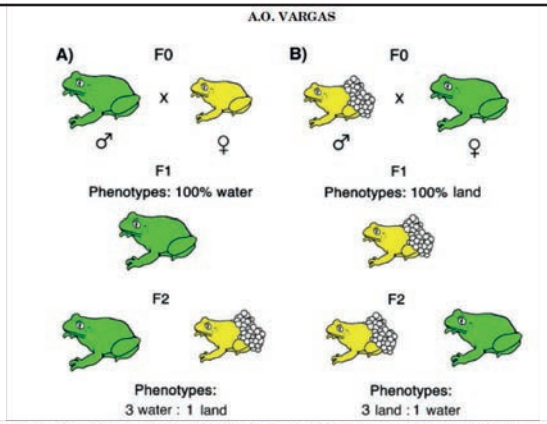
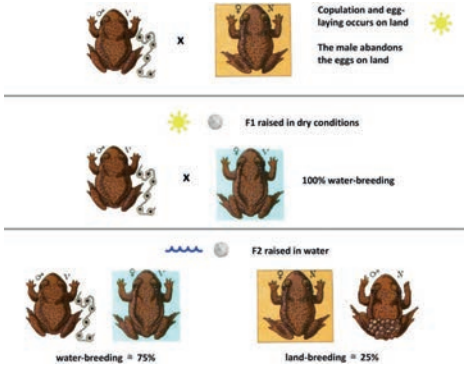


Fig. 2. Parent-of-origin effects in Kammerer's hybrid crosses. Kammerer reported that hybrid crosses of "water" and "land" toads rendered a typically Mendelian phenomenon of dominance, with 100% dominant phenotypes at F1 and a rough 3:1 ratio of dominant to recessive phenotypes at F2. However, Kammerer also reported a complication: either the land phenotype, or the water phenotype, would be dominant depending on which phenotype was present in the male used in the hybrid cross, such that if the male is a water toad, the water phenotype dominates (A), whereas if the male is a land toad, the land phenotype dominates (B). This parent-of-origin effect is currently well known in non-Mendelian genetics, in which the passage of an allele through the germ-line of one of the sexes only (paternal or maternal) determines the inactivation of the allele in the progeny. This occurs in genomic imprinting by DNA methylation as well as in experiments of environmental modification of epigenetic inheritance. Kammerer had no good reason to make up this complication, which did not contribute to his Lamarckian ideas.

An Epigenetic Perspective on the Midwife Toad Experiments of Paul Kammerer (1880-1926).
J Exp Zool B Mol Dev Evol. 2017 Jan;328(1-2):179-192.
Vargas AO, Krabichler Q, Guerrero-Bosagna C.



Environmental Impact on Biology

- Regional Disease Frequencies
- Low Frequency of Genetic Component of Disease (GWAS)
- Increases In Disease Frequencies
- Identical Twins and Variable Disease Frequency
- Environmental Exposures and Disease
- Evolutionary Regional Differences and Rapid Induction

Environmental Epigenetics (Twin Studies)

Twin methodology in epigenetic studies.
J Exp Biol. 2015 Jan 1;218(Pt 1):134-9.
Tan Q, Christiansen L, von Bornemann Hjelmborg J, Christensen K.

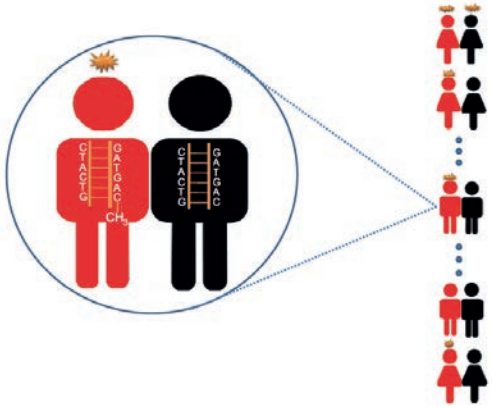
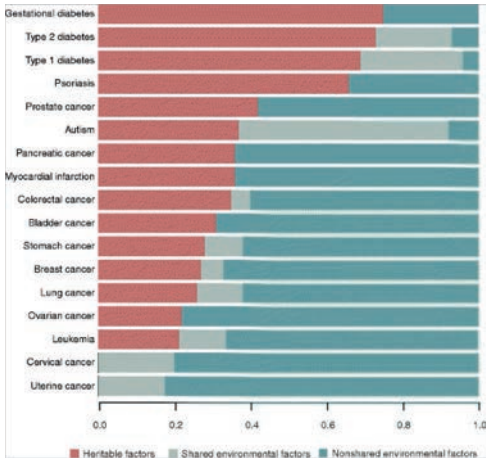


Table 1. Summary of epigenetic studies using MZ twin pairs discordant for disease or trait

Study	Year of publication	Sample size (pairs)	Coverage	Laboratory method	Statistical method	Discordant trait	Finding
Weksberg et al.	2002	10	*KCNQ1OT1* gene	Southern blotting	Descriptive	BWS	Loss of imprinting predisposes to BWS
Petronis et al.	2003	2	*DRD2* gene	Bis-seq	Descriptive	Schizophrenia	High epigenetic similarity in affected twins
Oates et al.	2006	1	*AXIN1* gene	Bis-seq	Descriptive	Caudal duplication	High methylation at promoter region of AXIN1 gene in affected twin
Mill et al.	2006	12	*COMT* gene	Bis-seq	Descriptive	Birth weight	Epigenetic variation in MZ twins may play a key role in the etiology of psychopathology
Kuratomi et al.	2008	1	Genome-wide	MS-RDA	Descriptive	Bipolar	Altered DNA methylation of *PPIEL* is associated with bipolar
Kaminsky et al.	2008	1	Genome-wide	Array	Descriptive	Risk-taking behaviour	Some DNA methylation differences may have developmental and behavioural implications
Mastroeni et al.	2009	1	Genome-wide	Immunohisto-chemistry	*t*-test	Alzheimer's disease	Low levels of DNA methylation observed in temporal neocortex neuronal nuclei of diseased twin
Javierre et al.	2010	5	Genome-wide	Array	Paired *t*-test	Systemic lupus erythematosus	Twin discordance is associated with widespread changes in DNA methylation
Baranzini et al.	2010	3	Genome-wide	Array	Descriptive	Multiple sclerosis	Very few changes in CpG methylation between siblings
Nguyen et al.	2010	3	Genome-wide	Array	SAM	Autism	Epigenetic contributions to autism confirmed
Harder et al.	2010	8	NF1 regions	Bis-seq	Paired *t*-test	NF1	DNA methylation of normal NF1 allele responsible for modification of NF1 phenotype
Tierling et al.	2011	1	Candidate regions	Bis-seq	Descriptive	BWS	KvDMR1 was exclusively hypomethylated in all cell types of the affected BWS twin
Souren et al.	2011	8	Candidate regions	Bis-seq	Paired *t*-test	BMI	DNA methylation variability at nine regions does not contribute to discordancy in BMI
Dempster et al.	2011	22	Genome-wide	Infinium array	Paired *t*-test	Bipolar	Further evidence for DNA methylation in the etiology of major psychoses
Gervin et al.	2012	27	Genome-wide	Infinium array	Paired *t*-test	Psoriasis	Epigenetic alteration potentially contributes to the development of psoriasis
Runyon et al.	2012	21	Selected loci	Bis-seq	Paired *t*-test	Asthma	Differential function of T cell subsets is regulated by changes in DNA methylation
Souren et al.	2013	17	Genome-wide	Infinium array	Rank test	Birth weight	No distinguishable genome-wide DNA methylation differences due to birth weight discordance
Wong et al.	2014	50	Genome-wide	Infinium array	Paired *t*-test	Autism	Numerous differentially methylated regions associated with autism

MZ, monozygotic; BWS, Beckwith–Wiedemann syndrome; NF1, neurofibromatosis type 1.

Epigenetics of discordant monozygotic twins: implications for disease.
Castillo-Fernandez JE, Spector TD, Bell JT.
Genome Med. 2014 Jul 31;6(7):60.



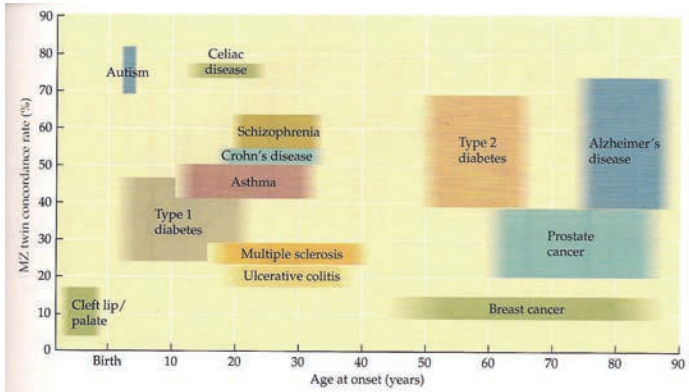


FIGURE 7.14 Age of disease onset and concordance of occurrence among monozygotic (MZ) twins for a number of pathologies. The concordance rate reflects the probability that both twins will be affected; a low rate indicates that often one twin has the disease and the other does not. Note that there is no correlation between age of onset and the degree of concordance. (After Petronis 2006.)

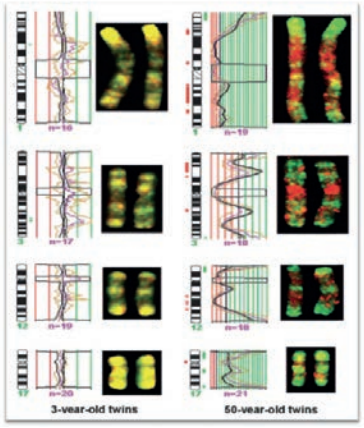


Fig. 5. Mapping chromosomal regions with differential DNA methylation in MZ twins by using comparative genomic hybridization for methylated DNA. Competitive hybridization onto normal metaphase chromosomes of the AIMS products generated from 3- and 50-year-old twin pairs. Examples of the hybridization of chromosomes 1, 3, 12, and 17 are displayed. The 50-year-old twin pair shows abundant changes in the pattern of DNA methylation observed by the presence of green and red signals that indicate hypermethylation and hypomethylation events, whereas the 3-year-old twins have a very similar distribution of DNA methylation indicated by the presence of the yellow color obtained by equal amounts of the green and red dyes. Significant DNA methylation changes are indicated as thick red and green blocks in the ideograms (Reproduced from Fraga et al. 2005)

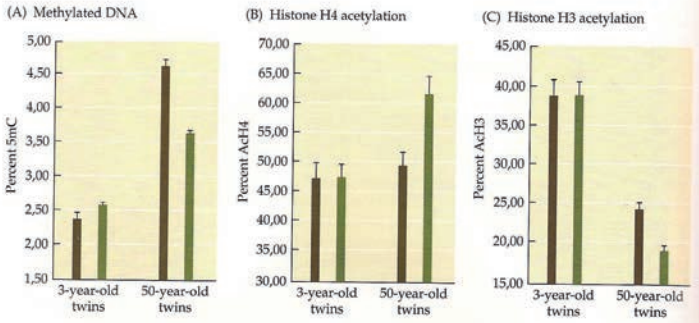
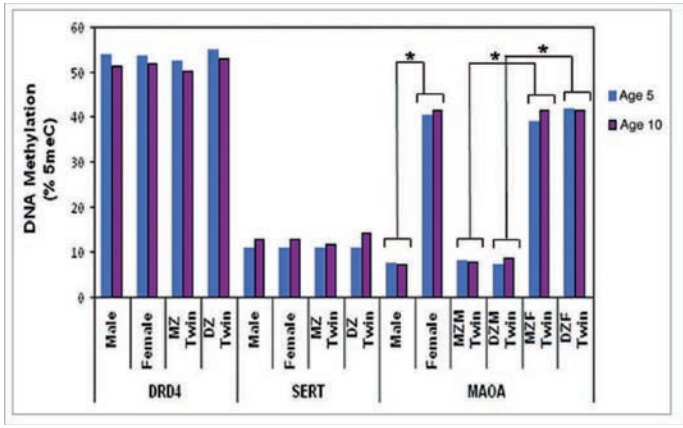


FIGURE 7.15 Monozygotic twins start off with identical amounts of (A) methylated DNA, (B) histone H4 acetylation, and (C) histone H3 acetylation. However, in older pairs, the patterns of methylation and acetylation have become discordant. (After Fraga et al. 2005.)

A longitudinal study of epigenetic variation in twins.

Wong CC, Caspi A, Williams B, Craig IW, Houts R, Ambler A, Moffitt TE, Mill J.

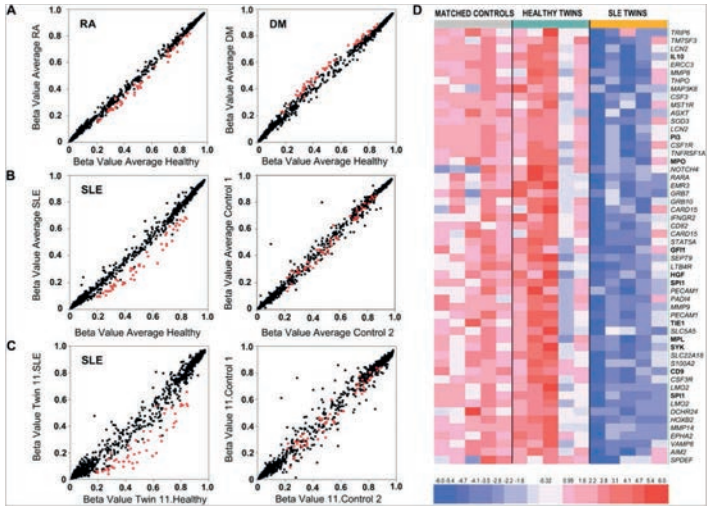
Epigenetics. 2010 Aug 4;5(6). [Epub ahead of print]



Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus.

Javierre BM, Fernandez AF, Richter J, Al-Shahrour F, Martin-Subero JI, Rodriguez-Ubreva J, Berdasco M, Fraga MF, O'Hanlon TP, Rider LG, Jacinto FV, Lopez-Longo FJ, Dopazo J, Forn M, Peinado MA, Carreño L, Sawalha AH, Harley JB, Siebert R, Esteller M, Miller FW, Ballestar E.

Genome Res. 2010 Feb;20(2):170-9.



Identical twins doubly exchanged at birth: a case report of genetic and environmental influences on the adult epigenome.

Segal NL, Montoya YS, Loke YJ, Craig JM.

Epigenomics. 2017 Jan;9(1):5-12.

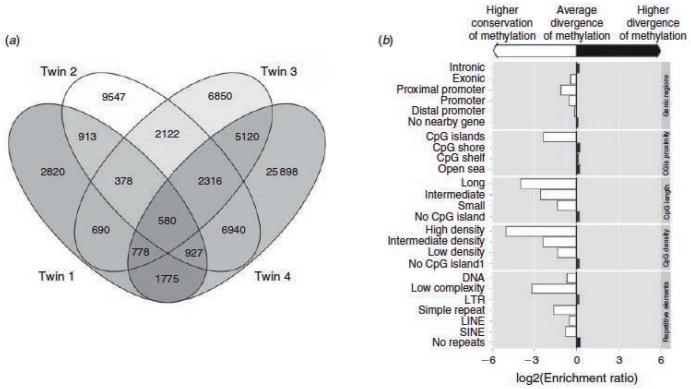
Executive summary

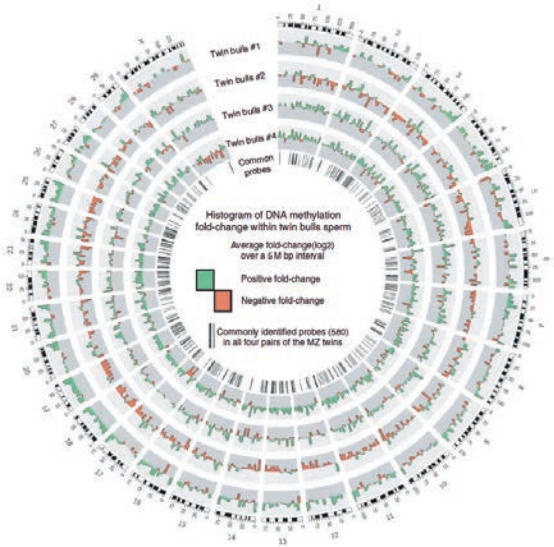
- Monozygotic twins reared apart (MZA) are good models for studying the influence of pre- and postnatal environments on epigenetics, while controlling for shared genetics.
- Genetic and intrauterine environmental factors appeared to have a stronger influence on DNA methylation than rearing environment, with the latter being very different for the two twin pairs.
- The largest effects of the rearing environment on DNA methylation within our two MZA pairs involve genes relevant to immune response and cell death.

Genome-wide analysis of sperm DNA methylation from monozygotic twin bulls.

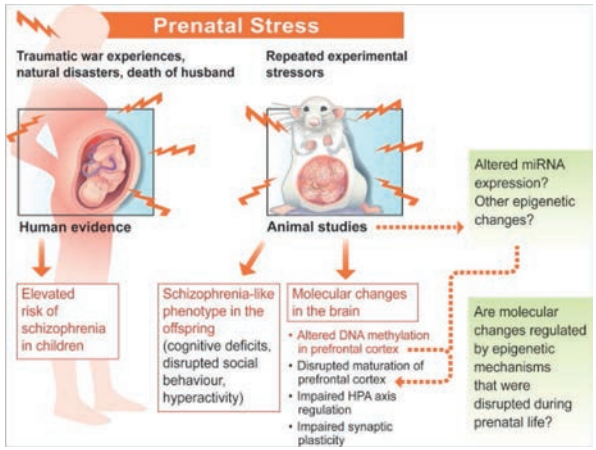
Reprod Fertil Dev. 2016 Jan 12 [Epub ahead of print]

Shojaei Saadi HA, Fournier É, Vigneault C, Blondin P, Bailey J, Robert C.





Environmental Epigenetics (Early Life History Exposures)



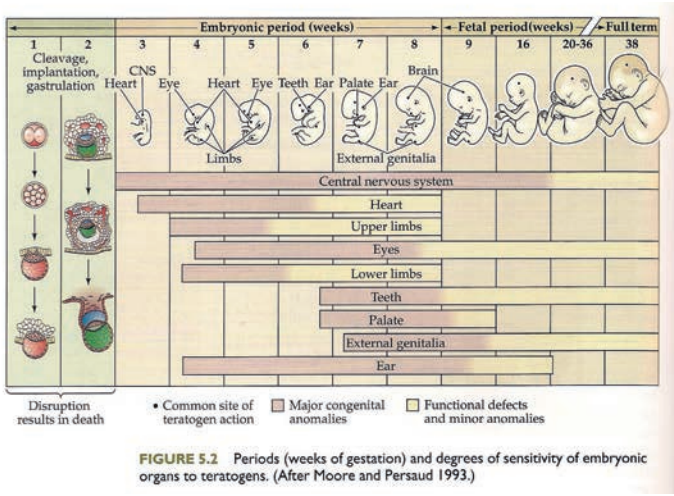


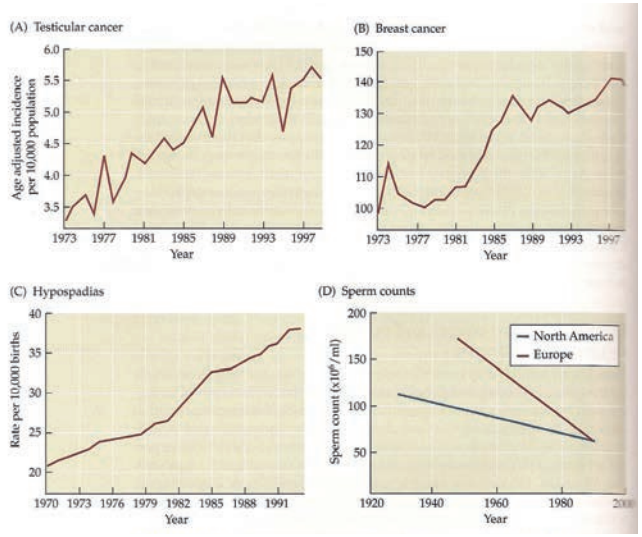
FIGURE 5.2 Periods (weeks of gestation) and degrees of sensitivity of embryonic organs to teratogens. (After Moore and Persaud 1993.)

