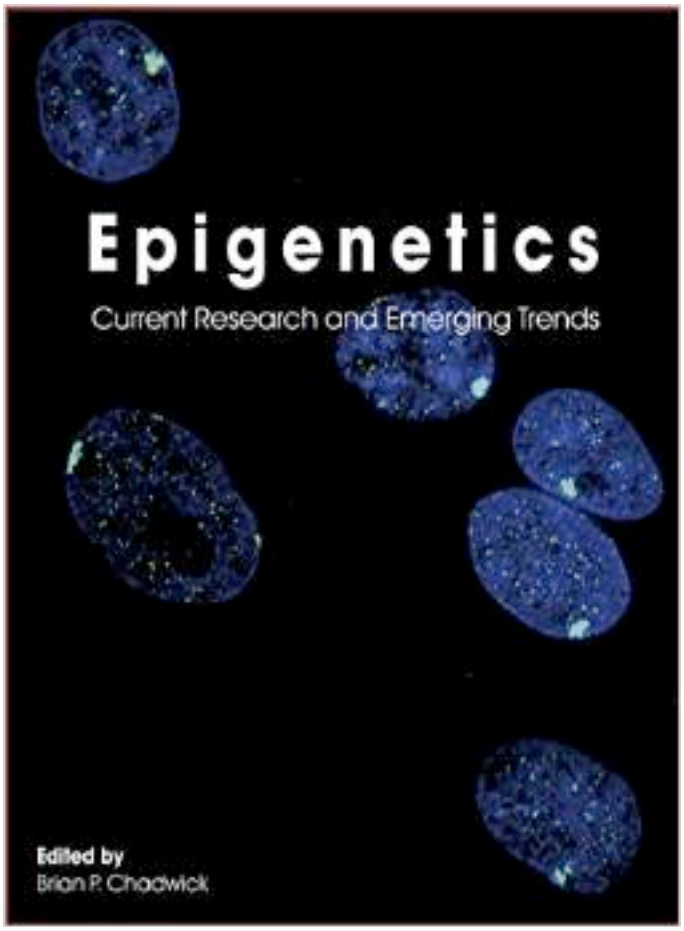

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Environment and the Epigenetic Transgenerational Inheritance of Disease

15

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Abstract

Environmental factors can lead to transgenerational epigenetic changes in generations originating from ancestors exposed to environmental insults such as toxicants, abnormal nutrition or stress. These epigenetic germline changes can in turn increase disease susceptibility in offspring of exposed ancestors. The focus of this chapter is on environmentally induced epigenetic transgenerational inheritance of disease. The molecular mechanisms that allow these inherited epimutations to increase disease susceptibility will be reviewed. Environmental exposure specificity and exposure-specific disease development are also discussed.

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 genetic phenomena (Van Speybroeck, 2002; Waddington, 1940). In the 1970s the first of the molecular factors enabling epigenetic processes – DNA methylation – was discovered (Holliday and Pugh, 1975). Other epigenetic modifications, like histone modification (Turner, 1998) and action of non-coding RNAs (Jodar *et al.*, 2013; Mattick, 2009) were later described. The complex integration of epigenetic modifications is referred to as the 'epigenome' with the first whole epigenome analysis accomplished in 2005 (Pokholok *et al.*, 2005). Epigenetics has dramatic impacts on developmental processes and disease aetiologies.

The definition of epigenetic transgenerational inheritance is 'germline-mediated inheritance of epigenetic information between generations in the *absence* of direct environmental influences, that leads to phenotypic variation' (Skinner, 2011). Multigenerational exposures on the other hand means that the observed effects are the result of direct exposure and may disappear when exposure is no longer present. The term transgenerational is often misused as multigenerational and transgenerational being the same, so the following has to be taken into consideration to use the term correctly. Direct environmental exposure of the parents (either male or female = F0 generation) will also affect the germline of either parent, Fig. 15.1. Therefore, the next generation (F1) derived from this germline is still considered exposed, so not truly transgenerational. The F2 generation offspring would be considered the first transgenerational unexposed generation. This is different when a pregnant female is exposed, because then the fetus and the fetus's germline are directly exposed as well. In this case the F3 generation is the first unexposed transgenerational offspring (Fig. 15.1).

