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Review

The epigenome as a target for heritable environmental disruptions of cellular function

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ABSTRACT

The environment is a well-established source of damaging or disrupting influences on cellular function. In the past, studies of the mechanisms by which such disruptions occur have focused largely on either direct toxic effects on cellular function at the protein or cell signaling level, or mutagenic effects that impact the genome. In recent years there has been a growing appreciation for the potential for environmental influences to disrupt the epigenome and mechanisms of epigenetic regulation within the cell. Indeed, because of the inherent lability of the epigenome, this represents a primary target for environmentally induced disruption. This review summarizes the manner in which the epigenome normally regulates cellular function, the effects of disruptions on this function, and the manner in which such disruptions may or may not be corrected within the organism and/or transmitted to subsequent generations.

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1. Cellular function is regulated by genetic and epigenetic mechanisms

Cellular function is based largely on the expression of genes. Thus, while in most Metazoan organisms it is the case that every cell possesses an essentially identical and complete copy of the genetic information inherited from an individual's parents, it has become clear that different cell types express different subsets of genes, and that cellular differentiation is based primarily on differential gene expression. Therefore, to understand the process of cellular differentiation and, to a large degree the process of cellular function, it is important to understand the mechanisms that regulate differential gene expression.

Because every cell type in a multicellular organism typically contains the same genetic information, cellular differentiation cannot be based on the presence or absence of specific portions of the genome. Indeed, it has been known for many years from nuclear transplantation experiments – first in frogs in the 1950s (Briggs and King, 1952) and 1960s (Gurdon, 1962), and more recently in mammals in the 1990s (Wilmut et al., 1997; Wakayama et al., 1998), that the nucleus of a differentiated somatic cell retains all of the genetic information necessary to direct the development of a complete new individual. These experiments proved that differentiation of different cell types does not occur by a mechanism involving elimination

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of irrelevant genetic information from each cell type. Therefore, differential gene expression in different cell types must result from the differential regulation of gene expression.

We now know that there are multiple molecular mechanisms that regulate differential gene expression. These include protein–DNA interactions – specifically transcription factors including activators and co-activators, as well as repressors and co-repressors. Transcription factors often interact directly or indirectly to regulate attraction or binding of RNA polymerase to a gene to activate (in the case of activators and co-activators) or inhibit (in the case of repressors and co-repressors) transcription. Beyond this mechanism, however, there are additional effects that regulate the structure of chromatin in a region containing the promoter of a gene. Thus, when chromatin is present in a highly condensed state, access to binding sites for transcription factors or to the transcription start site for RNA polymerase is typically hindered or completely blocked, whereas a decondensed chromatin structure is normally much more accessible to transcription factors and RNA polymerase and therefore favorable to the initiation of transcription.

Chromatin structure is regulated by multiple mechanisms. Post-translational modifications of specific amino acids in histones can directly influence the affinity that histone tails extending from core nucleosomes show for one another and this can favor condensation (when affinity among histone tails is high) or decondensation (when affinity among histone tails is low) of chromatin. In addition, modifications to histone residues can also influence binding of chromatin remodeling or modifying complexes that can exert additional regulation on the structure of chromatin and, hence, on potentiation of transcriptional activity in a specific region of the genome or of specific individual genes (Kramer et al., 1998).

An additional mechanism regulating chromatin structure is DNA methylation. In mammalian somatic cells, DNA methylation has the potential to occur as a modification of carbon 5 in the base cytosine when that cytosine is present in a dinucleotide with the sequence 5′-CpG-3′. The presence or absence of DNA methylation at a particular CpG dinucleotide in mammalian DNA has been shown to be heritable through both mitosis and meiosis, though DNA methylation can be altered by de novo methylation of a previously unmethylated CpG dinucleotide or by demethylation of a previously methylated CpG dinucleotide. Interestingly, the presence or absence of DNA methylation, especially in promoter regions of genes, has shown a very high correlation with repression or activation, respectively, of transcription of that gene (Cedar and Bergman, 2009). It has been shown that methylated DNA attracts methyl-DNA-binding proteins that can include or attract chromatin modifying complexes that tend to promote chromatin condensation and the establishment of a condensed, repressive chromatin structure (Bird and Wolffe, 1999; Urnov and Wolffe, 2001). Thus, a transcriptionally repressed gene tends to have methylated DNA and histone modifications in the promoter region that favor a condensed chromatin state which, in turn, is refractory to binding of activating transcription factors and RNA polymerase II, thus inhibiting transcription. In contrast, a transcriptionally active gene typically shows a lack of DNA methylation along with the presence of histone modifications in the promoter region that promote a decondensed chromatin state which, in turn, facilitates binding of transcription factors and RNA polymerase II necessary for initiation of transcription. In certain circumstances, DNA methylation can promote gene expression, as, for instance, when methylation interferes with the binding of repressors (Engel et al., 2006).