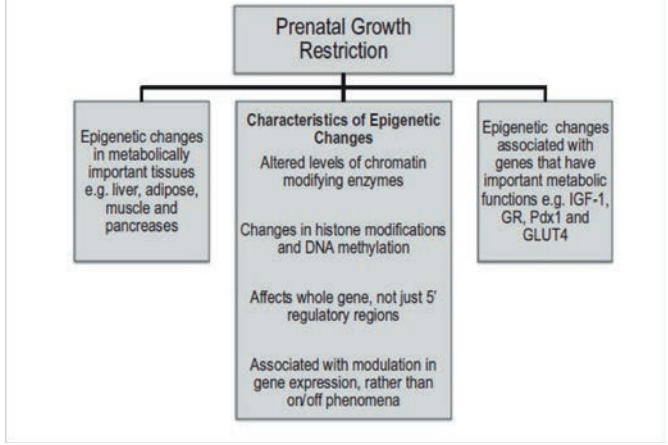
TABLE 5.1 Some agents thought to disrupt human fetal development[a]

DRUGS AND CHEMICALS	IONIZING RADIATION (X-RAYS)
Alcohol	**HYPERTHERMIA (FEVER)**
Aminoglycosides (Gentamycin)	**INFECTIOUS MICROORGANISMS**
Aminopterin	Coxsackie virus
Antithyroid agents (PTU)	Cytomegalovirus
Bromine	Herpes simplex
Cortisone	Parvovirus
Diethylstilbesterol (DES)	Rubella (German measles)
Lead	*Toxoplasma gondii* (toxoplasmosis)
Methylmercury	*Treponema pallidum* (syphilis)
Penicillamine	**METABOLIC CONDITIONS IN THE MOTHER**
Retinoic acid (Isotretinoin, Accutane)	Autoimmune disease (including Rh incompatibility)
Streptomycin	Diabetes
Tetracycline	Dietary deficiencies, malnutrition
Thalidomide	Phenylketonuria
Trimethadione	
Valproic acid	
Warfarin	

Source: Adapted from Opitz 1991.

[a]This list includes known and possible teratogenic agents and is not exhaustive.





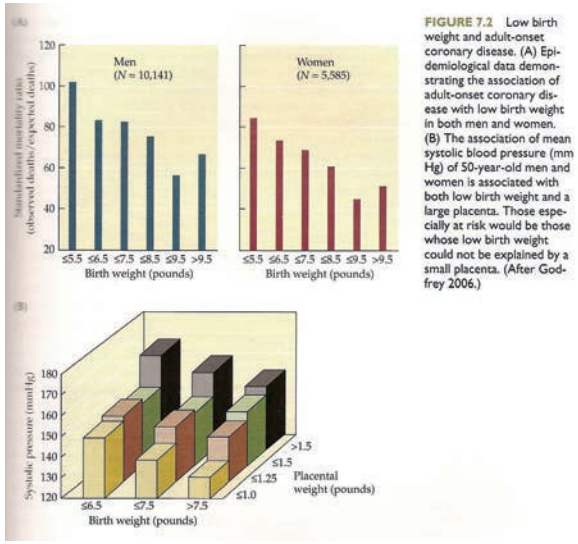


FIGURE 7.2 Low birth weight and adult-onset coronary disease. (A) Epidemiological data demonstrating the association of adult-onset coronary disease with low birth weight in both men and women. (B) The association of mean systolic blood pressure (mm Hg) of 50-year-old men and women is associated with both low birth weight and a large placenta. Those especially at risk would be those whose low birth weight could not be explained by a small placenta. (After Godfrey 2006.)

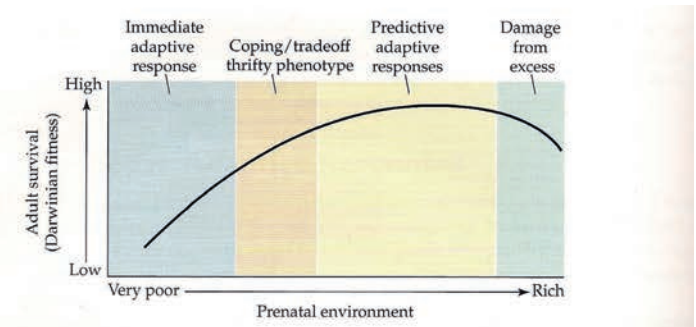


FIGURE 7.7 Developmental responses and fitness. Mutations or severe environmental disturbances can disrupt development and cause *immediate responses* to the injury or defect. Less extreme disturbances can lead to *predictive adaptive responses* (PARs), including the induction of thrifty phenotypes. Within the normal range of variation, an individual's developmental trajectory will be conferred by the actions of PARs. (After Gluckman and Hanson 2006b.)

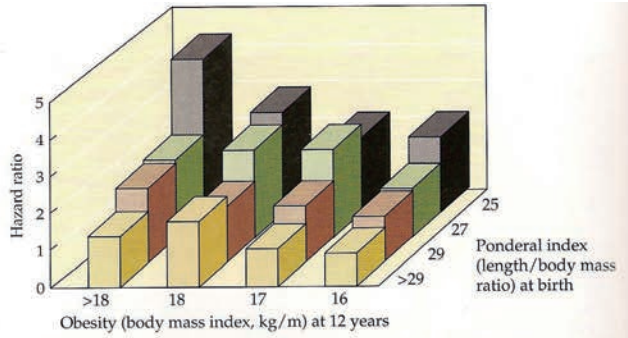


FIGURE 7.10 Data showing that the risk of coronary heart disease is increased by both small birth size and greater fatness in childhood. The graph shows that these conditions interact such that the highest risk of heart disease is in those people who were born at low birth weight (i.e., in conditions that may have caused a thrifty phenotype) but subsequently experienced ample nutrition. (After Eriksson et al. 2001.)

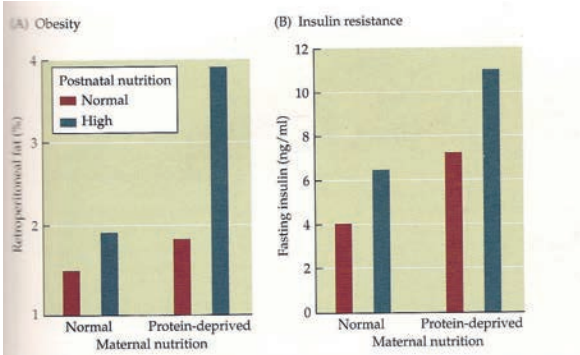


FIGURE 7.11 Experimental evidence for the fetal origin of adult disease in rats. Rat fetuses were exposed to either normal or protein-deficient maternal diets. After birth, the pups were given either normal or high nutrition. They were then measured for (A) obesity (percent fatty tissue in the abdominal area between the peritoneal cavity and the skin) and (B) insulin resistance (as measured by the amount of insulin in their blood after fasting). Obesity and insulin resistance were greater in the pups whose mothers were fed a protein-deficient diet. High nutrition after birth heightened these differences. (After Vickers et al. 2000.)

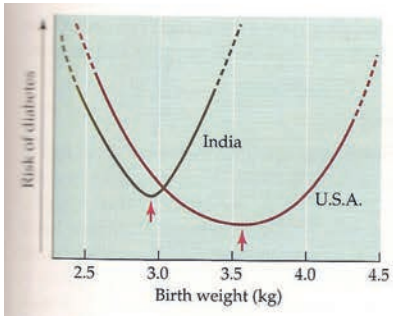
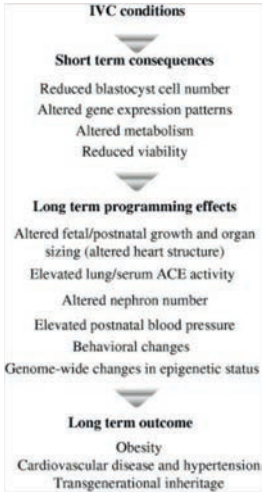


FIGURE 7.13 The risk of adult-onset diabetes is related to birth weight in both India and the United States. The U-shaped curve indicates that the risk for this disease increases at both very high and very low birth weights. However, the optimum birth weight is different for the two populations (arrows), suggesting that developmental plasticity may have resulted in adaptation to distinct adult nutritional milieus. (After Gluckman and Hansen 2005.)

Environmental Epigenetics (Cell Culture Effects)

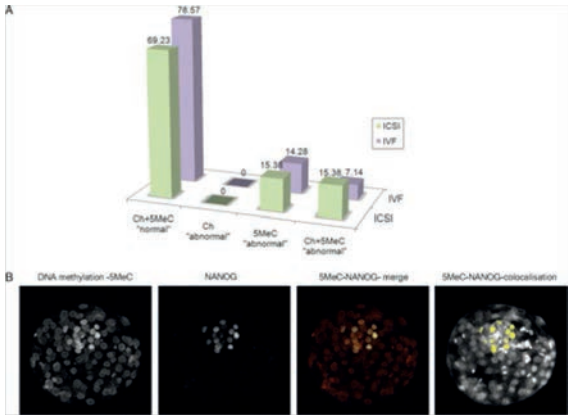


Long-term programming of postnatal growth and physiology can be induced irreversibly during the preimplantation period of development by adverse preconceptional environment in vitro (for example suboptimal in vitro culture).

Evaluation of epigenetic marks in human embryos derived from IVF and ICSI.

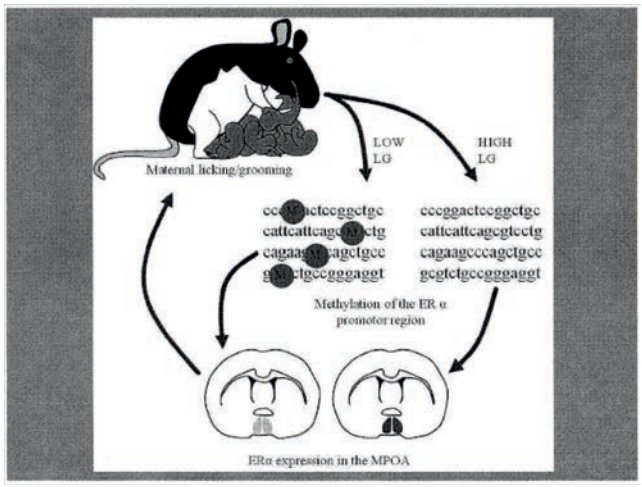
Santos F, Hyslop L, Stojkovic P, Leary C, Murdoch A, Reik W, Stojkovic M, Herbert M, Dean W.

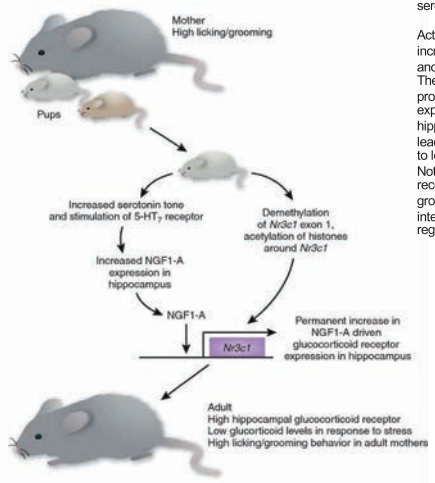
Hum Reprod. 2010 Sep;25(9):2387-95.



Epigenetic lineage asymmetry is conserved in human embryos. (A) Assessment of epigenotypes in blastocysts arising from ART procedures. Blastocysts generated by IVF (n = 14) or ICSI (n = 13) were stained and are portrayed in the discontinuous plot from double normal to double abnormal scores. In contrast to other embryo stages obtained throughout the study, blastocysts showed an overwhelmingly significant positive correlation with normal epigenotype scores (Table III, P < 0.001). (B) High morphology score expanded blastocysts were labelled for a lineage identification marker, NANOG, followed by staining for 5MeC. ICM cells were consistently hypermethylated and positive for NANOG. Co-localisation software, Image J, was used to analyse potential association between the epigenetic mark and the pluripotency factor. Bright yellow cells identified most, but not all, of the cells of the ICM, highlighting the intrinsic heterogeneity associated with this compartment. In the expanded blastocyst NANOG is highly specific for the ICM and hence is justifiably used as a lineage marker.

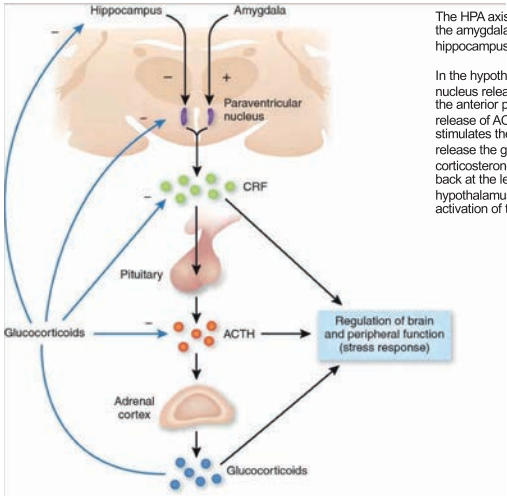
Environmental Epigenetics (Early Life History Brain Effects)





High licking and grooming of rat pups increases serotonin tone in the brain.

Acting via 5-HT7 receptors and cyclic AMP, this increases expression of NGF1-A, which binds to and activates the promoter of the Nr3c1 gene. The pups also show demethylation of the Nr3c1 promoter, which facilitates long-term gene expression. The result is high levels of hippocampal glucocorticoid receptor expression, leading to enhanced feedback inhibition and thus to low cortisol levels in response to stress. Notably, females with high levels of glucocorticoid receptor expression show high licking and grooming behaviors on their offspring, and thus intergenerational transmission of epigenetic regulation of the Nr3c1 gene.



The HPA axis is under the excitatory control of the amygdala and inhibitory control of the hippocampus.

In the hypothalamus, the paraventricular nucleus releases CRF, which is transported to the anterior pituitary, where it causes the release of ACTH into the blood stream. ACTH stimulates the adrenal cortex to synthesize and release the glucocorticoids cortisol (humans) or corticosterone (rodents). Glucocorticoids feed back at the level of the hippocampus, hypothalamus and pituitary to dampen excess activation of the HPA axis.

Lasting epigenetic influence of early-life adversity on the BDNF gene.

Roth TL, Lubin FD, Funk AJ, Sweatt JD.

Biol Psychiatry. 2009 May 1;65(9):760-9.

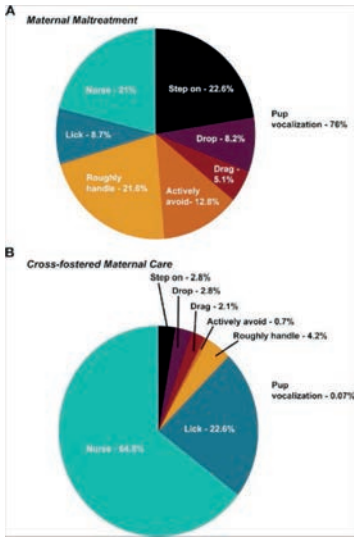


Figure 1. Infants experienced an adverse caregiving environment. (A) Qualitative assessment of the percent occurrence of pup-directed behaviors in the maltreatment condition indicates that pups experienced predominately abusive behaviors, which resulted in considerable audible pup vocalization. (B) In sharp contrast, pups experienced significant amounts of normal maternal care behaviors in the cross-fostered maternal care condition. Statistical analyses of the maternal behaviors (abusive vs. normal care) are provided in Supplement 3.

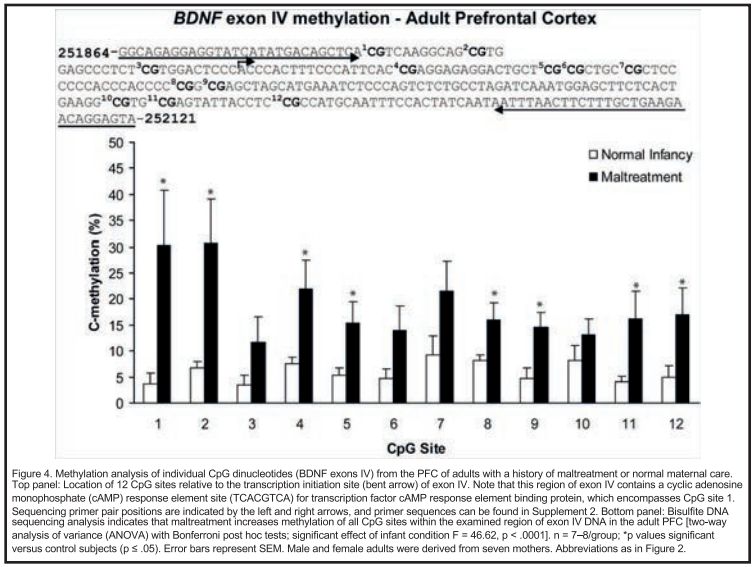
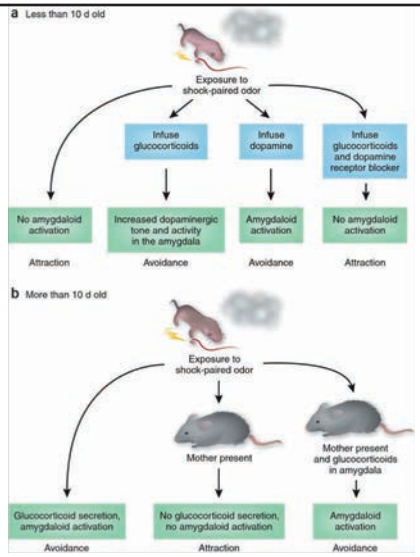


Figure 4. Methylation analysis of individual CpG dinucleotides (BDNF exons IV) from the PFC of adults with a history of maltreatment or normal maternal care. Top panel: Location of 12 CpG sites relative to the transcription initiation site (bent arrow) of exon IV. Note that this region of exon IV contains a cyclic adenosine monophosphate (cAMP) response element site (TCACGTCA) for transcription factor cAMP response element binding protein, which encompasses CpG site 1. Sequencing primer pair positions are indicated by the left and right arrows, and primer sequences can be found in Supplement 2. Bottom panel: Bisulfite DNA sequencing analysis indicates that maltreatment increases methylation of all CpG sites within the examined region of exon IV DNA in the adult PFC [two-way analysis of variance (ANOVA) with Bonferroni post hoc tests; significant effect of infant condition $F = 46.62$, $p < .0001$]. $n = 7–8$/group; *p values significant versus control subjects ($p \leq .05$). Error bars represent SEM. Male and female adults were derived from seven mothers. Abbreviations as in Figure 2.



Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ.

Nat Neurosci. 2009 Mar;12(3):342-8.

Table 1 Demographic characteristics and psychiatric diagnoses

	Abused suicide	Nonabused suicide	Control
Male/female	12/0	12/0	12/0
Age (years)	34.2 ± 10	33.8 ± 11	35.8 ± 12
PMI (h)	24.6 ± 5.8	39.0 ± 25.7	23.5 ± 6.0
pH	6.3 ± 0.24	6.5 ± 0.29	6.5 ± 0.22
Childhood abuse/neglect	12/0 (100%)	0/12 (0%)	0/12 (0%)
Mood disorder	8/12 (67%)	8/12 (67%)	0/12 (0%)
Alcohol/drug abuse disorder	9/12 (75%)	6/12 (50%)	5/12 (42%)

Data are presented as mean ± s.d.