The Agouti mouse model would be an example of multigenerational inheritance (Blewitt *et al.*, 2006; Waterland *et al.*, 2007). When pregnant Agouti mice are exposed to a methyl donor in their diet, they will experience increased methylation on an allele of their Agouti gene, which will lead to a coat colour change in their offspring. But this change will not be passed on to future generations. Instead the normal process of epigenetic reprogramming in the germline and early

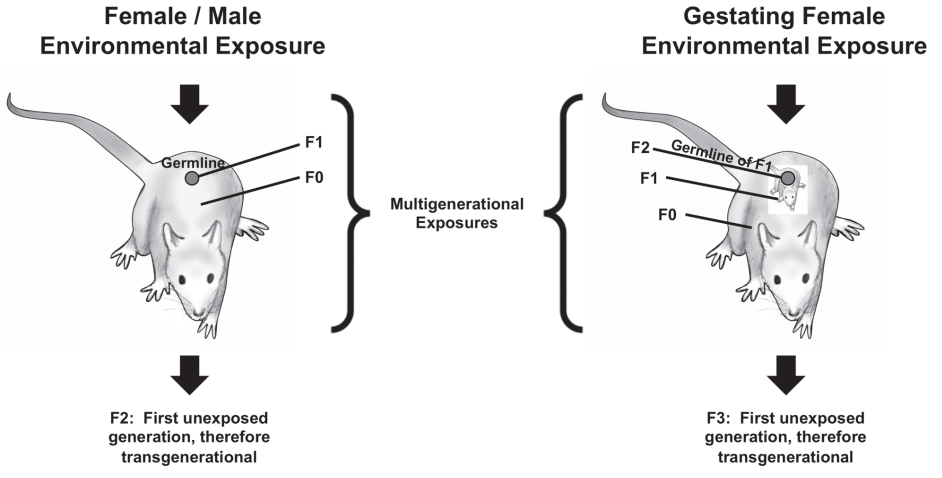

Figure 15.1 Environmentally induced transgenerational epigenetic inheritance: schematic of environmental exposure and affected generations.

embryo will return the DNA methylation state to its original setting. Similarly, exposure of human pregnant women to diethylstilbestrol (DES) did result in abnormalities and increased disease risk in only their direct offspring and grandchildren. In an animal model these multigenerational effects of DES have been shown to be caused by epigenetic alterations (Bromer *et al.*, 2009; Harris and Waring, 2012; Li *et al.*, 2014).

There are a large number of examples of transgenerational inheritance in the literature. The authors' laboratory has examined several environmental toxicants (e.g. DDT, vinclozolin, methoxychlor, plastics, hydrocarbons) and shown transgenerational inheritance of disease after exposure to these toxicants (Anway *et al.*, 2005; Guerrero-Bosagna *et al.*, 2013; Manikkam *et al.*, 2012a; Skinner *et al.*, 2013), Table 15.1. Other examples of transgenerational inheritance are caused by caloric restriction or high fat diet. The Överkalix study by Bygren *et al.* (2014) shows how cardiovascular mortality can be influenced by childhood food supply. Effects will reach into the second generation. Maternal high fat diet in mice (Dunn and Bale, 2011) will increase body size and reduce insulin sensitivity in F3 generation female offspring. A paternal prediabetic condition in mice will be inherited transgenerationally as shown by impaired glucose tolerance and decreased insulin sensitivity (Wei *et al.*, 2014). A number of studies from other laboratories have shown transgenerational inheritance after exposure to environmental factors such as stress and toxicants. Wolstenholme *et al.* (2012) showed that ancestral exposure to bisphenol A caused changes in social behaviour in juvenile mice and changes in expression of neural genes like oxytocin and vasopressin. Franklin *et al.* (2011) found that the stress of maternal separation in mice will transgenerationally impair social interaction and cognition but will also make the mice more stress resilient. Transgenerational inheritance has been shown in a variety of organism: vertebrates (Anway *et al.*, 2005), invertebrates like *C. elegans* (Kelly, 2014) or *Artemia* (Norouzitallab *et al.*, 2014) and plants (Zheng *et al.*, 2013). Table 15.1 shows a select list of studies of transgenerational inheritance after various environmental exposures.

Phenoptypic diversity of transgenerationally inherited diseases

The transgenerational inheritance of germline epigenetic modifications and its effects on disease, reproduction and behaviour has been a focus in the author's laboratory for the past decade and it was one of the first laboratories to study transgenerational effects on the epigenome. The initial research involved studying impacts of the agricultural agents vinclozolin and methoxychlor (Anway *et al.*, 2005) on pregnant rats. These

Table 15.1 Examples of transgenerational inheritance and its specific effects

Exposure	Effects	Reference
Environmental toxicants		
Vinclozolin	Impaired male fertility; prostate, kidney disease, tumours, immune and reproductive pathologies	Anway *et al.* (2005, 2006)
Vinclozolin	Gender-specific changes in anxiety-like behaviour	Skinner *et al.* (2008)
Vinclozolin	Immune and reproductive pathologies	Nilsson *et al.* (2008)
Methoxychlor	Impaired male fertility	Anway *et al.* (2005)
Permethrin/DEET	Prostate, kidney disease	Manikkam *et al.* (2012b)
Dioxin	Prostate, kidney disease; reduced fertility, negative effects on pregnancy outcome	Manikkam *et al.* (2012c), Bruner-Tran (2011)
BPA/phthalates	Prostate, kidney disease; obesity	Manikkam *et al.* (2013)
Hydrocarbon mixture (jet fuel)	Prostate, kidney disease; obesity; immune and reproductive pathologies	Tracey *et al.* (2013)
Vinclozolin, permethrin/DEET, plastics, dioxin, jet fuel	Polycystic ovaries, reduced primordial follicle pool	Nilsson *et al.* (2012)
DDT	Obesity	Skinner *et al.* (2013)
Phthalate	Disruption of testicular germ cell organization and spermatogonial stem cell function	Doyle *et al.* (2013)
Tributyltin	Increase in fat depot size	Chamorro-Garcia *et al.* (2013)
BPA	Changes in social behaviour and neural gene expression; increased post-implantation loss, decreased litter size; decrease in sperm count and motility	Wolstenholme *et al.* (2012), Salian *et al.* (2009)
Other exposures		
Caloric restriction	Cardiovascular mortality	Bygren *et al.* (2014)
High-fat diet	Increased body size; reduced insulin sensitivity	Dunn and Bale (2011)
Folate	Congenital malformations	Padmanabhan *et al.* (2013)
Stress	Reduced social interaction; increased stress resilience	Franklin *et al.* (2011)
Traumatic stress	Change in behavioural and metabolic responses	Gapp *et al.* (2014)
Drought	DNA methylation changes	Zheng *et al.* (2013)
Heat/salt stress	Accelerated flowering, increased salt tolerance	Suter and Widmer (2013)
Prediabetes	Impaired glucose tolerance; reduced insulin sensitivity	Wei *et al.* (2014)
Smoking	Abnormal pulmonary function	Rehan (2013)
Alcohol consumption	Deficit in POMC (proopiomelanocortin) neuronal function	Govorko (2012)
Heat stress	Increased Hsp70 production and tolerance to heat stress	Norouzitallab *et al.* (2014)

early findings were fortuitous observations in animals bred beyond the originally planned F1 generation. Effects in the F2 generation animals (increased spermatogenic apoptosis) were found to persist through the F3 and F4 generation and in outcrossed offspring, thus showing true transgenerational inheritance in a non-Mendelian manner with 90% of the male population affected at each generation.