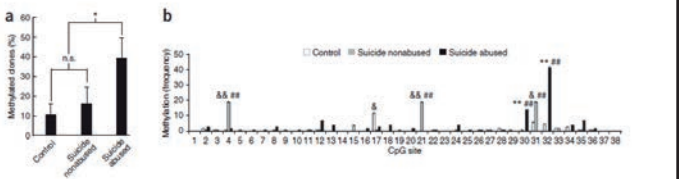
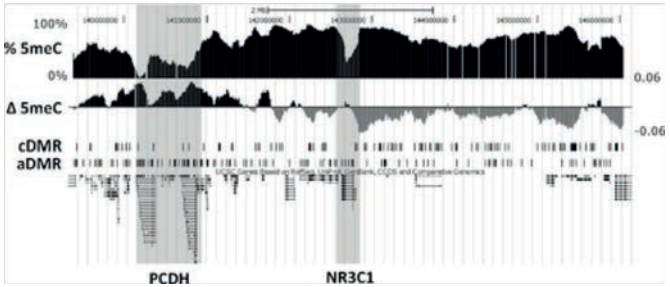
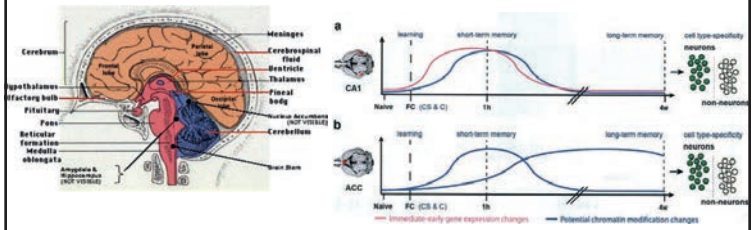


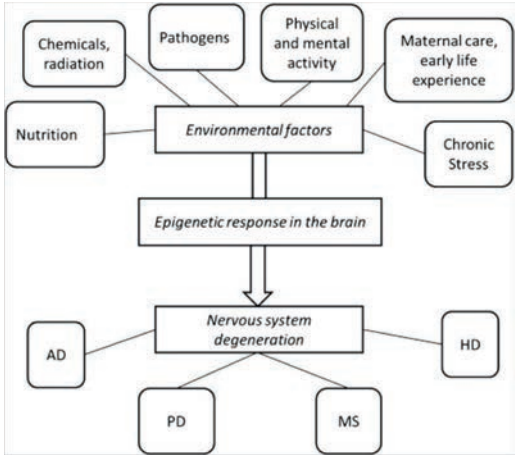
Figure 2 Methylation of the *NR3C1* promoter in the hippocampus. Twenty clones were sequenced for each subject for methylation mapping. (**a**) Mean ± s.e.m. percentage of methylated clones for suicide victims with a history of childhood abuse ($n = 12$), suicide victims without a history of childhood abuse ($n = 12$) and controls ($n = 12$). The methylation percentage was calculated as the number of clones with at least one methylated CpG site divided by the total number of clones (* indicates $P \leq 0.05$; n.s. indicates not statistically significant). (**b**) Methylation of the *NR3C1* promoter region, showing the frequency of methylation observed at each CpG site for suicide victims with a history of childhood abuse, suicide victims with no history of childhood abuse and control subjects (*$P < 0.05$, **$P < 0.001$, abused suicides versus controls; &$P < 0.05$, &&$P < 0.001$, non-abused suicides versus controls; #$P < 0.05$, ##$P < 0.001$, abused suicides versus non-abused suicides; Bonferroni *post hoc* comparisons).

Suderman M, et al. (2012) Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. Proc Natl Acad Sci U S A. 16;109 Suppl 2:17266-72

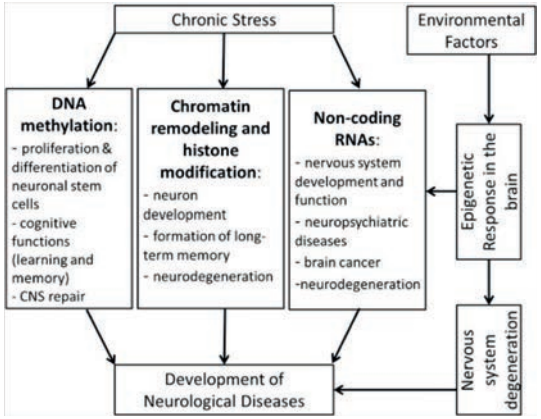


Effect of stress on human biology: Epigenetics, adaptation, inheritance, and social significance.
J Cell Physiol. 2018 Mar;233(3):1975-1984.
Denhardt DT.



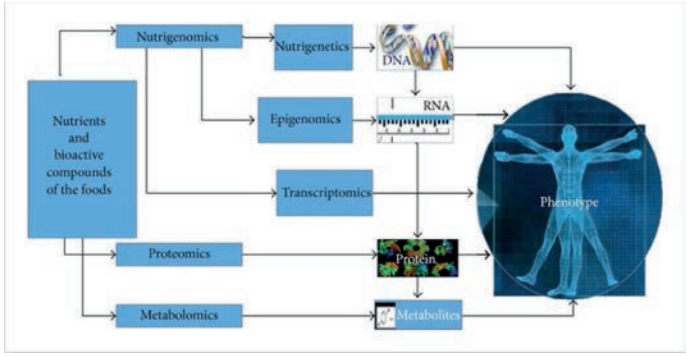


Role of epigenetic regulation as a central regulatory interface that translates the relationship between brain and behavior into patterns of gene expression. Different environmental factors such as nutrition, physical and mental activity, maternal care and early life experience, exposure to chronic stress, chemicals and radiation, and pathogens can alter the epigenetic pattern in the brain. These processes in turn may either delay or accelerate neurodegenerative processes in the nervous system and contribute to the onset and progression of neurological conditions, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and multiple sclerosis (MS).



Stress might be a main contributing factor to enhanced risk and accelerated progression of neurodegenerative diseases. The intermediate link between stress and neurodegeneration represent epigenetic components, such as DNA methylation, chromatin remodeling and histone modification, and non-coding RNAs in the nervous system.

Environmental Epigenetics (Nutrition Effects)



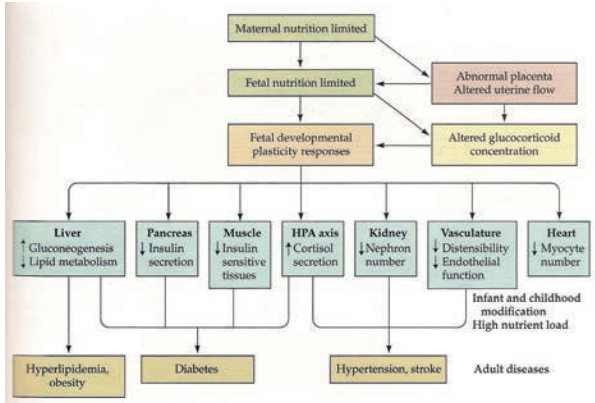
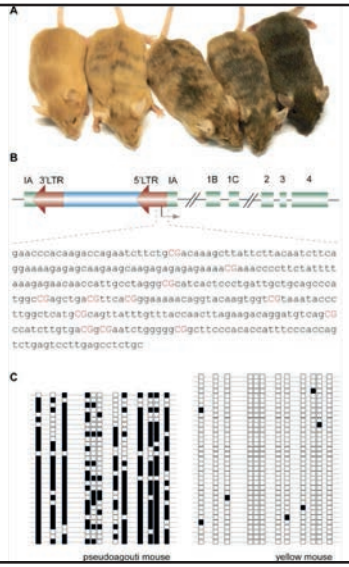


FIGURE 7.6 The thrifty phenotype hypothesis. Altered maternal nutrition leads to a cascade of consequences mediated by the phenotypic plasticity of the fetal organs. The combination of biochemical and anatomical changes in these organs produces a phenotype that stores excess energy as fats and sacrifices kidney growth for brain growth. This phenotype is beneficial under environments of low nutrition, but high nutrition during infancy and/or childhood can result in adult obesity, diabetes, and hypertension. (After De Boo and Harding 2006.)

Table 3. Effect of maternal dietary modifications on imprinted gene, IGF2

Species	Modification during development	Time winnow for the modification	Observations in offspring	Reference
Human	Famine (Dutch Famine cohort)	Periconceptional period	Decrease in methylation of CpG dinucleotides in the IGF2 nearly 60 years after	[35]
Human	Supplementary folic acid use	Periconceptional period	Higher methylation of the IGF2 differentially methylated region (DMR)	[62]
Rat	Maternal low protein diet (8% vs 20 %) and high fat diet (45% vs 10%) after weaning	2 weeks prior to mating – gestation – lactation	Increase in adipose tissue Igf2 mRNAs by the low protein prenatal diet	[38]
Rat	Maternal low protein diet (9% vs 19% control)	Preimplantation period	Decrease in H19 imprinted gene expression in male blastocysts; reduction in H19 and Igf2 expression in male fetal liver at day 20 of gestation	[36]
Mouse	Methyl deficiency (methionine, choline, folic acid and vitamin B12)	60-day post-weaning	Loss of imprinting of Igf2	[37]



Phenotypic variation in A^{vy} mice, A^{vy} allele structure, and complex CpG methylation at the IAP.

A. Phenotypes of isogenic A^{vy} littermates range from pure yellow and obese (left) through mottled yellow/agouti to lean fully agouti, called pseudoagouti (right). **B.** Schematic of the A^{vy} locus, with the sequence of the amplified region, which includes portions of the 5′LTR and pseudoexon 1A (−240 to +92 relative to the cryptic promoter, which is marked by an arrow). The start point of Avy transcription [11] is marked by an arrow. CpG dinucleotides are displayed in red. **C.** Representative bisulphite allelic sequencing profiles of individual alleles from yellow and pseudoagouti mice. Each single row represents a single allele, and each box a CpG (white: unmethylated; black: methylated).

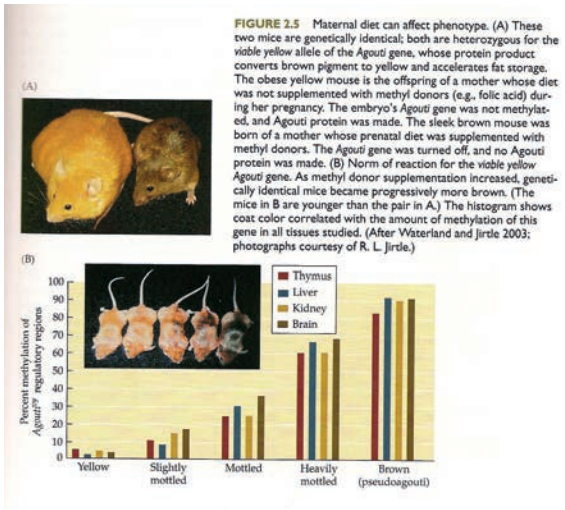
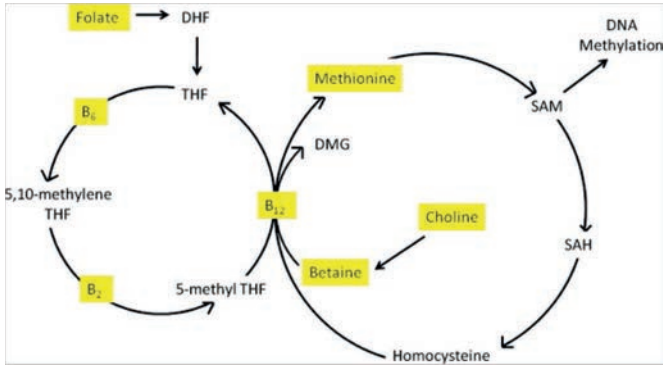
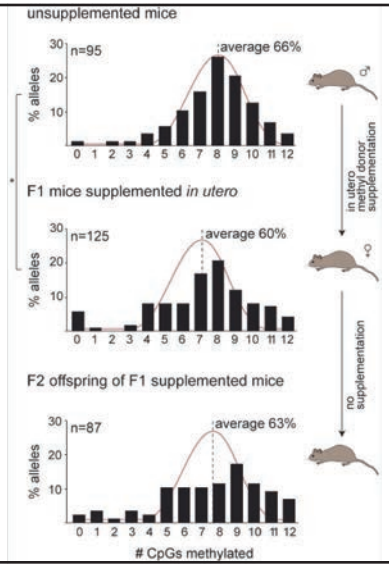
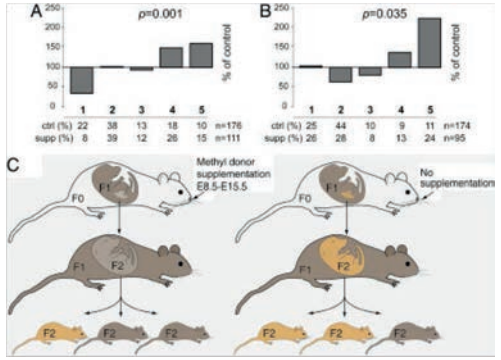


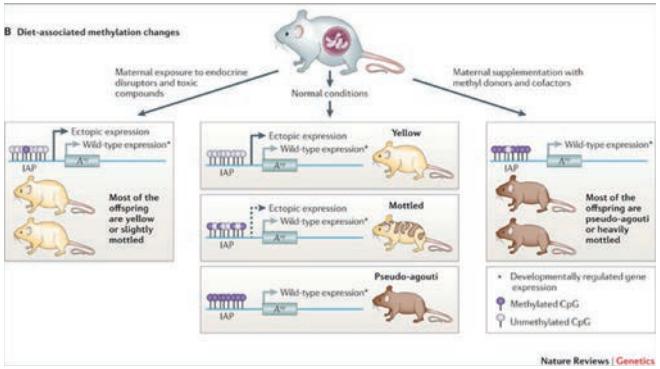
FIGURE 2.5 Maternal diet can affect phenotype. (A) These two mice are genetically identical; both are heterozygous for the *viable yellow* allele of the *Agouti* gene, whose protein product converts brown pigment to yellow and accelerates fat storage. The obese yellow mouse is the offspring of a mother whose diet was not supplemented with methyl donors (e.g., folic acid) during her pregnancy. The embryo's *Agouti* gene was not methylated, and Agouti protein was made. The sleek brown mouse was born of a mother whose prenatal diet was supplemented with methyl donors. The *Agouti* gene was turned off, and no Agouti protein was made. (B) Norm of reaction for the *viable yellow Agouti* gene. As methyl donor supplementation increased, genetically identical mice became progressively more brown. (The mice in B are younger than the pair in A.) The histogram shows coat color correlated with the amount of methylation of this gene in all tissues studied. (After Waterland and Jirtle 2003; photographs courtesy of R. L. Jirtle.)

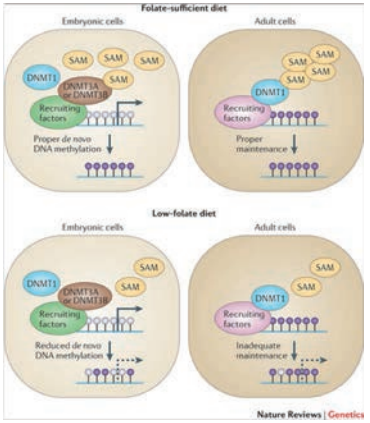






Midgestation and germ-line effects of methyl donor supplementation. (A) F_1 phenotypes. Shown is the effect of methyl donor supplementation on mid-gestation supplemented offspring. Dams were supplemented with methyl donors from E8.5 to E15.5, and offspring phenotypes were scored as in Fig. 1B. Results are expressed as the percentage of supplemented offspring with the same phenotype as unsupplemented controls, and the percentage of offspring of each coat color is shown beneath each graph. (B) F_2 phenotypes. Shown is the heritable/grandparental effect of methyl donor supplementation. Phenotypes of F_2 offspring from pseudoagouti female mice shown in A are expressed as a percentage of control offspring (offspring from pseudoagouti dams that had never been supplemented) with the same phenotype. (C) A schematic diagram illustrating the effect of methyl donor supplementation in the germ line. Epigenetic changes to A^{vy} in primordial germ cells exposed to methyl donors during differentiation (Left) are maintained throughout gametogenesis and embryogenesis. Thus, pseudoagouti F_1 mice that are genetically and phenotypically identical but were subject to different diets *in utero* (Left vs. Right), can produce phenotypically different F_2 offspring.





Epigenetic transitions play crucial roles in development and in the differentiation of stem cells and primordial germ cells. Concordantly, the regulating enzymes are generally highly expressed in these pluripotent cells6. For example, the de novo methyltransferases, DNMT3A and DNMT3B, are highly expressed in the early embryonic cells in which de novo methylation is acquired. Low amounts of external methyl donor groups from dietary sources can reduce the concentrations of the universal methyl donor, S-adenosylmethionine (SAM), and can readily affect de novo DNA methylation. Also, aberrant gains of methylation may occur in early embryonic cells owing to other external triggers. In adult cells, the maintenance of DNA methylation is performed mainly by the maintenance methyltransferase, DNMT1, in a process that seems less sensitive to diet-induced changes in the abundance of methyl donors.

Table 1
Studies providing for folate impacts on DNA methylation

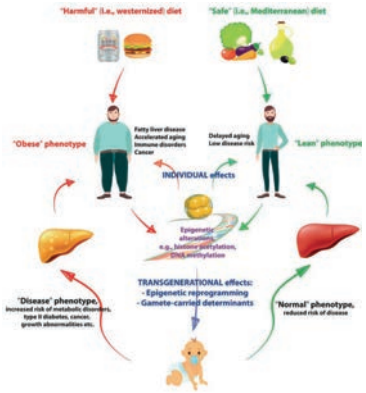
Authors	Study population/tissue	Methylation measure
Human		
Ba et al. (2011) [47]	Pregnant women, maternal blood and cord blood	Gene-specific
Christensen et al. (2011) [50]	Primary breast tumors	Epigenome-wide
Hoyo et al. (2011) [49]	Pregnant women, cord blood leukocytes	Gene-specific
Vineis et al. (2011) [46]	Lung cancer cases and controls, leukocytes	Gene-specific
Stidley et al. (2010) [45]	Smokers, sputum	Gene-specific
Hervouet et al. (2009) [37]	Cells cultured from glioblastomas	Global, Gene-specific
van den Donk et al. (2007) [43]	Colon tumors from MTHFR variants	Gene-specific
Pufulete et al. (2005) [35]	Healthy adult colonic mucosa	Global
Pufulete et al. (2005) [34]	Adults with CRC, leukocytes and colonic mucosa	Global
Shelnutt et al. (2004) [30]	Adult women, leukocytes	Global
van Engeland et al. (2003) [44]	Colon tumors	Gene-specific
Rampersaud et al. (2000) [36]	Postmenopausal women, leukocytes	Global
Mouse		
McKay et al. (2011) [42]	Various adult tissues	Gene-specific
McKay et al. (2011) [31]	Adult small intestine	Global
Wakefield et al. (2010) [38]	Various adult tissues	Gene-specific
Rat		
Ly et al. (2011) [32]	Adult mammary tissue	Global
McKay et al. (2011) [41]	Adult small intestine	Gene-specific
Sie et al. (2011) [33]	Adult colonic mucosa	Global
Burdge et al. (2009) [40]	Adult liver and adipose tissue	Gene-specific
Kim et al. (1997) [39]	Adult liver and blood	Global, gene-specific

Increased cancer incidence in Holocaust survivors and the implications for survivors of other extreme events.
Expert Rev Anticancer Ther. 2018 Nov;18(11):1059-1062.
Keinan-Boker L.