The authors' initial research on epigenetic transgenerational inheritance showed a rise in spermatogenic cell apoptosis in the F3 generation

after exposure to vinclozolin in the F0 generation. In later experiments, after treatment of the F0 generation with an array of additional toxicants (Table 15.1), a number of other problems were detected, for example prostate and kidney disease (Anway *et al.*, 2006; Anway and Skinner, 2008; Manikkam *et al.*, 2012a–c, 2013; Tracey *et al.*, 2013), mammary tumours (Anway *et al.*, 2006), immune and reproductive pathologies (Anway *et al.*, 2006; Nilsson *et al.*, 2008; Tracey *et al.*, 2013), obesity (Skinner *et al.*, 2013; Tracey *et al.*, 2013), as well as behavioural effects (Skinner *et al.*, 2008). Therefore, between the authors' and other laboratories (Chamorro-Garcia *et al.*, 2013; Doyle *et al.*, 2013), evidence has been given that a variety of diseases and behavioural abnormalities will occur transgenerationally after exposure to a large number of environmental toxicants and other environmental exposures, (Table 15.1). The disease phenotypes observed in these experiments often depend on the specific exposure of the F0 generation. For example, increased obesity risk is inherited transgenerationally after ancestral exposure to DDT, plastic compounds, hydrocarbons and methoxychlor (Manikkam *et al.*, 2013, 2014; Skinner *et al.*, 2013) but not others. Jet fuel hydrocarbons will induce an elevated rate of luteal ovarian cyst formation in F3 females (Manikkam *et al.*, 2012a; Tracey *et al.*, 2013), a phenotype not observed with other exposures. On the other hand, some ovarian disorders like polycystic ovaries and reduction of the primordial follicle pool size have been shown to be inherited transgenerationally after exposure of the F0 generation to all the toxicants studied so far in the authors' laboratory (Nilsson *et al.*, 2012). The explanation for this phenomenon may be that some developmental mechanisms, in this case ovarian follicle development, are more sensitive to gene expression changes and will be affected more often than other cells and tissues.

Germline epimutations and specificity of environmental toxicants

The term epimutations is defined as differential presence of epigenetic marks that lead to altered genome activity, see Glossary. It is a prerequisite for environmentally induced epigenetic transgenerational inheritance to have epimutations in the germline, because the germ cells (sperm and egg) are the only cells that can transmit information from the parents to their offspring. DNA methylation is the most thoroughly investigated molecular mechanism involved in the transmission of epigenetic changes via the germ cells to future generations, but other factors like histone modification and actions by non-coding RNAs appear to also be involved. Normally, environmentally induced methylation changes will be reset by a genome wide DNA methylation reprogramming after fertilization in the early embryo (Hackett and Surani, 2013; Smith *et al.*, 2012). This phenomenon allows embryonic stem cells to be developed, but also one would not want parental epigenetic changes to be established in the offspring. These epigenetic reprogramming events take place at two developmental periods, during germ cell specification at gonadal sex determination and in the early embryo. After this DNA methylation erasure parent-specific imprints are established (imprinted genes) (Constancia *et al.*, 1998). It has been found that certain regions in the genome called differential DNA methylation regions (DMRs) (Skinner *et al.*, 2010) appear to behave as imprinted-like genes in the way that their methylation pattern persists. 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 may not be involved. Differentially methylated sites connected with transgenerational inheritance are called 'imprinted-like' (Jirtle and Skinner, 2007).

When epigenetic information is passed on to future generations via germ cells, the epigenome of the developing embryonic stem cells would be expected to have an altered epigenome that will change the epigenetic and transcriptome programming of all subsequent somatic cells developed (Guerrero-Bosagna *et al.*, 2013). All developing tissues will have cell and tissue specific effects on the transgenerational transcriptome. Therefore, one would expect a different transcriptome stemming from the same germline epigenome in

each somatic cell type. Those tissues that have a sensitivity to alterations in their transcriptomes may also show increased sensitivity to disease development, which will be inherited because of the causative epigenetic changes in the germline (Skinner *et al.*, 2010, 2012).

The first examples of transgenerational epimutations come from studies from the authors' laboratory. The studies investigated Vinclozolin, a dicarboximide fungicide, used especially in vineyards and on fruits and vegetables (Anway *et al.*, 2005). A genome-wide promoter analysis was applied to look for DNA modifications in the sperm DNA and approximately 50 differential DNA methylated regions (epimutations) were identified in vinclozolin lineage F3 generation sperm DNA versus control lineage (Guerrero-Bosagna *et al.*, 2010).

After having established a specific set of epimutations in vinclozolin lineage animals, the authors' laboratory pursued the question if treatment with different environmental toxicants would also lead to germline epimutations. Experiments similar to the original vinclozolin experiment were performed with a number of additional toxicants: dioxin (Manikkam *et al.*, 2012c), a mixture of permethrin and DEET (Manikkam *et al.*, 2012b), bisphenol A and phthalates (Manikkam *et al.*, 2013) and jet fuel (hydrocarbons) (Tracey *et al.*, 2013). All these toxicants were found to promote transgenerational inheritance of disease and sperm epimutations. Intriguingly, it was observed that each toxicant produced an exposure specific set of epigenetic changes in the sperm DNA and comparison between the individual exposures demonstrated negligible overlap (Manikkam *et al.*, 2012a). The possibility of using these unique 'epimutation signatures' as a diagnostic tool in the future is speculated.

Molecular basis of transgenerationally inherited disease

Transgenerational inheritance of environmentally induced epigenetic changes requires transmission through the germ line from parents to future generations. 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 (Guerrero-Bosagna *et al.*, 2013; Nilsson *et al.*, 2012). These cell and tissue specific epimutations promote tissue-specific alterations in transcriptomes (Skinner *et al.*, 2012). These aberrant transcriptomes could then lead to physiological abnormalities and disease.

Experiments have shown that F0 generation exposure to environmental toxicants will affect and change the transcriptomes of multiple tissues in future generations (Skinner *et al.*, 2012). It was observed that the epigenomic changes of one cell type could be very different from another cell type in the body, despite the fact that they all result from a common germ line. The authors' laboratory examined the transcriptomes of 11 different tissue types from adult male and female animals who were of the vinclozolin lineage mentioned earlier. They found that (a) there were (not-unexpectedly) gene expression differences between control and vinclozolin lineage animals and (b) there was only minor overlap in the differentially expressed genes between tissues. These findings warranted a closer look at the genomic locations of epimutations and differentially expressed genes. Regions of the genome were found to have statistically over-represented clusters of gene expression changes. These regions in the genome were called epigenetic control regions (ECR). These epigenetic control regions are 2–5 MB in size and have gene clusters of statistically over-represented regulated genes. These ECR are often located close to epimutations (Skinner *et al.*, 2012) and to regions of long non-coding (nc) RNA expression (Kung *et al.*, 2013). It is well known that long ncRNAs play a role in regulation of distal gene transcription and epigenetic regulation. Further research will reveal if the long ncRNAs have a part in the management of the genes located in the epigenetic control regions.

Several studies in the authors' laboratory have elucidated at least partially how the molecular mechanisms of environmentally induced transgenerational inheritance may lead to tissue specific disease appearance. 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 (Nilsson *et al.*, 2008). In order to study this phenomenon as related to the vinclozolin lineage animals, granulosa cells were isolated from ovarian follicles from young female rats before disease onset. The epigenomes and transcriptomes of these granulosa cells (control and vinclozolin lineage) were analysed (Nilsson *et al.*, 2012). Granulosa cells from vinclozolin lineage rats had differences in both the epigenome and the transcriptome compared to control lineage. Closer examination revealed that specific signalling pathways were affected, and some of the affected genes had been shown previously to be associated to polycystic ovarian syndrome and primary ovarian insufficiency. Similar results were obtained when the molecular basis of vinclozolin lineage male infertility in rats was examined. As above, changes in the epigenome and transcriptome were found in testicular Sertoli cells of F3 generation rats (Guerrero-Bosagna *et al.*, 2013). Several of the differentially regulated genes were known to be involved in male infertility.