3. Conclusions

Studies show unequivocally that restricted diet in lab animals reduces cancer risk. Observational studies in non-Jewish European populations yielded mixed results which may be attributed to differing study types, definitions of exposure, the selection of control subjects, the nature of the exposure etc. In contrast, most studies of Holocaust survivors clearly indicated an inverse effect despite differing methodologies. Although these findings need consolidation, there are grounds for believing that exposure to hunger and stress under extreme situations may cause a cascade of epigenetic, hormonal, and biological changes that eventually modify cancer risk. Thus, exposed individuals should be regarded as a high risk group for cancer. Holocaust survivors are one example of such a group; these conclusions may be generalized to many populations around the globe, including, for example, the survivors of the civil war in Syria.

The Impact of Nutrition and Environmental Epigenetics on Human Health and Disease.
Int J Mol Sci. 2018 Nov 1;19(11).
Tiffon C.



Graduate Students

Grant Proposal

Outline:
- Title
- Abstract
- Specific Aims
- Background
- Preliminary Results
- Experimental Design and Methods
- References

(5-10 pp. single spaced typed limit)

Key Points:
- Specific aims should be focused and concise and clarify hypothesis
- Be as concise and direct as possible
- Work significance of proposal into grant when appropriate
- Use only critical preliminary results

Additional Information:
- Propose short-range studies to address long-range goals
- Write grant for 3 to 4 year period to complete studies
- Feasibility of success is critical, ask right type of question
- Experimental design needs to address hypothesis

Score/Rating:
Factors involved: Type question addressed, organization of thoughts, preliminary results, feasibility, reasonable completion expectations, focus of aims and proposed studies.

Score		
1.0 - 1.5	**Outstanding**	Funded
1.5 - 2.0	**Excellent**	Probably Funded
2.0 - 2.5	**Good**	Accepted, but not Fund
2.5 - 3.0	**Satisfactory**	
3.0 - 3.5	**Adequate**	
3.5 - 4.0	**Fair**	
4.0 - 5.0	**Acceptable**	

Review:
NIH Study Section style review with all students/fellows participating in the review. Primary and secondary reviewers will be selected and all grants will be critiqued.

Spring 2019 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 11 and 12 (March 19 & 26)

Environmental Epigenetics

- Environmental Impacts on Biology
- Environment and Phenotype Variation
- Environmental Factors
- Environmental Epigenetics and Twin Studies
- Early life Exposures and Developmental Effects
- Nutrition and Epigenetics
- Environmental Toxicants and Epigenetics
- Environmental Induced Epigenetic Transgenerational Inheritance

Required Reading

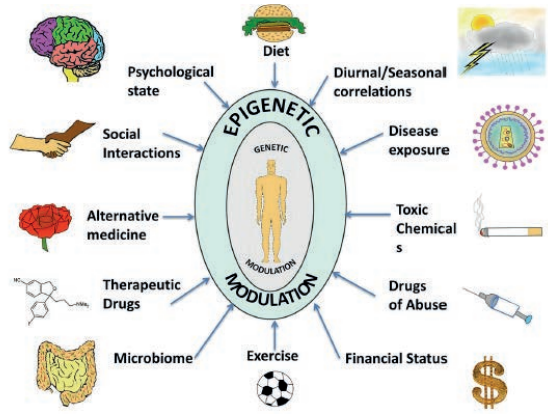
Nilsson E, Sadler-Riggleman I, Skinner MK (2018) Environmentally Induced Epigenetic Transgenerational Inheritance of Disease. Environmental Epigenetics. 4(2):1-13, dvy016.

Books (Reserve in Library)

Scott F. Gilbert and David Epel (2009) Ecological Developmental Biology. Sinauer Associates Inc. Sunderland, Massachusetts

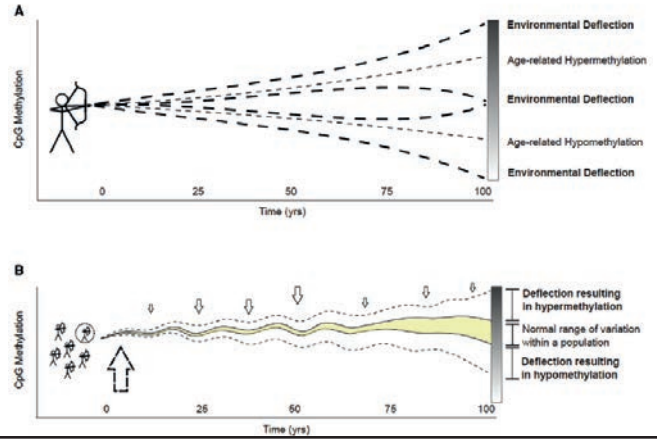
E-Book: Craig and Wong (2011) Epigenetics: A Reference Manual. Caister Academic Press. ISBN-13: 978-1904455882

Environmental Epigenetics (Toxicant Exposures)



Environmental Deflection: The Impact of Toxicant Exposures on the Aging Epigenome.

Kochmanski J, Montrose L, Goodrich JM, Dolinoy DC.
Toxicol Sci. 2017 [Epub ahead of print]



Influences of Environmental Toxicants on the Human Epigenome
Epigenetics of Lifestyle, (2012), Pp. 161-174 (14)
Jorge A. Alegría-Torres, Valentina Bollati and Andrea Baccarelli

Table 1: Epigenetic effects of environmental contaminants.

POLLUTING AGENT	EFFECT	SETTING	REFERENCES
Particulate matter	Reduced telomere length in circulating blood leukocytes	Elderly smokers and traffic pollutants exposure	[14-16]
	Demethylation of *NOS2* gene and changes in the expression of miRNAs	Foundry workers long-term exposure to PM_{10}	[25-28]
Lead	LINE-1 hypomethylation	Cumulative exposure	[29, 30]
Arsenic	DNA methylation imbalance and hypermethylation of *p53* and *p16* promoters	Exposure to toxic level of arsenic in drinking water	[35-38]
Benzene	Hypermethylation in *p15* and hypomethylation of the *MAGEA1* cancer-antigen gene associated with increased risk of acute myelogenous leukemia	Gasoline station attendants and traffic police officers	[39, 40]
Polycyclic aromatic hydrocarbons	Hypermethylation of Alu, LINE-1 and *IL6*, hypomethylation of *p53* and *HIC1*, shortening of telomere length	Coke oven workers	[45, 47]
Persistent organic pollutants	Global DNA hypomethylation	Inuits from Greenland exposed to Environmental contaminants	[48]

Table 1 | **Chemicals and pollutants that affect health and induce epigenetic alterations**

Compound	Species	Ontogenic stage	Epigenetic alteration	Tissues or cell types affected	Phenotypic alterations	Refs
Tobacco smoke	Human	Adult life	Locus-specific DNA methylation and histone modifications; chromatin remodelling machinery	Lung, blood	Lung cancer?	60,61,143
Particulate air pollution	Human, Mouse	Adult life	DNA methylation	Blood, sperm	Unknown	54,69
Asbestos	Human	Adult life	DNA methylation	Pleural tissues	Susceptibility to different diseases	57
Bisphenol A (BPA)	Mouse	Embryonic development	Locus-specific DNA methylation	Systemic	Coat colour distribution of agouti viable yellow (A^{vy}) mice	99
Diethylstilbestrol (DES)	Mouse	Embryonic development	DNA methylation	Gonads	Male sexual function	144,145
Metal ions (such as chromium, cadmium, nickel, arsenic and methylmercury)	Multiple species	Embryonic development, adult life	DNA methylation; histone modifications (for nickel)	Multiple tissues	Increased susceptibility to diseases such as cancer	Reviewed in REFS 146,147
Vinclozolin	Mouse, rat	Embryonic development	DNA methylation	Male germ cells	Altered gonad development and spermatogenesis in the male offspring	81,82
Methoxychlor	Mouse	Embryonic development, adult life	DNA methylation	Male germ cells	Altered male reproductive system	84
Silica	Human	Adult life	DNA methylation	Blood	Silicosis	148
Benzene	Human	Adult life	DNA methylation	Blood	Increased risk of AML	55
Di- and trichloroacetic acid, trichloroethylene	Mouse	Adult life	Locus-specific DNA methylation	Liver	Increased risk of hepatic cancer	Reviewed in REF. 147

AML, acute myeloid leukaemia

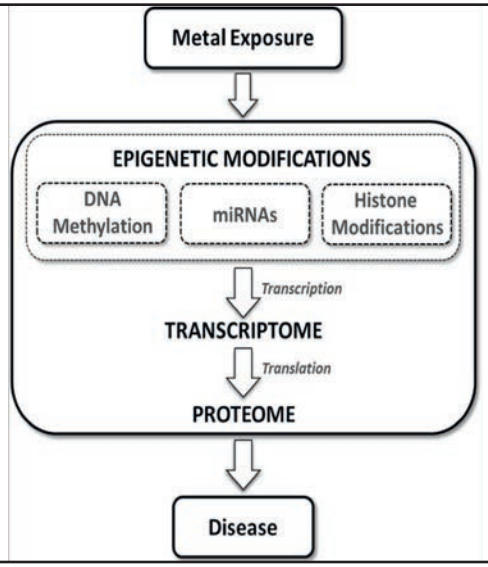


Table 1 Effects of environmental chemicals on DNA methylation

Exposure	↑/↓*	Genes	Type	Tissue	References
Arsenic	↓	Global	Rat	Liver	Zhao *et al.* [19]
	↑	*p53*	*In vitro*	A549 cells	Mass and Wang [20]
	↑↓	Multiple genes	*In vitro*	Human kidney cells	Zhong and Mass [21]
	↑	*p16, p53*	Human	PBL	Chanda *et al.* [22]
	↑	Global	Human	PBL	Pilsner *et al.* [23,24]
	↑	*p16*	Human	PBL	Zhang *et al.* [25]
Cadmium	↓	Global	*In vitro*	Rat liver cells	Takiguchi *et al.* [26]
Nickel	↑	*ATF-1, HIF-1, Rb*	*In vitro*	G12 cell line	Lee *et al.* [27]
	↑	*p16*	Mouse	Histiocytomas	Govindarajan *et al.* [28]
Chromium	↑	*p16*	Human	Lung	Kondo *et al.* [29]
Methylmercury	↑	*BDNF*	Mouse	Hippocampus	Onishchenko *et al.* [30]
TCE, DCA, TCA	↑	*c-jun, c-myc*	Mouse	Liver	Tao *et al.* [31]
Air pollution	↓	Global (*Alu, LINE-1*)	Human	Buffy coat	Tarantini *et al.* [32]
	↓	*iNOS*			
Benzene	↓	Global (*Alu, LINE-1*)	Human	Blood	Bollati *et al.* [33]
	↑	*p15*			
	↓	*MAGE*			
Vinclozolin	↑	Gene-specific	Rat	Testis	Anway *et al.* [34]
DES	↓	Global	Mouse	Uterus	Li *et al.* [35]
BPA	↓	Agouti gene, *CabpIAP*	Mouse	Embryo	Dolinoy *et al.* [36]
POPs	↓	*Alu, LINE*	Human	Blood	Rusiecki *et al.* [37]

ATF-1, activating transcription factor 1; *BDNF*, brain-derived neurotrophic factor; BPA, bisphenol A; DCA, dichloroacetic acid; DES, diethylstilbestrol; *HIF-1*, hypoxia-inducible factor-1; *LINE-1*, long interspersed nuclear element-1; *MAGE*, melanoma antigen-1; PBL, peripheral blood leukocyte; *Rb*, retinoblastoma; TCE, trichloroethylene.
* Increase (↑) or decrease (↓) in DNA methylation.

Table 2 Effects of environmental chemicals on histones

Exposure	↑/↓*	Modification	Type	Tissue	References
Nickel	↓	Acetylation	*In vitro*	Liver, brain	Ke *et al.* [38]
	↑	H3K9 dimethylation			
	↑	H2A and H2B monoubiquitination			
	↓	H4K12 acetylation	*In vitro*	Yeast cells	Broday *et al.* [39]
	↓	H4K4 acetylation	*In vitro*	Mammalian cells	
	↑	H3K9 monomethylation and dimethylation	*In vitro*	G12 cell line	Chen *et al.* [40]
	↓	Acetylation of histone H2B	*In vitro*	HAE and NRK cell lines	Golebiowski and Kasprzak [41]
	↓↑	H2B ubiquitination	*In vitro*	HAEo and HPL1D cell lines	Karaczyn *et al.* [42]
Arsenic	↑	H3K9 dimethylation	*In vitro*	A549 cell line	Zhou *et al.* [43]
	↓	H3K27 trimethylation			
	↑	H3K4 trimethylation			

HAE, human airway epithelial; NRK, normal rat kidney.
* Increase (↑) or decrease (↓) in histone modification.

An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis.

Ren X, McHale CM, Skibola CF, Smith AH, Smith MT, Zhang L.

Environ Health Perspect. 2011 Jan;119(1):11-9

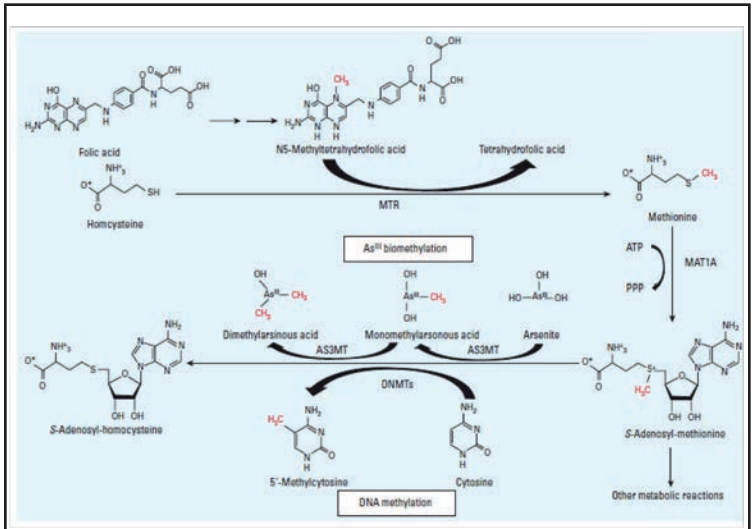


Table 3. Summary of studies exploring epigenetic effects of mercury

Species	Tissue/cell type	Chemical	Effect	Reference
Polar bear	Brainstem	MeHg	Reduced global DNA methylation in male bears but not in female bears	[67]
Mink	Occipital cortex	MeHg	Reduced global DNA methylation, reduced DNMT activity	[68]
Chicken	Cerebrum	MeHg	No effect on global DNA methylation or DNMT activity	[68]
Yellow perch	Telencephalon	MeHg	No effect on global DNA methylation	[68]
Earthworm	Whole	Hg	Reduced global DNA methylation	[71]
Mouse	Brain hippocampus	MeHg	Suppression of the *Bdnf* promoter via hypermethylation, increased histone H3K27 trimethylation, and decreased histone H3 acetylation	[73]
Mouse	Embryonic stem cells	Hg	Reversible alterations to heterochromatin Hypermethylation of *Rnd2* gene	[75]
Mouse	Embryonic stem cells	Hg	Reduction of total histone protein levels and H3K27 monomethylation	[74]
Rat	Liver	MeHg	Reduced *Dnmt1* and *Dnmt3b* mRNA expression, decreased CpG methylation at *Cdkn2a* promoter, and no effect on global DNA methylation or SAM abundance	[70]
Rat	Primary cultures of embryonic cortical neural stem cells	MeHg	Decreased global DNA methylation, and downregulation of *Dnmt3b* mRNA	[69]
Human	Blood	Hg	Hypermethylation of the *GSTM1/5* promoter	[76]
Human	Buccal cells	MeHg	Hypomethylation of *SEPP1* gene among males	[77]

MeHg = methylmercury; DNMT = DNA methyltransferase; SAM = S-adenosylmethionine.

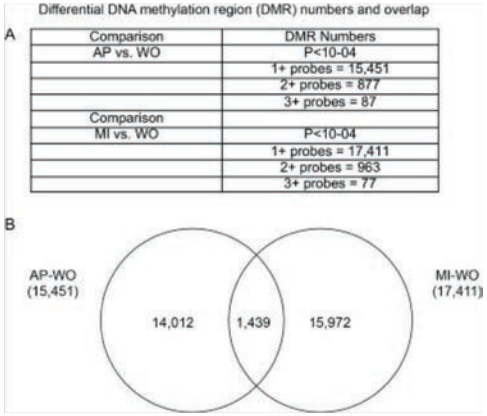
Epigenetic programming alterations in alligators from environmentally contaminated lakes.

Gen Comp Endocrinol. 2016 Nov 1;238:4-12.

Guillette LJ Jr, Parrott BB, Nilsson E, Haque MM, Skinner MK.

Representative examples of endocrine actions of various environmental contaminants on wildlife and documented health perturbations. American alligators from three sampling sites in Florida, USA; AP: Lake Apopka, MI: Merritt Island National Wildlife Refuge, and WO: Lake Woodruff National Wildlife Refuge. Endocrine activity associated with specific contaminants are presented. Health effects previously documented (Guillette, 2006; Hotz et al., 2014) are listed.

Site	Associated contaminants	Endocrine activity	Health perturbations in alligators
Lake Apopka	p,p'-DDE	Xenoestrogen (Warembourg et al., 2016), Antiandrogen (Wilson et al., 2008)	Altered sex steroid hormone concentrations (Guillette et al., 1994, 1996; Moore et al., 2010)
	p,p'-DDD	Xenoestrogen (Zhuang et al., 2012)	Reduced penis size (Guillette et al., 1996)
	trans-nonachlor	Xenoestrogen (Kelce et al., 2010)	Abated response to gonadotropin stimulation (Moore et al., 2010, 2012)
	Dieldrin	Altered hormone metabolism (Fowler et al., 2007), Xenoestrogen (Soto et al., 1994)	Increased presence of polyovular follicles (Guillette et al., 1994)
	Mirex	Xenoestrogen (Foster et al., 2014; Wiemer, 1987)	Decreased robustness of sexually dimorphic gene expression (Milnes et al., 2008)
	Toxaphene	Xenoestrogen (Hodges et al., 2000; Schippe et al., 2004)	Decreased hatch rate (Milnes et al., 2008; Woodward et al., 1993)
Merritt Island	Elevated Li, Fe, Ni, Sr, Sb, Pb, Bi, Y, As, S	Thyroid hormone changes (Aprioski and Catalani 2011; Lauran, 2005)	Reduced length (Trillmich et al., 2014)
		HPG axis abnormalities (Rossi et al., 2016) Metalloestrogen (Aprioski and Catalani, 2011; Aquino et al., 2012; Darbre, 2006; Faroon, 2013; Ganne et al., 2014; Martin-Diaz et al., 2008) Decreased testosterone (Hamase, 2005; Kachpanno et al., 2015; Romo et al., 2015)	Decreased hatch rate (Lance et al., 2006)
Lake Woodruff	Reference site	None	None

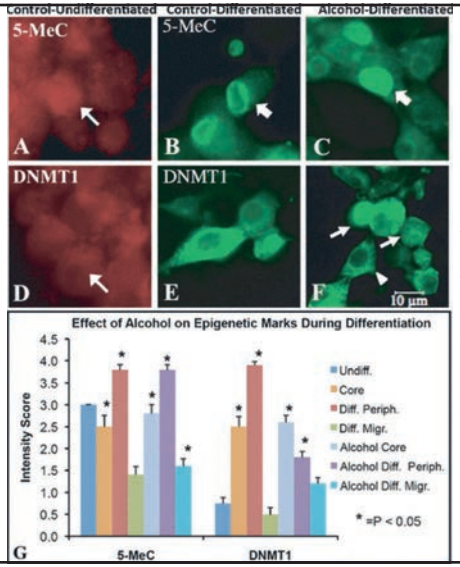


DMR numbers and overlap. (A) The comparison AP vs. WO and MI vs. WO with $p < 10^{-4}$ for single oligo probe (1+), two adjacent oligo probes (2+) and $\geqslant 3$ adjacent oligo probes (3+) detection. (B) Venn diagram overlap for single (1+) probe overlap. The DMR had to have overlapping sequence at the same genomic locations to be considered overlapped.