Impact on evolution

The fact that studies have shown that environmental factors can induce transgenerational changes in organisms leads to the assumption that epigenetic transgenerational inheritance may also play a role in evolution. Evolutionary change through DNA mutations is a slow process and not always suitable for an aggressively changing environment. Epigenetic inheritance would provide a quick-response solution and adaptation in a rapidly shifting setting.

Darwin (1871) considered sexual selection one of the important processes driving evolution. He defined sexual selection as either male–male competition or females' choice of a male mate (mate selection). One of the authors' studies has shown that F3 generation rats from the vinclozolin lineage mentioned earlier have a significant change in mate preference behaviour. Female rats of both control and vinclozolin F3 generation lineages were found to preferentially chose males from the control group instead of males from the vinclozolin lineage (Crews *et al.*, 2007). Considering that sexual selection is a major element in evolutionary biology, the fact that environmental factors can change mate preference in a transgenerational way would support a critical role of epigenetic transgenerational inheritance in evolution. Generally, epigenetic changes in the germline that are passed on to offspring can produce inherited changes in gene expression in all cells and tissues, and if those gene expression changes result in an increased phenotypic diversity, one might expect that natural selection will act upon these phenotypic variations and lead to evolutionary change.

The rats used in the mate preference study are from a lineage that had been shown to have epimutations in the sperm genome that alters epigenomes in adult tissues transgenerationally (Skinner *et al.*, 2010, 2012). The mate preference study investigated male and female brain regions and showed distinct differentially expressed gene sets in each region (Skinner *et al.*, 2014b). Gene bionetworks were found in various brain regions that statistically correlated to the altered mate preference behaviours (Skinner *et al.*, 2014b). Observations indicate the environmentally induced changes in mate preference appear to impact a major determinant of evolution, sexual selection.

A more recent experiment investigated the phylogenetic relationships of epimutations and genetic mutations in Darwin's finches in the Galapagos Islands (Skinner *et al.*, 2014a). The epigenetic changes, epimutations, among five different species demonstrated a statistically significant correlation with the phylogenetic association of these species. Interestingly, the number of epigenetic changes were generally greater than genetic mutations and more correlated with the phylogenetics (Skinner *et al.*, 2014a). Although additional research is needed to establish a functional role of environmental epigenetics in evolution, the ability of environment to promote the epigenetic transgenerational inheritance of phenotypic variation will clearly have a significant impact on natural selection.

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, pigs, mice, rats and humans. Ancestral exposure to environmental influences such as toxicants or nutritional stress can transmit changes in the epigenome to their descendants. These epimutations caused by individual exposures are required to be transmitted through the germline. They subsequently will induce somatic cell changes in the epigenome and transcriptome in the offspring. There are several mechanisms that will be involved in the creation of epigenetic change, DNA methylation being the most thoroughly researched. A major mechanism involved in the epigenetic transgenerational inheritance of disease is through changes in DNA methylation which are transferred through the germline to somatic cells causing the observed phenotypical changes. Therefore, these ancestral exposures to environmental toxicants can lead to transgenerational changes in the epigenome and transcriptome of future generation and lead to increased incidence of disease. The potential role of these ancestral exposures and epigenetic transgenerational inheritance in disease aetiology needs to be seriously considered.

Since it has been shown in initial experiments that the majority of the environmental influences tested have a unique pattern of epigenetic changes in the offspring, one might consider the possibility for epigenetic diagnostics in the future, testing for ancestral exposure and associated disease risk in a population.

Glossary

Epigenetics: molecular factors/processes around the 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 direct environmental influences, that leads to phenotypic variation.

Epimutation: differential presence of epigenetic marks that lead to altered genome activity.

F0, F1, F2, F3: various generations – parents, offspring, grand-offspring, great-grand-offspring.

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