Alcohol Alters DNA Methylation Patterns and Inhibits Neural Stem Cell Differentiation.

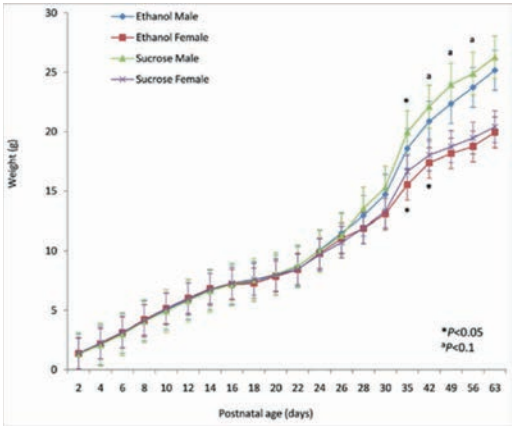
Zhou FC, Balaraman Y, Teng M, Liu Y, Singh RP, Nephew KP.

Alcohol Clin Exp Res. 2011 Jan 11. doi: 10.1111/j.1530-0277.2010.01391.x. [Epub ahead of print]



Immunostaining for 5-Methyl-cytidine (5-MeC) and DNMT1 epigenetic marks in neural stem cells during differentiation upon alcohol treatment. (A–F) shows translocation of epigenetic marks during differentiation. The 5-MeC immunoreactivity distributed throughout the nucleus (A, arrow) in undifferentiated neural stem cells (NSCs), but relocated to the perinucleus (B, arrow) upon differentiation. Following alcohol treatment, 5-MeC not only was found in the perinucleus (C, arrow) but also regained nuclear-wide distribution (C, arrowhead). In undifferentiated NSCs, DNMT1 was expressed in the nucleus and cytoplasm (D, arrow); however, following differentiation, DNMT1 expression became exclusively cytoplasmic around nucleus (E). With alcohol treatment, DNMT1 was distributed in both the cytoplasm (F, arrowhead), and nucleus (F, arrows) which is normally seen in undifferentiated NSCs. Scale bars: A–F = 10 µm. (A, D) Alexa633 (red fluorescence), and (B, C, E, F) Alexa488 (Green fluorescence). (G) shows, through semiquantitative measurement, that 5-MeC and DNMT1 were dynamically changed from the undifferentiated control neurosphere (undiff.) to the differentiated Control and Alcohol groups in core and periphery (Periph.) of neurospheres and in migrated (Migr.) cells (n = 10 to 12, *p < 0.05). The methods and statistics are described in the Methods.

Knezovich JG, Ramsay M. (2012) The effect of preconception paternal alcohol exposure on epigenetic remodeling of the h19 and rasgrf1 imprinting control regions in mouse offspring. Front Genet; 3:10.



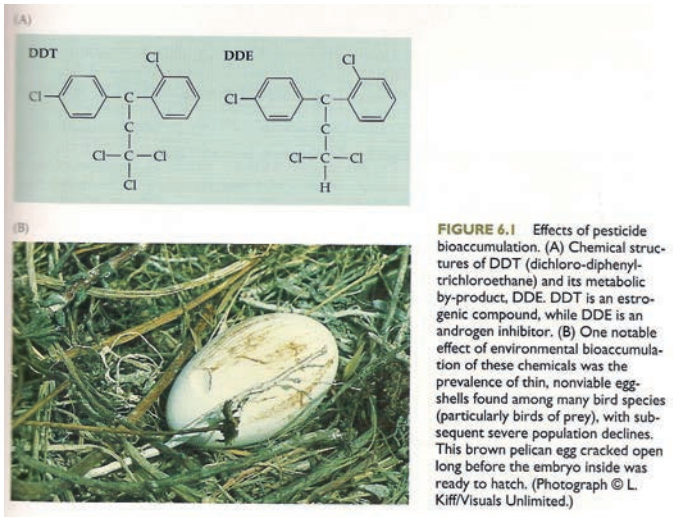
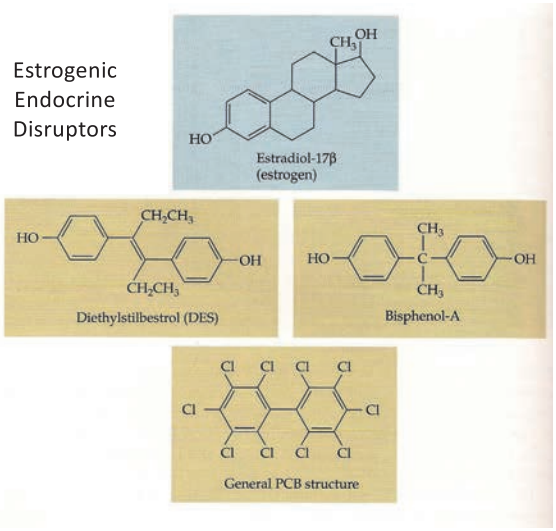
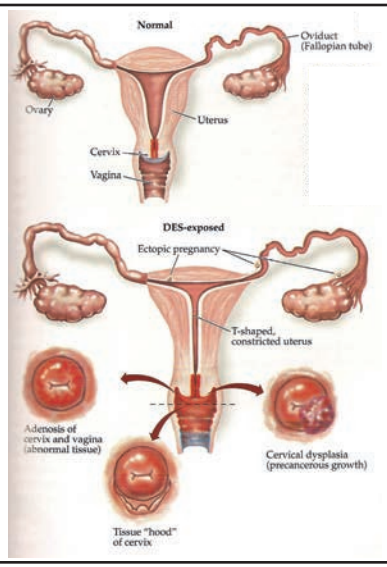
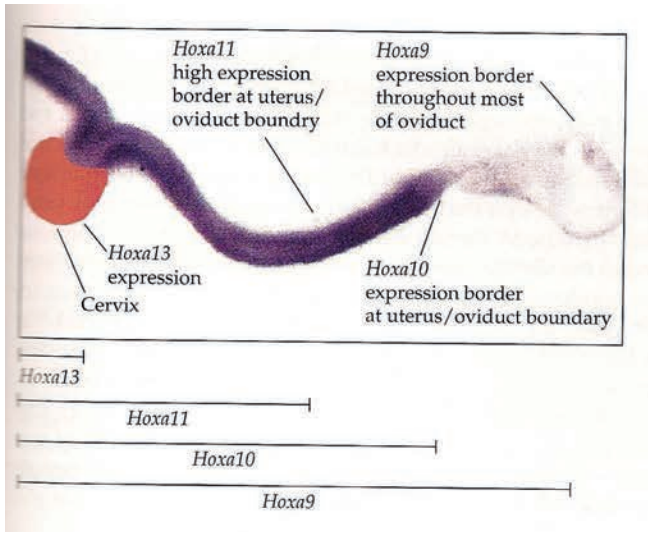


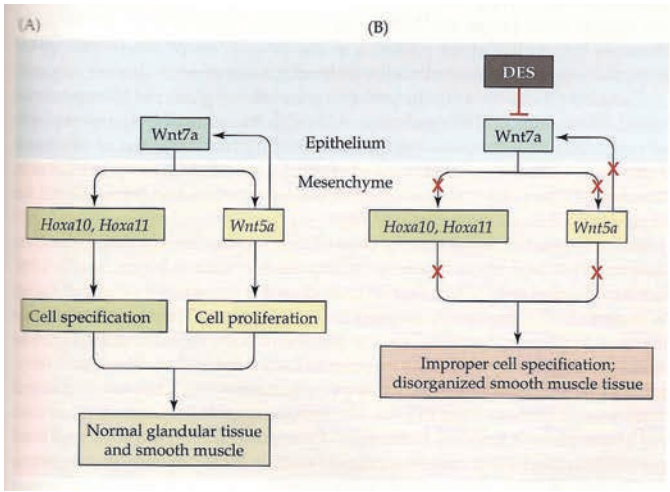
FIGURE 6.1 Effects of pesticide bioaccumulation. (A) Chemical structures of DDT (dichloro-diphenyl-trichloroethane) and its metabolic by-product, DDE. DDT is an estrogenic compound, while DDE is an androgen inhibitor. (B) One notable effect of environmental bioaccumulation of these chemicals was the prevalence of thin, nonviable eggshells found among many bird species (particularly birds of prey), with subsequent severe population declines. This brown pelican egg cracked open long before the embryo inside was ready to hatch. (Photograph © L. Kiff/Visuals Unlimited.)

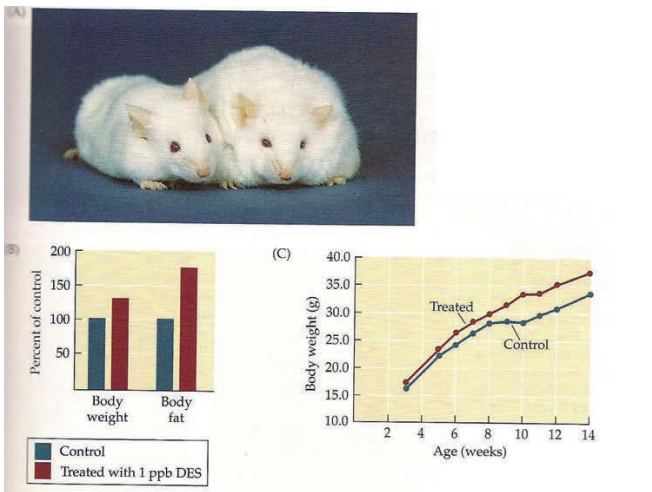
Estrogenic
Endocrine
Disruptors




Normal
Oviduct (Fallopian tube)
Ovary
Uterus
Cervix
Vagina
DES-exposed
Ectopic pregnancy
T-shaped, constricted uterus
Adenosis of cervix and vagina (abnormal tissue)
Cervical dysplasia (precancerous growth)
Tissue "hood" of cervix


Hoxa11
high expression border at uterus/ oviduct boundry
Hoxa9
expression border throughout most of oviduct
Hoxa13
expression
Cervix
Hoxa10
expression border at uterus/oviduct boundary
Hoxa13
Hoxa11
Hoxa10
Hoxa9


(A)
(B)
DES
Wnt7a
Epithelium
Wnt7a
Mesenchyme
Hoxa10, Hoxa11
Wnt5a
Hoxa10, Hoxa11
Wnt5a
Cell specification
Cell proliferation
Improper cell specification; disorganized smooth muscle tissue
Normal glandular tissue and smooth muscle


(A)
(B)
Percent of control
200
150
100
50
Body weight
Body fat
Control
Treated with 1 ppb DES
(C)
Body weight (g)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
Treated
Control
2
4
6
8
10
12
14
Age (weeks)

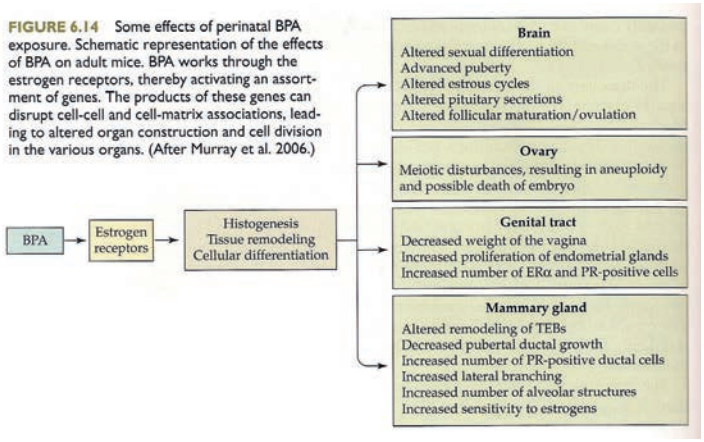


FIGURE 6.14 Some effects of perinatal BPA exposure. Schematic representation of the effects of BPA on adult mice. BPA works through the estrogen receptors, thereby activating an assortment of genes. The products of these genes can disrupt cell-cell and cell-matrix associations, leading to altered organ construction and cell division in the various organs. (After Murray et al. 2006.)

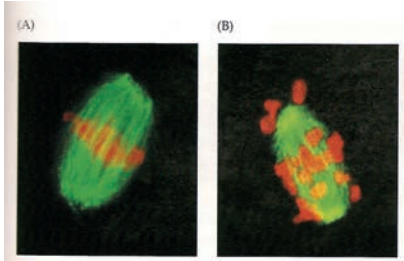


FIGURE 6.15 Bisphenol A causes meiotic defects in maturing oocytes. (A) During the first meiotic metaphase, chromosomes (stained red) normally line up in a paired fashion at the center (equator) of the spindle. (B) Brief exposure to BPA causes chromosomes to align randomly on the spindle. Different numbers of chromosomes then enter the egg and polar body, resulting in chromosomal aneuploidy (incorrect number of chromosomes in the daughter cells of meiosis) and infertility. (From Hunt et al. 2003; photographs courtesy of P. Hunt.)

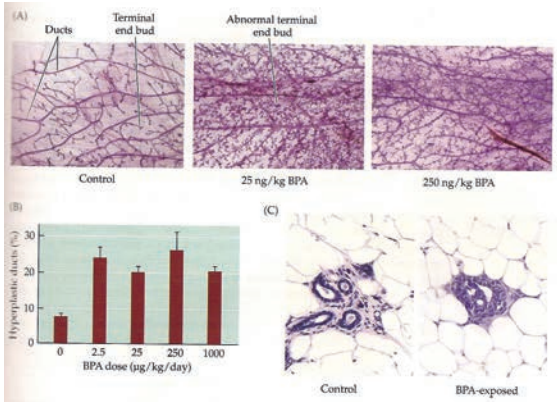


FIGURE 6.16 Bisphenol A induces mammary lesions in mice. (A) Photomicrographs of mammary glands show profound differences between a control animal and animals exposed perinatally to nanogram levels of BPA. The increase in terminal end buds and branching in the BPA-exposed mammary glands predisposes the animal to cancer later in life. (B) The percentage of mammary glands showing intraductal hyperplasia (indicating a cancer-prone state) is significantly increased at postnatal day 50 in BPA-treated animals. (C) Later in life, BPA-exposed animals displayed significant cancer in situ. (From Murray et al. 2007 and Soto et al. 2008; photographs courtesy of Ana Soto.)

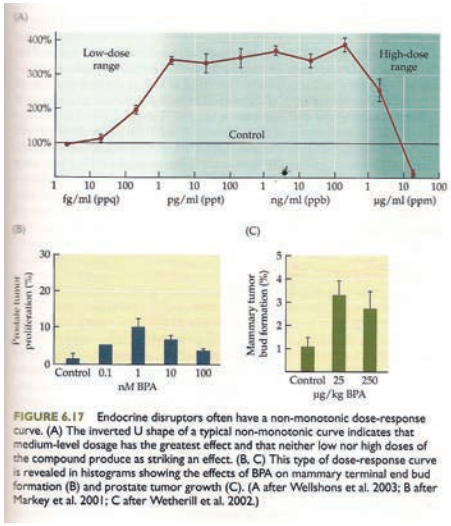
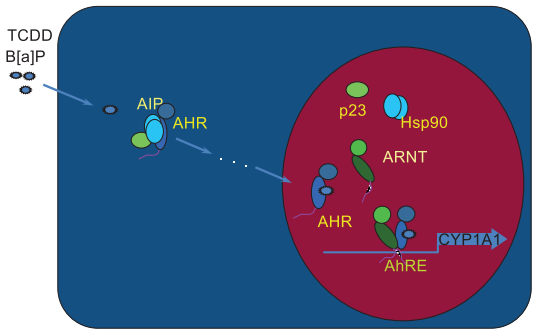


FIGURE 6.17 Endocrine disruptors often have a non-monotonic dose-response curve. (A) The inverted U shape of a typical non-monotonic curve indicates that medium-level dosage has the greatest effect and that neither low nor high doses of the compound produce as striking an effect. (B, C) This type of dose-response curve is revealed in histograms showing the effects of BPA on mammary terminal end bud formation (B) and prostate tumor growth (C). (A after Wellshons et al. 2003; B after Markey et al. 2001; C after Wetherill et al. 2002.)

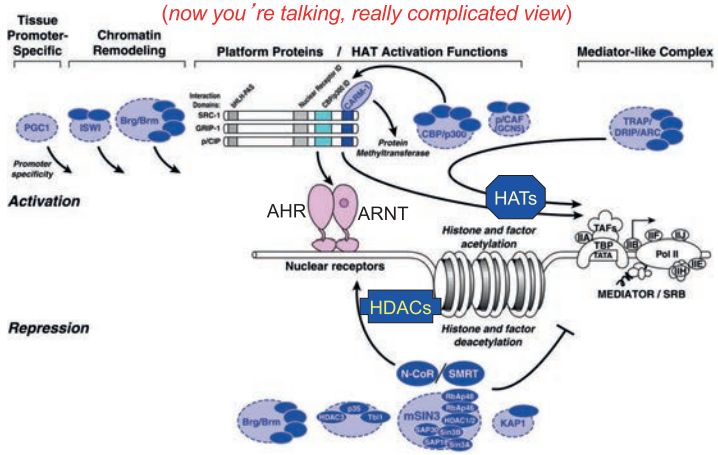
Ah Receptor-Dependent Target Gene Expression

(*simplified view*)



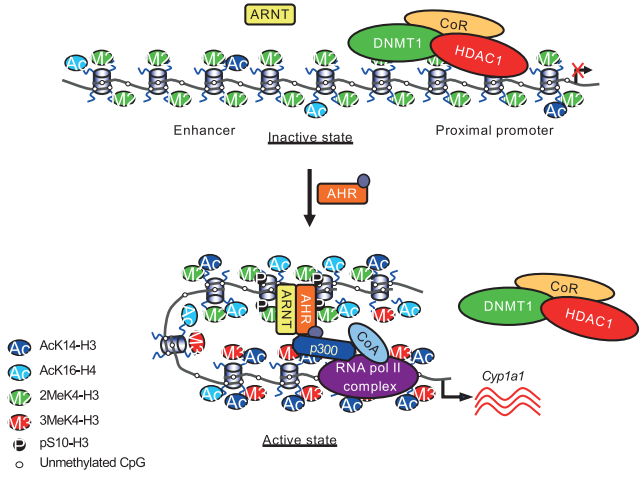
Ah Receptor-Dependent Target Gene Expression

(*now you're talking, really complicated view*)



Aranda, A. and Pascual, A. (2001) Physiol.Rev. 81:1269-1304

Epigenetic activation of the *Cyp1a1* promoter



Stoccoro A, Karlsson HL, Coppedè F, Migliore L. (2012) Epigenetic effects of nano-sized materials. Toxicology. 2012 Dec 10. doi: 10.1016/j.tox.2012.12.002. [Epub ahead of print]

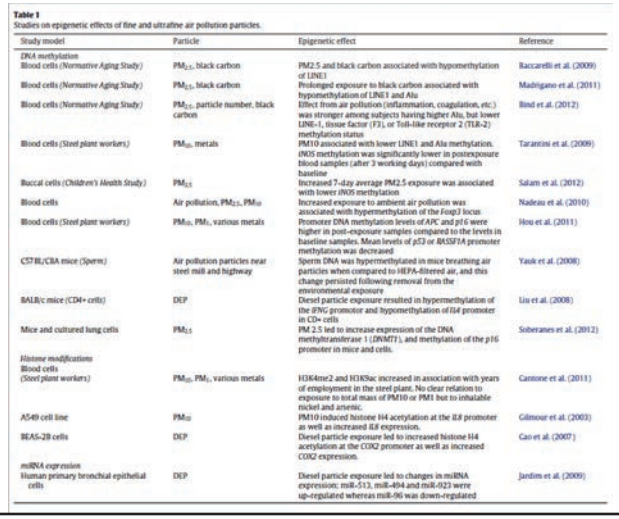


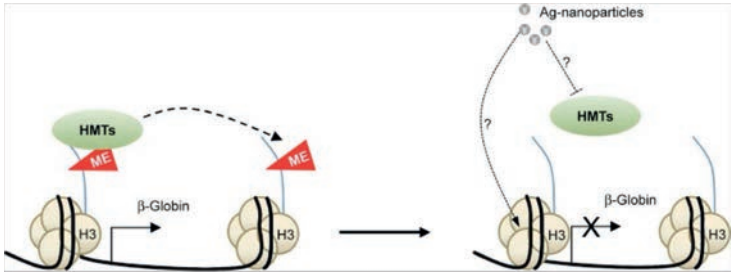
Table 1
Studies on epigenetic effects of fine and ultrafine air pollution particles.

Study model	Particle	Epigenetic effect	Reference
DNA methylation			
Blood cells (Normative Aging Study)	$PM_{2.5}$, black carbon	PM2.5 and black carbon associated with hypomethylation of LINE1	Baccarelli et al. (2009)
Blood cells (Normative Aging Study)	$PM_{2.5}$, black carbon	Prolonged exposure to black carbon associated with hypomethylation of LINE1 and Alu	Madrigano et al. (2011)
Blood cells (Normative Aging Study)	$PM_{2.5}$, particle number, black carbon	Effect from air pollution (inflammation, coagulation, etc.) was stronger among subjects having higher Alu, but lower LINE-1, tissue factor (F3), or Toll-like receptor 2 (TLR-2) methylation status	Bind et al. (2012)
Blood cells (Steel plant workers)	PM_{10}, metals	PM10 associated with lower LINE1 and Alu methylation. iNOS methylation was significantly lower in postexposure blood samples (after 3 working days) compared with baseline	Tarantini et al. (2009)
Buccal cells (Children's Health Study)	$PM_{2.5}$	Increased 7-day average PM2.5 exposure was associated with lower iNOS methylation	Salam et al. (2012)
Blood cells	Air pollution, $PM_{2.5}$, PM_{10}	Increased exposure to ambient air pollution was associated with hypermethylation of the Foxp3 locus	Nadeau et al. (2010)
Blood cells (Steel plant workers)	PM_{10}, $PM_{2.5}$, various metals	Promoter DNA methylation levels of APC and p16 were higher in post-exposure samples compared to the levels in baseline samples. Mean levels of p53 or RASSF1A promoter methylation was decreased	Hou et al. (2011)
C57BL/CBA mice (Sperm)	Air pollution particles near steel mill and highway	Sperm DNA was hypermethylated in mice breathing air particles when compared to HEPA-filtered air, and this change persisted following removal from the environmental exposure	Yauk et al. (2008)
BALB/c mice (CD4+ cells)	DEP	Diesel particle exposure resulted in hypermethylation of the IFNG promotor and hypomethylation of Il4 promoter in CD+ cells	Liu et al. (2008)
Mice and cultured lung cells	$PM_{2.5}$	PM 2.5 led to increase expression of the DNA methyltransferase 1 (DNMT1), and methylation of the p16 promoter in mice and cells.	Soberanes et al. (2012)
Histone modifications			
Blood cells (Steel plant workers)	PM_{10}, $PM_{2.5}$, various metals	H3K4me2 and H3K9ac increased in association with years of employment in the steel plant. No clear relation to exposure to total mass of PM10 or PM1 but to inhalable nickel and arsenic.	Cantone et al. (2011)
A549 cell line	PM_{10}	PM10 induced histone H4 acetylation at the IL8 promoter as well as increased IL8 expression.	Gilmour et al. (2003)
BEAS-2B cells	DEP	Diesel particle exposure led to increased histone H4 acetylation at the COX2 promoter as well as increased COX2 expression.	Gao et al. (2007)
miRNA expression			
Human primary bronchial epithelial cells	DEP	Diesel particle exposure led to changes in miRNA expression; miR-513, miR-494 and miR-923 were up-regulated whereas miR-96 was down-regulated	Jardim et al. (2009)

The effect of exposure to nanoparticles and nanomaterials on the mammalian epigenome.

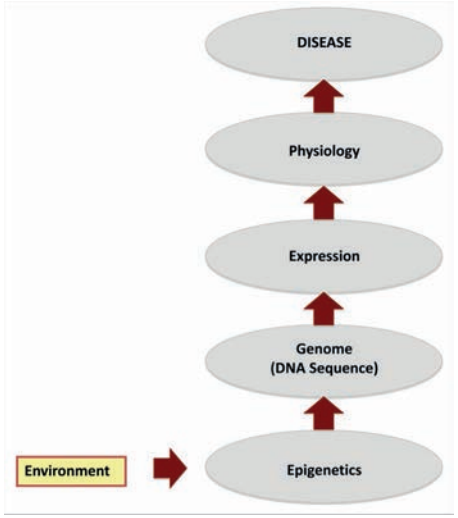
Int J Nanomedicine. 2016 Nov 25;11:6297-6306.

Sierra MI, Valdés A, Fernández AF, Torrecillas R, Fraga MF.



Model explaining the possible molecular mechanisms underlying the effect of Ag-nanoparticles on the regulation of the β-globin gene in mouse erythroleukemia cells. Exposure to Ag-nanoparticles induces β-globin repression through still not fully understood molecular mechanisms that might involve inhibition of specific histone methyl transferases and direct binding of the nanoparticles to histones.

Abbreviations: ME, methylation; H3, histone H3; HMTs, histone methyltransferases; Ag, silver.

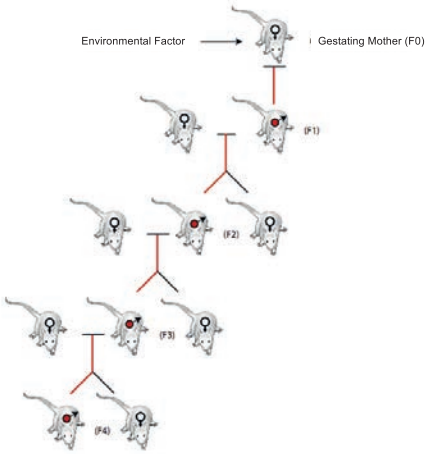


Environmental Epigenetics (Transgenerational Inheritance)

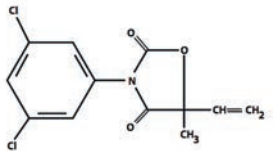
Epigenetic Transgenerational Inheritance Definition

- Germ line transmission of epigenetic marks in the absence of any continued direct environmental exposure to promote the generational inheritance of disease and phenotypic variation
- Distinct from direct exposure somatic or germ line epigenetic alterations not permanently programmed in the germ line

Transgenerational Inheritance of Disease

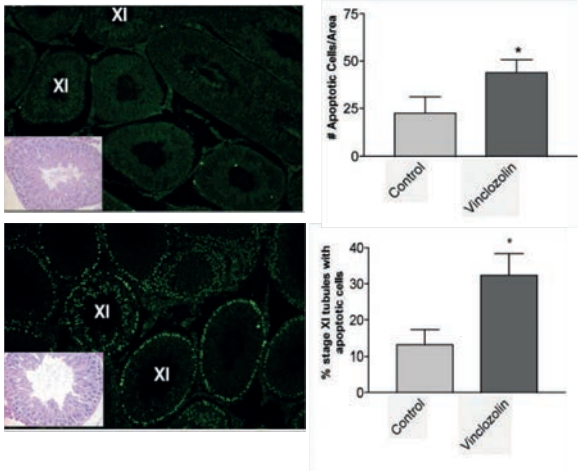


Model Endocrine Disruptor: Vinclozolin

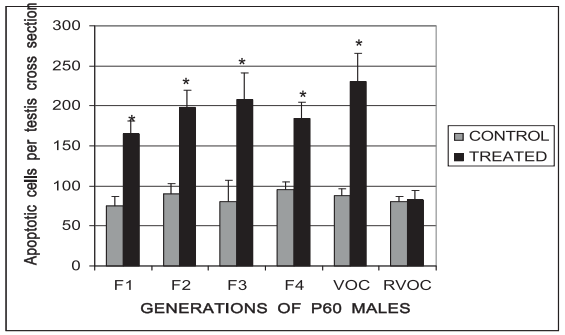


- Vinclozolin is a systemic fungicide (e.g. Wine Industry)
- Vinclozolin and its metabolites are anti-androgenic
- Late embryonic/early postnatal exposure causes abnormal reproductive tract development and gonadal function

Vinclozolin Effects on Spermatogenic Cell Apoptosis



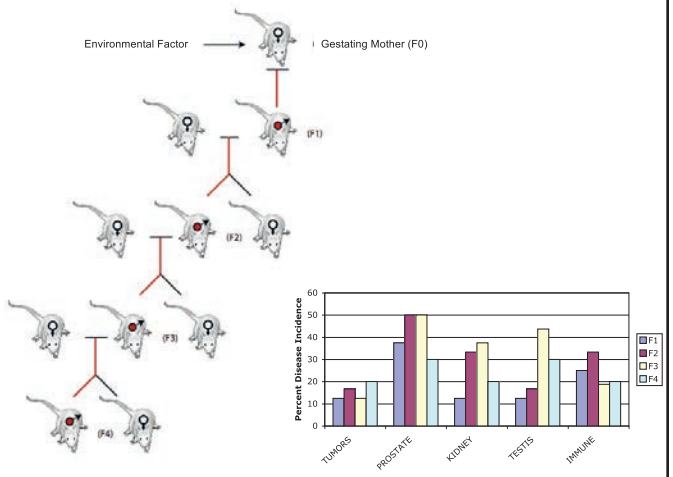
Germ Cell Apoptosis



* P<0.05

Vinclozolin

Transgenerational Inheritance of Disease

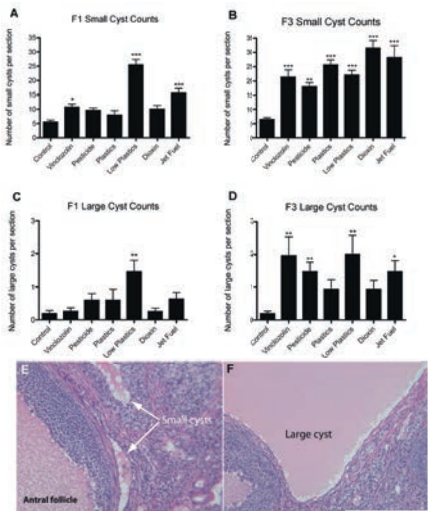


Compound Specificity

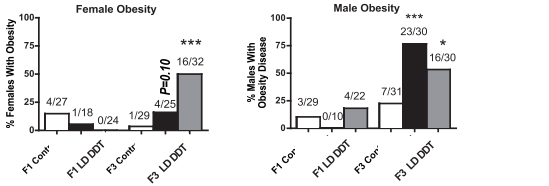
Environmental Compound Specificity

(Exposure Groups)	F1	F3
A. Vinclozolin [+ control for transgenerational effects]	Yes	Yes
B. Flutamide [anti-androgenic compound]	Yes	No
C. TCDD/Dioxin	Yes	Yes
D. Plastics [Bisphenol-A, Phthalate-DEHP & DBP]	Yes	Yes
E. Jet Fuel [JP8] (Hydrocarbon Mixture)	Yes	Yes
F. Pesticide & Insect Repellent [Permethrin & DEET]	No	Yes
G. DDT	Yes	Yes
H. Methoxychlor	Yes	Yes

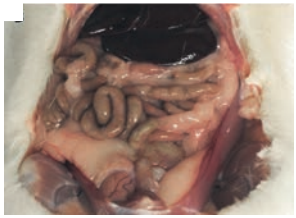
Polycystic Ovarian Disease



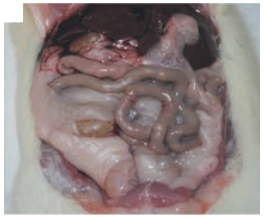
DDT Induced Transgenerational Obesity



Non-obese



Obese



TRANSGENERATIONAL DISEASE ETIOLOGY

MALE		FEMALE
▪ Spermatogenic Defect (>90%)	▪ Kidney disease (~30-40%)	▪ Pre-eclampsia-like during late pregnancy (~10%)
▪ Male infertility (complete ~10%, severe 20%)	▪ Increase in mammary tumor formation (~10-20%)	▪ Premature Ovarian Failure POF (>90%)
▪ Prostate disease (~50%)	▪ Behavior (Mate Preference, Anxiety, & Stress) (>90%)	▪ Ovarian Polycystic Ovarian Disease (>90%)
	▪ Obesity (~10-50%)	▪ Female Premature Pubertal Onset (>90%)

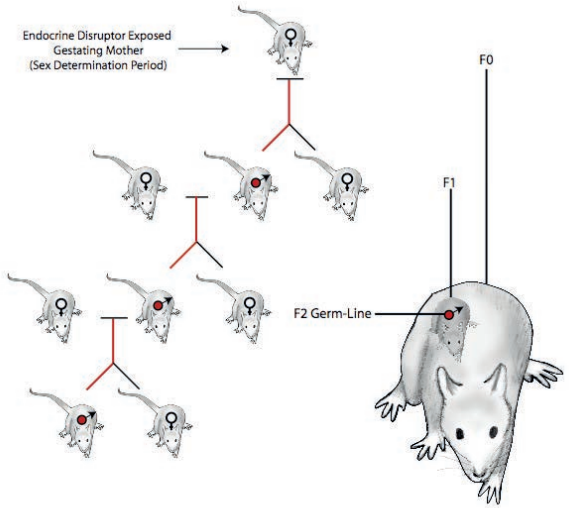
ENVIRONMENTALLY INDUCED EPIGENETIC TRANSGENERATIONAL INHERITANCE

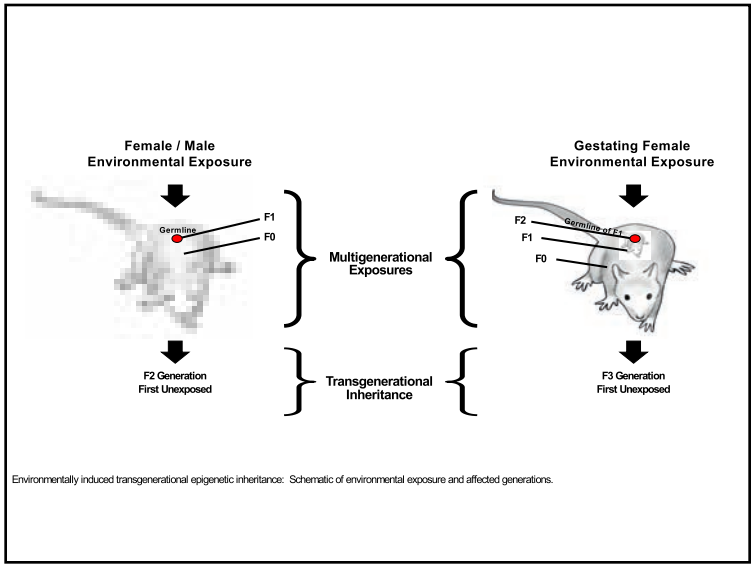
Environmental Toxicants

Agricultural Fungicide (Vinclozolin)	Insect Repellants (Permethrin & DEET)
Agricultural Pesticide (Methoxychlor)	Pesticide (DDT)
Industrial Contaminant (Dioxin/TCDD)	Industrial Toxicant & Biocide (Tributyltin)
BPA & Phthalates (Plastic Compounds)	Hydrocarbons (Jet Fuel JP8)
Herbicides (Atrazine)	Heavy Metals (Mercury)

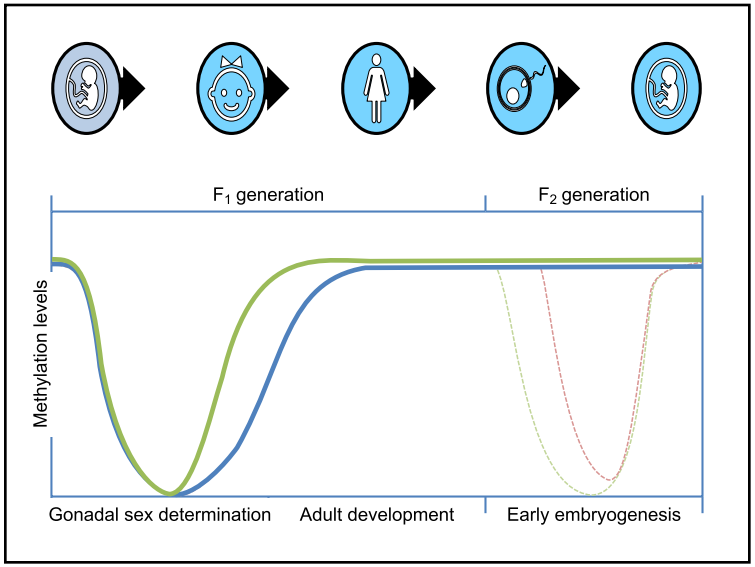
Other Types Exposures

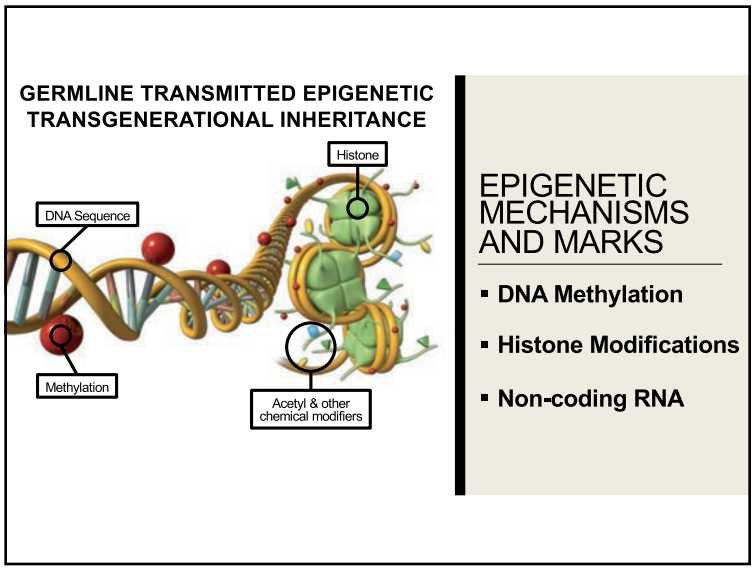
Nutrition (High Fat or Caloric Restriction)	Smoking & Alcohol
Temperature & Drought (Plant Health & Flowering)	Stress (Behavioral)





Environmentally induced transgenerational epigenetic inheritance: Schematic of environmental exposure and affected generations.

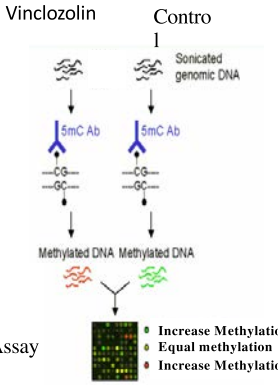




EPIGENETIC MECHANISMS AND MARKS

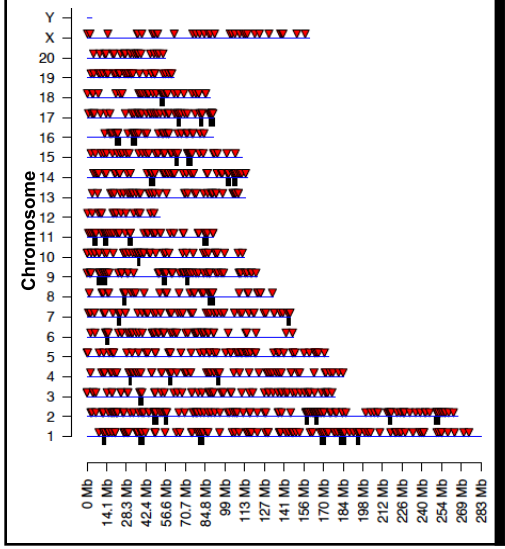
- **DNA Methylation**
- **Histone Modifications**
- **Non-coding RNA**

Comparative Methylation, MeDIP Chip or MeDIP Seq
F3 Generation Sperm DNA pools



MeDIP-Chip Assay
or
MeDIP-Seq Assay

Next Generation Sequencing (linker bar codes)

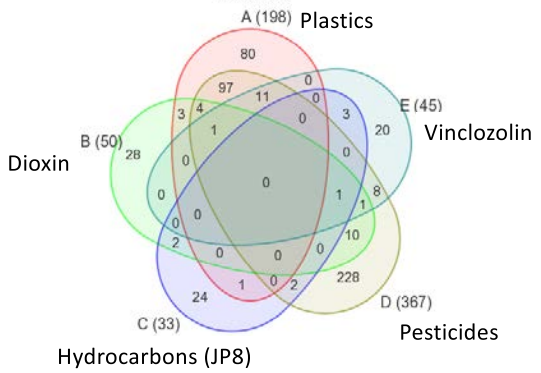


F3 Generation Sperm Genome Wide Epimutations (MeDIP-Seq)

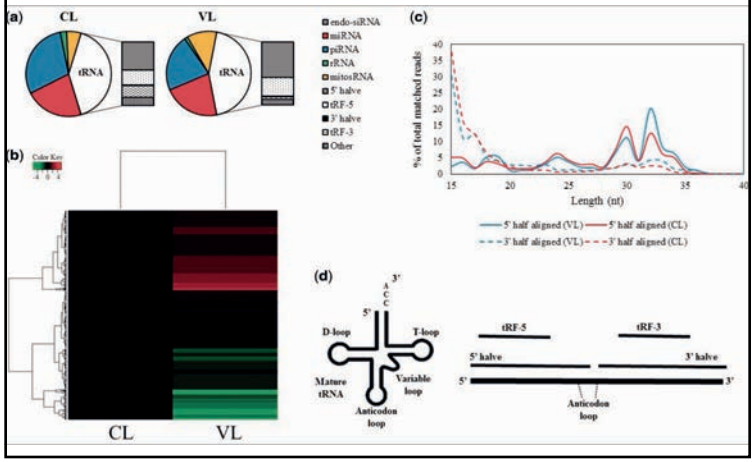
1075 p<10-4 DMR Sites

Transgenerational Epimutations & Clusters

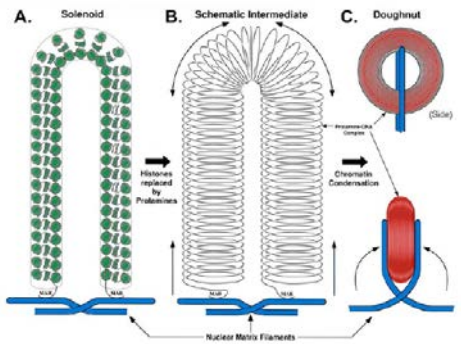
Exposure Specific
Transgenerational (F3) Sperm Epigenome Alterations



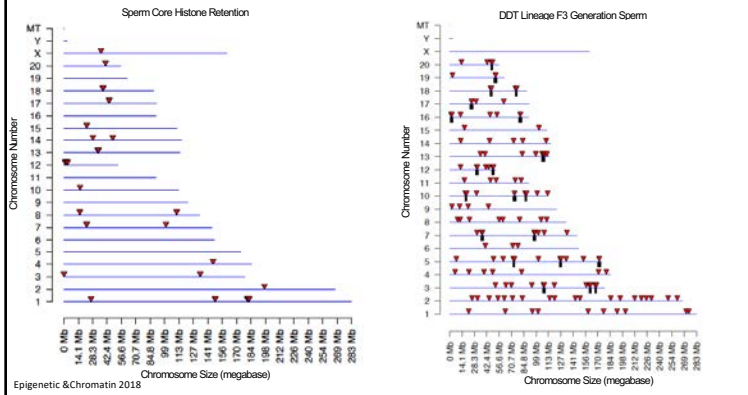
Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs.
Environ Epigenet. 2016;2(1).
Schuster A, Skinner MK, Yan W.



Chromatin Structure – Composition and Function during Spermiogenesis
Khochbin, S. and Ward, W.S



Environmentally Induced New Transgenerational Histone Retention

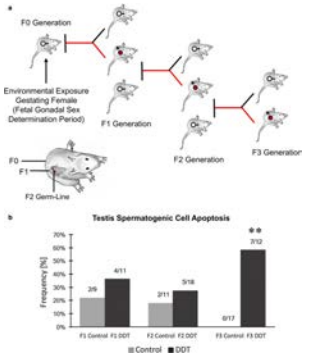


Epigenetic &Chromatin 2018

Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational inheritance of disease.

Epigenetics Chromatin. 2018 Feb 27;11(1):8.

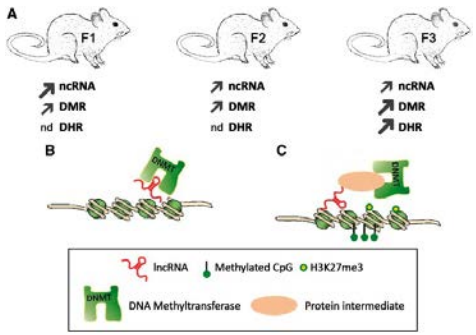
Skinner MK, Ben Maamar M, Sadler-Riggleman I, Beck D, Nilsson E, McBirney M, Klukovich R, Xie Y, Tang C, Yan W.



Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention mediate vinclozolin-induced epigenetic transgenerational inheritance of disease.

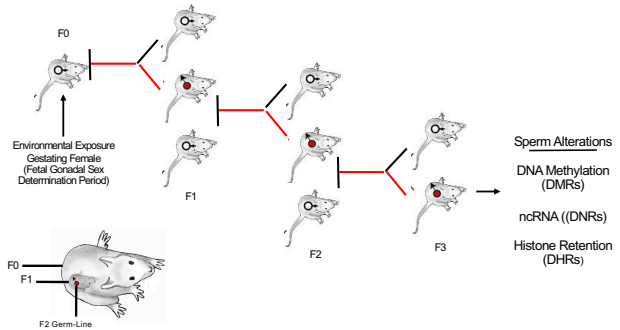
Environ Epigenet. 2018 Apr 26;4(2):dvy010.

Ben Maamar M, Sadler-Riggleman I, Beck D, McBirney M, Nilsson E, Klukovich R, Xie Y, Tang C, Yan W, Skinner MK.

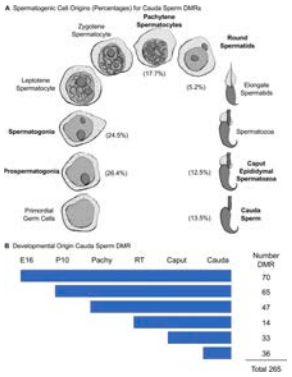


The regulatory mechanisms of DNA methylation by lncRNAs. **(A)** Summary of the different epimutations in the F1, F2, and F3 generation exposed rat sperm with larger/thicker arrows reflecting larger numbers of epimutations than thinner arrows and nd (negligible detected). **(B)** lncRNA/DNMT interaction prevents locus-specific DNA methylation locally *in cis*.**(C)** lncRNA interacts with DNMT1 (methyltransferase 1) indirectly through a protein intermediate.

Epigenetic Transgenerational Inheritance of Sperm Epimutations



Developmental origins of transgenerational sperm DNA methylation epimutations following ancestral DDT exposure.
Dev Biol. 2019 Jan 15;445(2):280-293.
Ben Maamar M, Nilsson E, Sadler-Riggleman I, Beck D, McCarrey JR, Skinner MK.



Transgenerational (F3) Sperm Epigenome Mapping
(Germline transmitted epimutations)

(Epigenetic Biomarkers for Ancestral Exposures)

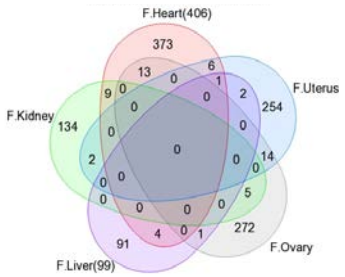
Transgenerational Sperm Epigenome Alterations
(>1000 differential DNA methylation sites)

Genomic Features-CpG Deserts (<10%) & Motifs (EDM1/2)
(Susceptibility epigenetic transgenerational mark)

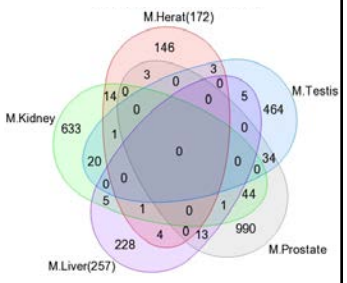
Genome Activity Alterations?
(transcriptome)

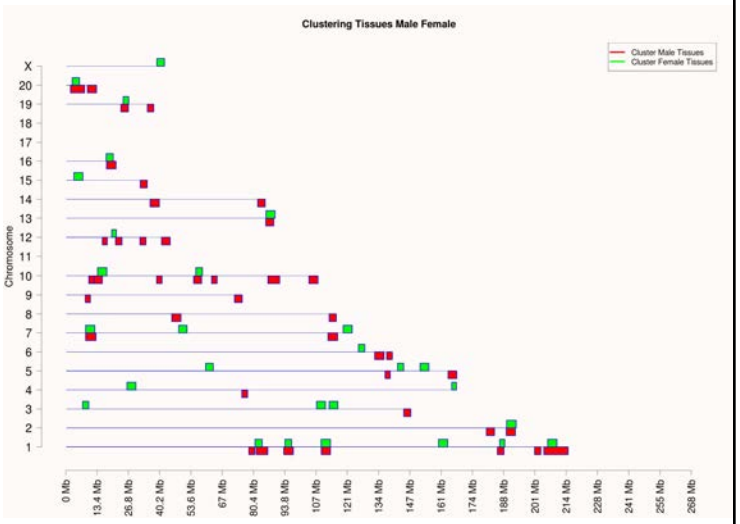
Tissue Specific Transgenerational Transcriptomes (F3)

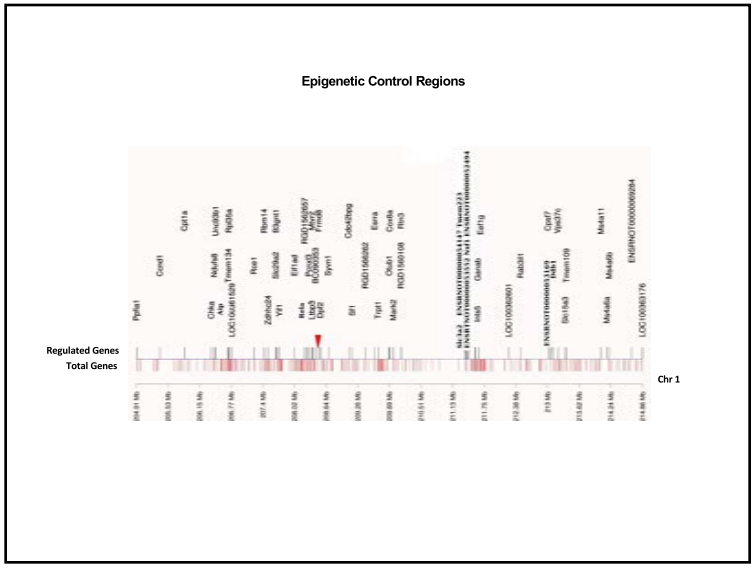
Female



Male



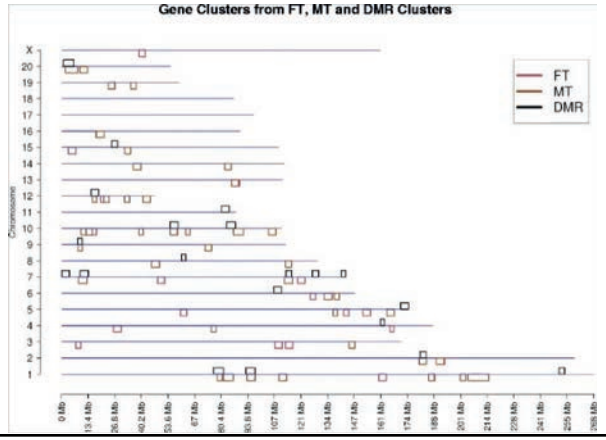


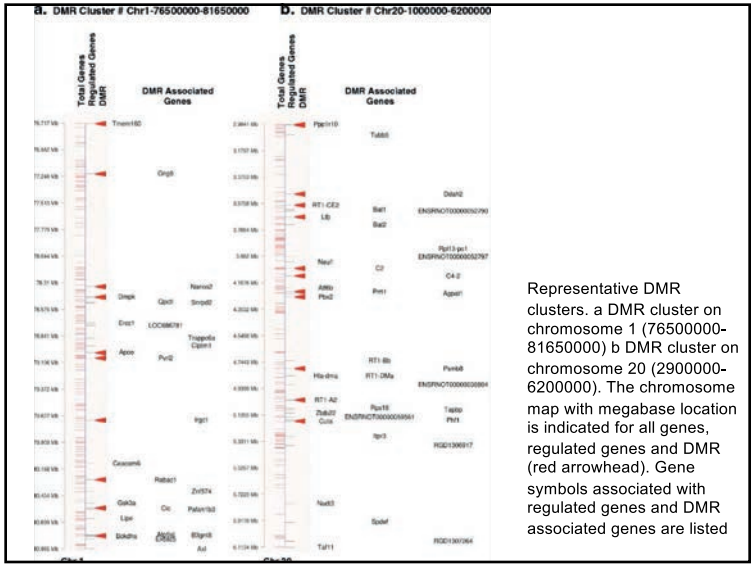


Genomic Clustering of differential DNA methylated regions (epimutations) associated with the epigenetic transgenerational inheritance of disease and phenotypic variation.

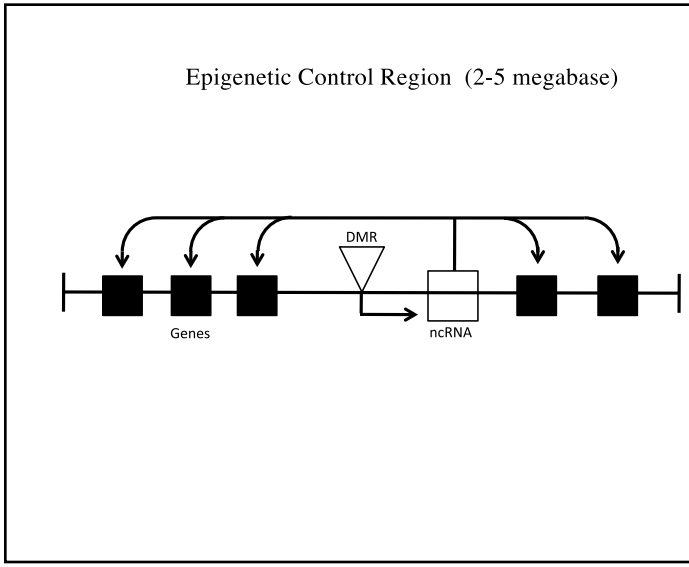
BMC Genomics. 2016 Jun 1;17:418.

Haque MM, Nilsson EE, Holder LB, Skinner MK.

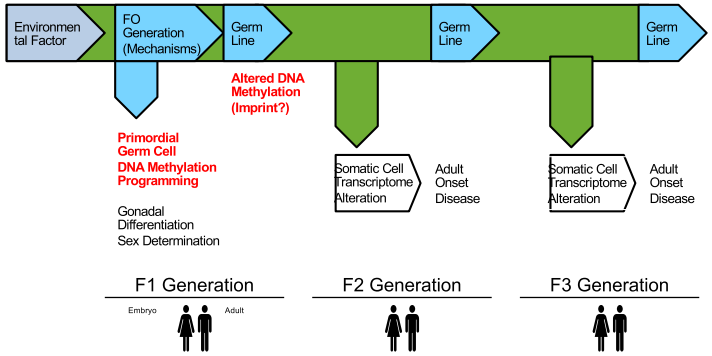




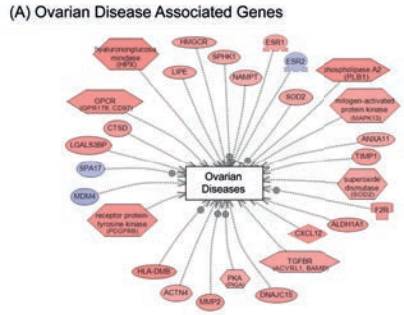
Representative DMR clusters. a DMR cluster on chromosome 1 (76500000-81650000) b DMR cluster on chromosome 20 (2900000-6200000). The chromosome map with megabase location is indicated for all genes, regulated genes and DMR (red arrowhead). Gene symbols associated with regulated genes and DMR associated genes are listed

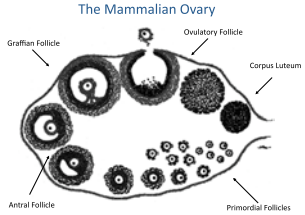


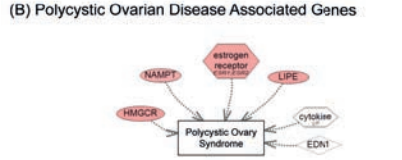
ROLE OF GERM LINE IN EPIGENETIC TRANS-GENERATIONAL INHERITANCE

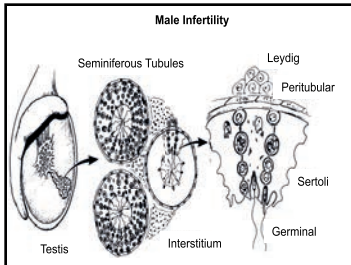


Ovarian
Granulosa Cell
Vinclozolin Lineage
F3 Generation
Transgenerational
Epigenome
(>100 DMR)
Transcriptome
(~500 genes)

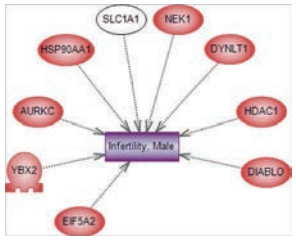


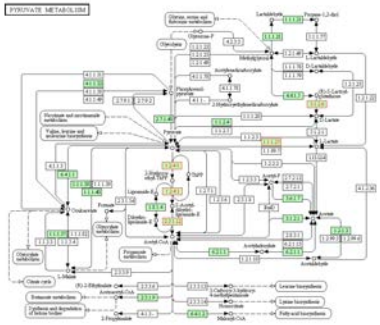






Testis
Sertoli Cell
F3 Generation
Transgenerational Transcriptome
(~400 genes)





EPIGENETIC TRANSGENERATIONAL INHERITANCE

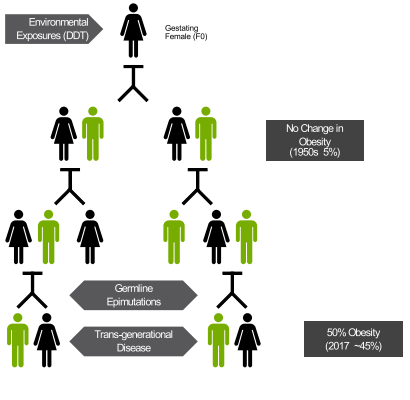
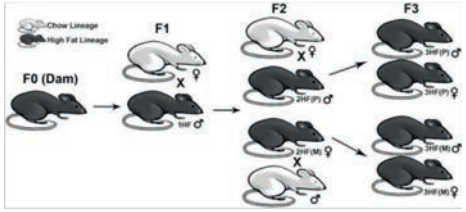


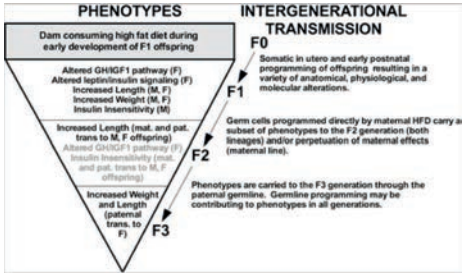
TABLE 1. Environmental exposures that induce transgenerational reproductive disease phenotypes.

Toxicants	Reproductive disease	References
Vinclozolin	Decreased sperm count, testis apoptosis, testis abnormalities,[a] prostate abnormalities, oocyte loss, ovarian cysts, altered mate selection. Epigenetic changes observed.	[51, 55–57, 63, 69, 78]
Methoxychlor	Ovarian cysts. Epigenetic changes observed.	[55, 75, 80]
TCDD/dioxin	Puberty onset, oocyte loss, ovarian cysts, fertility defect.[b] Epigenetic changes observed.	[72, 77, 78]
Plastics mixture (bisphenol-A, phthalate-DEHP, and DBP)	Testis abnormalities, puberty onset, oocyte loss, ovarian cysts. Epigenetic changes observed.	[67, 68]
Jet fuel (JP8)	Testis apoptosis, oocyte loss. Epigenetic changes observed.	[65, 74]
Permethrin and DEET	Testis abnormalities, puberty onset, oocyte loss, ovarian cysts. Epigenetic changes observed.	[69]
DDT	Decreased sperm count, testis apoptosis, ovarian cysts. Epigenetic changes observed.	[63, 64]
Bisphenol A	Decreased sperm count, fertility defect	[79, 89, 90]
Phthalates	Decreased sperm count, testis abnormalities, puberty onset, fertility defect	[66]
Tributyltin		[91]
Benzo[a]pyrene	Testis abnormalities	[70]
Other types exposures		
Folate (nutrition)		[92]
High-fat diet (nutrition)		[93, 94]
Caloric Restriction (nutrition)		[95–98]
Temperature and drought (plant flowering and health)	Abnormal flowering, fertility defect. Epigenetic changes observed.	[99–102]
Stress (behavioral)		[103, 104]
Smoking (health)		[105, 106]
Alcohol (health)		[107]

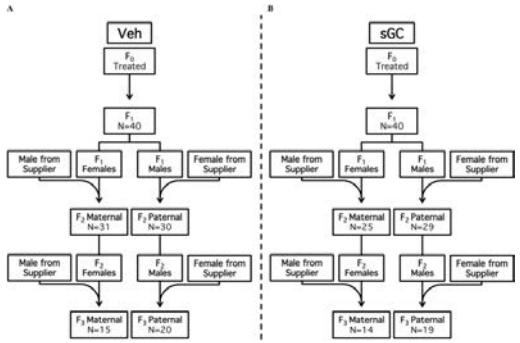
[a] Includes seminiferous tubule atrophy, tubule vacuoles, and germ cell agenesis.
[b] Fertility defect indicates reduced numbers of offspring.

Dunn GA, Bale TL. (2011) Maternal high-fat diet effects on third-generation female body size via the paternal lineage. Endocrinology. 152(6):2228-36.

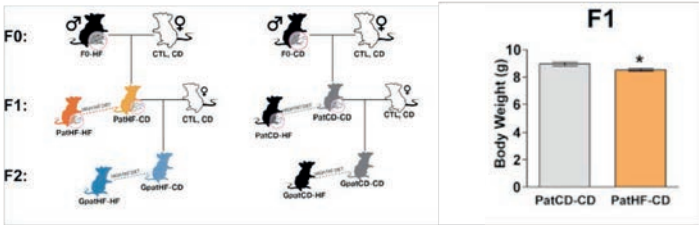


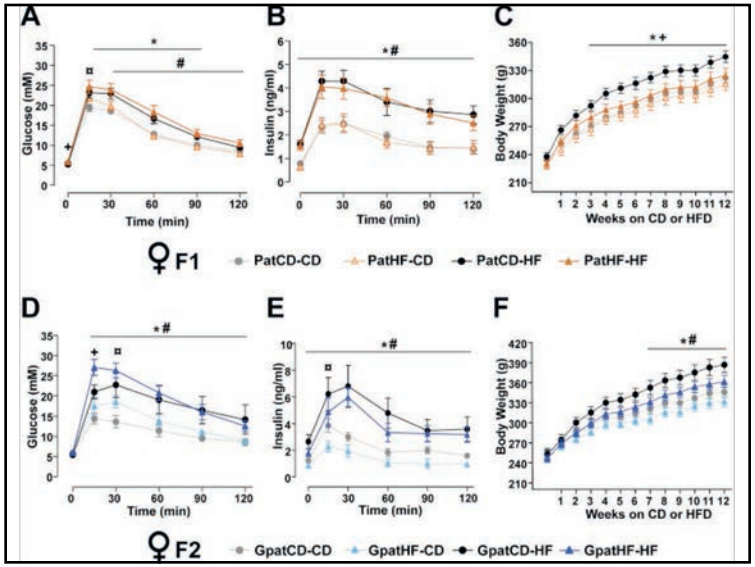


Prenatal Glucocorticoid Exposure Modifies Endocrine Function and Behaviour for 3 Generations Following Maternal and Paternal Transmission.
Sci Rep. 2017 Sep 18;7(1):11814.
Moisiadis VG, Constantinof A, Kostaki A, Szyf M, Matthews SG.



High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring.
Mol Metab. 2015 Dec 25;5(3):184-97.
de Castro Barbosa T, Ingerslev LR, et al.





Bruner-Tran KL, Osteen KG. (2011) Developmental exposure to TCDD reduces fertility and negatively affects pregnancy outcomes across multiple generations. Reprod Toxicol. 31(3):344-50.

Table 2
Impact of developmental TCDD exposure on reproductive outcome in MPV-free C57BL/6 mice over multiple generations.

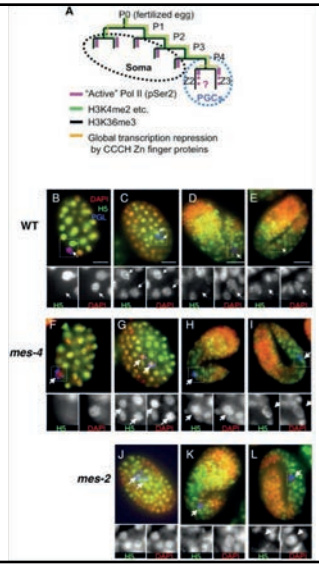
Exposure	Pregnancy rate	Pregnancy outcome	
		Full-term	Preterm
Vehicle control[a]			
conF1	10/10 (100%)	10/10[b]	0/10
conF3	12/12 (100%)	12/12	0/12
TCDD *in utero*[c]			
F1	11/28 (39%)	7/11	4/11
F3	8/14 (57%)	6/8	2/8

[a] Pregnant mice were exposed to corn oil vehicle (control) on E15.5 and control offspring (conF1 mice) mated at 10–12 weeks of age. Offspring of conF1 mice (conF2 mice) were mated at a similar age, as were the conF3 mice.
[b] Only a subset of conF1–F3 offspring were used to obtain additional generations of unexposed mice.
[c] Pregnant mice were exposed to 10 μg/kg TCDD in corn oil vehicle on E15.5 and singly exposed offspring (F1 mice) were mated at 10–12 weeks of age. Offspring of singly exposed F1 mice (F2 mice) were mated at a similar age, as were the F3 mice.

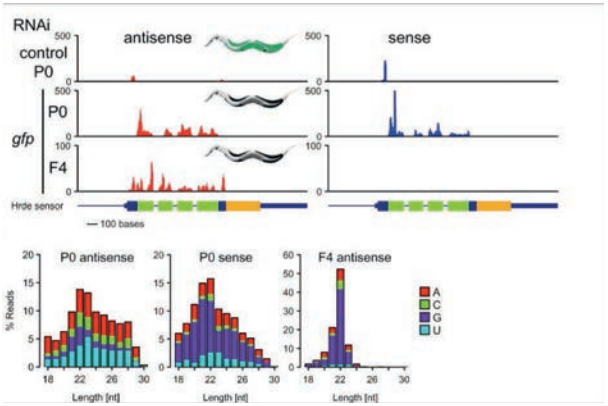
Trans-generational epigenetic regulation of C. elegans primordial germ cells.

Furuhashi H, Takasaki T, Rechtsteiner A, Li T, Kimura H, Checchi PM, Strome S, Kelly WG.

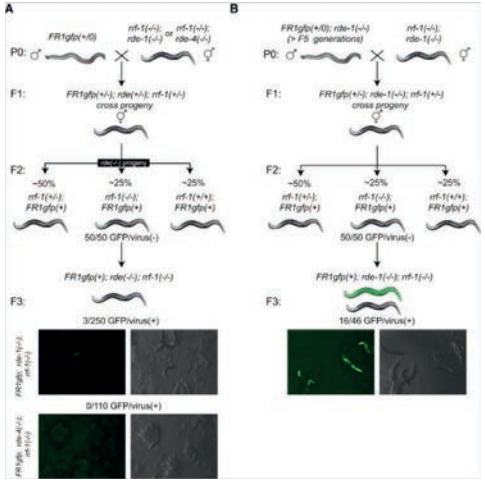
Epigenetics Chromatin. 2010 Aug 12;3(1):15.



Ashe A, et al. (2012) piRNAs can trigger a multigenerational epigenetic memory in the germline of C. elegans. Cell. 6;150(1):88-99.

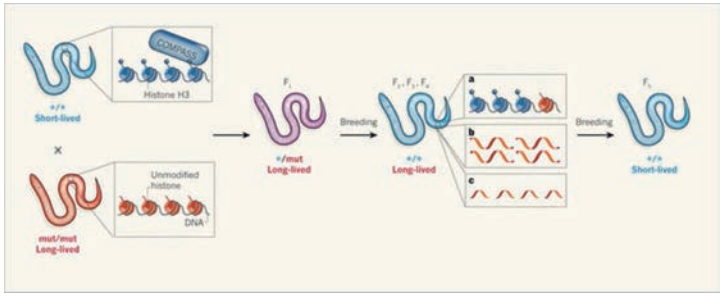


Rechavi O, Minevich G, Hobert O. (2011) Transgenerational inheritance of an acquired small RNA-based antiviral response in C. elegans. Cell. 9;147(6):1248-56.

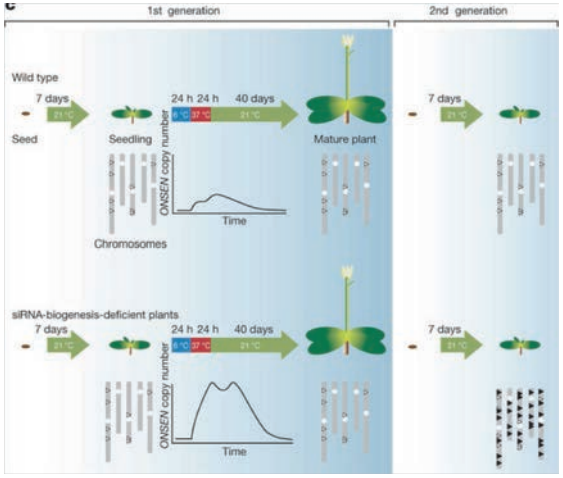


Mango SE. (2011) Ageing: generations of longevity. Nature. 16;479(7373):302-3.

The lifespan of some organisms can be extended by mutations that alter how DNA is packaged in their cells. A study reveals that this effect can last for generations, even in descendants that are genetically normal.



Ito H, et al. (2011) An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. Nature. 7;472(7341):115-9.



Small RNAs Reflect Grandparental Environments in Apomictic Dandelion.

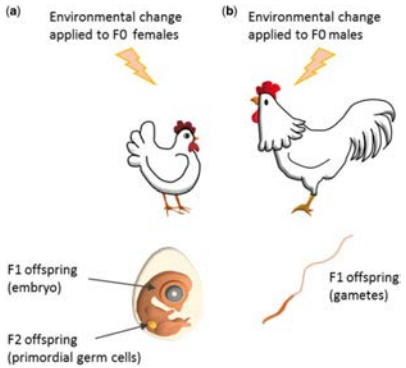
Mol Biol Evol. 2017 Aug 1;34(8):2035-2040.
Morgado L, Preite V, Oplaat C, Anava S, Ferreira de Carvalho J, Rechavi O, Johannes F, Verhoeven KJF.

Abstract

Plants can show long-term effects of environmental stresses and in some cases a stress "memory" has been reported to persist across generations, potentially mediated by epigenetic mechanisms. However, few documented cases exist of transgenerational effects that persist for multiple generations and it remains unclear if or how epigenetic mechanisms are involved. Here, we show that the composition of small regulatory RNAs in apomictic dandelion lineages reveals a footprint of drought stress and salicylic acid treatment experienced two generations ago. Overall proportions of 21 and 24 nt RNA pools were shifted due to grandparental treatments. While individual genes did not show strong up- or downregulation of associated sRNAs, the subset of genes that showed the strongest shifts in sRNA abundance was significantly enriched for several GO terms including stress-specific functions. This suggests that a stress-induced signal was transmitted across multiple unexposed generations leading to persistent changes in epigenetic gene regulation.

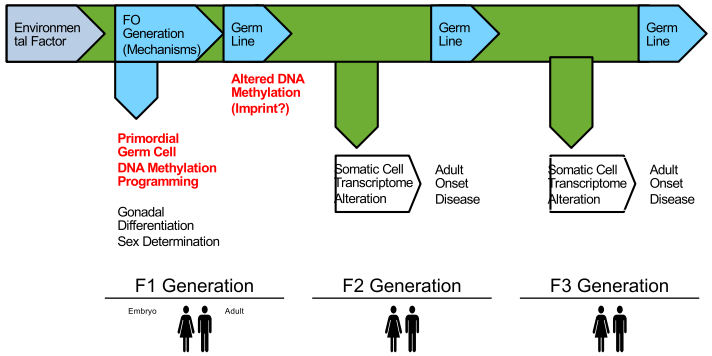
Transgenerational epigenetic inheritance in birds.

Environ Epigenet. 2018 Apr 26;4(2):dvy008.
Guerrero-Bosagna C, Morisson M, Liaubet L, Rodenburg TB, de Haas EN, Košťál Ľ, Pitel F.



The maternal environment directly impacts F1 and F2 offspring while the paternal environment only impacts F1 offspring. (**a**) A change in the maternal environment can affect egg components and thus may impact F1 individuals. However, as these F1 developing offspring bear the PGCs that will lead to differentiated gametes, the change in maternal environment may also impact F2 individuals. Thus only the effects observed on the F3 individuals will be considered as transgenerational effects. (**b**) A change in the paternal environment only affects its own gametes that will lead to the F1 generation. The effects observed on the F2 individuals will be considered as transgenerational effects

ROLE OF GERM LINE IN EPIGENETIC TRANS-GENERATIONAL INHERITANCE



Epigenetic Alterations Promote Genetic Instability

Genetic Mutation	Epigenetic Alteration	DNA Sequence Alteration
Point Mutation (SNP)	DNA Methylation (CpG)	Susceptibility C ➤ T Conversion
Copy Number Variation (CNV)	Hypomethylation (Repeats)	Susceptibility Repeat Element Alteration (CNV)
Transposon Migration	Hypomethylation DNA	Susceptibility Transposon Migration
Translocation	DNA Methylation and Histone Alterations	Susceptibility Translocation at Break Point
Telomere Length	DNA Methylation Alteration	Alteration in Telomere Length

Environmentally Induced Epigenetic Transgenerational Inheritance of Sperm Epimutations Promote Genetic Mutations

Skinner MK, Guerrero-Bosagna C, Haque M.
Epigenetics 2015; 10:8, 762-771

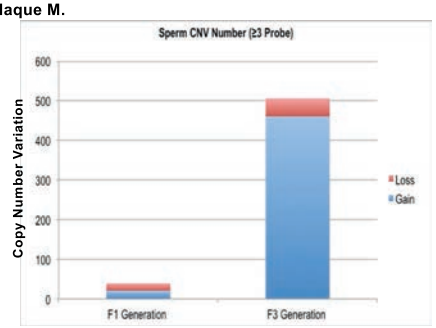
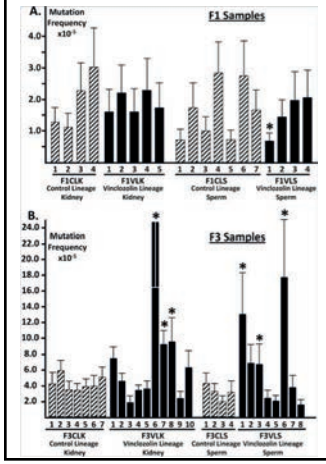


Table 1. (A) Vinclozolin F3 Generation Sperm Genome-wide CNV and Epimutations

Parameters	F1 Generation Sperm CNV	F3 Generation Sperm CNV	F3 Generation Epimutation Sperm
Number (Single Probe)	540(294 Gain / 246 Loss)	4912(4648 Gain / 264 Loss)	9932
Number (≥3 Probe)	39(21 Gain / 18 Loss)	506(461 Gain / 45 Loss)	191
Mean Size (base)	11,633	12,637	2,131
Mean CpG Density (CpG/100 bp)	1.1	1.0	0.9

Tertiary Epimutations - A Novel Aspect of Epigenetic Transgenerational Inheritance Promoting Genome Instability.

PLoS One. 2016 Dec 19;11(12):e0168038.
McCarrey JR, Lehle JD, Raju SS, Wang Y, Nilsson EE, Skinner MK.



Mutation frequencies in F1 and F3 generation samples.
(A) Mutation frequencies in kidney and sperm samples from F1 generation control- and vinclozolin-lineage animals. There were no statistically significant differences among the mutation frequencies detected in kidney or sperm samples from F1 generation control- and vinclozolin-lineage samples, except for one of the vinclozolin-lineage sperm samples (F1VL– marked with an asterisk) which showed a mutation frequency that was significantly lower than the mean of the F1 generation control-lineage samples ($p = 0.00352$). (B) Mutation frequencies in kidney and sperm samples from F3 generation control- and vinclozolin-lineage animals. A subset of both kidney and sperm samples from F3 vinclozolin-lineage descendants showed mutation frequencies that were not significantly different than the mean of the corresponding F3 generation control-lineage samples, although several of the F3 generation vinclozolin-lineage samples trended higher than the mean of the corresponding F3 generation control-lineage samples. However another subset of both kidney and sperm samples from F3 generation vinclozolin-lineage descendants showed mutation frequencies that were significantly higher than the mean of the corresponding F3 control-lineage samples. These mutation frequencies are marked with astrices, and include those found in the following samples: F3VLK6 ($p = 0.00342$), F3VLK7 ($p = 0.00131$), F3VLK8 ($p = 0.0222$), F3VLS1 ($p = 0.00185$), F3VLS2 ($p = 0.03611$)) and F3VLS6 ($p = 0.00018$). F1 = samples from F1 generation descendants, F3 = samples from F3 generation descendants, CL = samples from control-lineage descendants, VL = samples from vinclozolin-lineage descendants, K = kidney samples, S = sperm samples.