Mechanisms that regulate gene expression in ways that do not involve any change in the primary DNA sequence are known as epigenetic mechanisms (McCarrey, 2003). Collectively, the particular set of epigenetic mechanisms functioning to program gene expression in a particular cell type is known as the epigenome of that cell type. Whereas, as noted above, the genome remains essentially constant in all cell types of the body, the epigenome is distinct in different cell types. Indeed, because cellular differentiation is based on differential gene expression, and different patterns of gene expression characteristic of each different cell type are programmed by epigenetic mechanisms, it is the epigenome of a cell that determines its identity. Thus while all cell types within an individual typically share a common genome, they possess distinct epigenomes. Evidence suggests that specific states of epigenetic programming in a cell are heritable (Cedar and Razin, 1990; Lande-Diner and Cedar, 2005). The mechanism of heritability of certain epigenetic mechanisms, such as DNA methylation, is better understood than that for other epigenetic mechanisms, such as histone modifications, but there is evidence that all of these mechanisms are indeed heritable through either mitosis or meiosis (Cedar and Bergman, 2009).

2. Cellular function can be disrupted by environmental effects

It has long been known that various environmental effects can adversely affect cellular function. Different environmental agents can exert disruptive effects on cellular function at different levels. Many toxic agents can disrupt cellular function at the level of protein function or signaling mechanisms within individual cells and/or between cells or within tissues. Reports of such effects increased dramatically during the 20th Century commensurate with the increase in the production of synthetic chemicals – especially those derived from petroleum and natural gas (Gross, 2007). Many of these agents appear to induce defects manifest particularly in the nervous system (Li et al., 2007; Phelps, 2007). More recently, there has been significant focus on adverse cellular effects caused by oxidative stress, especially due to the actions of reactive oxygen species (ROS). ROS can induce defects in diverse cellular functions or components including membrane lipid integrity, activity of specific "ROS-susceptible" proteins and accumulation of toxic protein aggregates and/or induction of apoptotic cell death, among others (Avery, 2011). Such effects have been linked to the etiology of specific diseases including Alzheimer's disease, Friedreich's ataxia and cancer (Roberts et al., 2009). Mechanistically, it is known that ROS and other toxic agents can target various of the major macromolecules in the cell, including lipids, proteins and/or nucleic acids (Imlay, 2008; Thorpe et al., 2004; Cabiscol et al., 2000). Typically, toxic effects of environmental agents on cellular function have been studied in the context of the cells directly exposed to the toxic agent. However, certain toxic effects can induce cellular defects that are heritable.

3. Environmental disruptions of the genome lead to genetic mutations

A second, well-documented disruptive effect of certain environmental agents on cellular function involves mutagenesis (Fig. 1A). Thus, various mutagenic agents can cause a variety of genetic aberrations. These can be large-scale aberrations such as polyploidy (changes in the number of copies of the complete genome – i.e. an entire set of chromosomes – within individual cells or an entire organism) or aneuploidy (abnormal numbers of chromosomes within individual cells or an entire organism) or other types of gross chromosomal defects including translocations (rearrangements of large pieces of genetic material among different chromosomes) or inversions (rearrangements of the orientation of a large piece of genetic material within an individual chromosome). Other large-scale mutations include deletions, insertions, duplications and/or amplifications of a large segment of genetic material (typically within a single chromosome). Alternatively, these disruptions

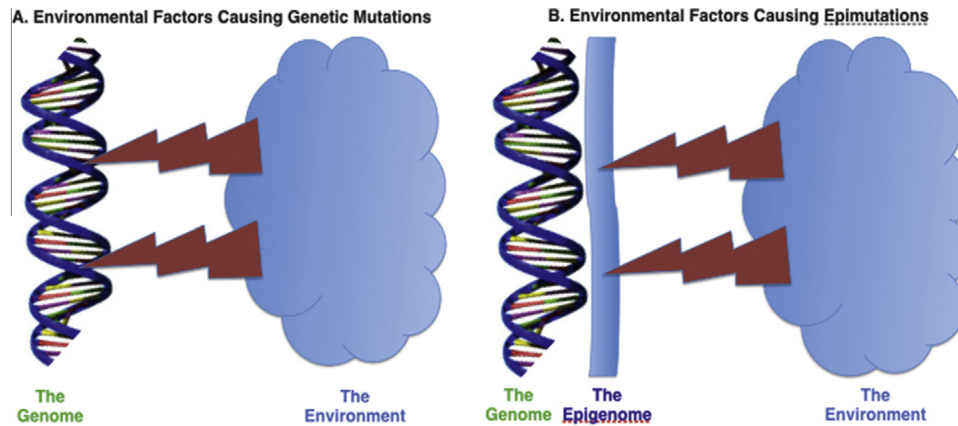

Fig. 1. Routes of environmental disruption of cellular function. Two types of environmental disruption are depicted. (A) Environmental factors causing genetic mutations represents the direct effect of the environment on the genome of the cell resulting in genetic mutations that may or may not lead to phenotypic effects. If deleterious, such phenotypic effects can include production of a defective RNA or protein product encoded by the mutated gene or abnormal regulation of expression of the mutated gene. (B) Environmental factors causing epimutations represents the direct effect of the environment on the epigenome of the cell leading to epimutations. If deleterious, such epimutations can lead to abnormal regulation of expression of the epimutated gene or abnormal maintenance of other chromatin functions in that same region of the genome.

can be small-scale aberrations such as point mutations including deletions, additions or changes in one or a few bases of DNA sequence. Importantly, point mutations are the cause of a majority of genetic diseases known to afflict humans (Crow, 2000), though the general incidence of such diseases is typically low (Wallace, 2010).

A majority of mutations, especially point mutations, do not impact cellular function. These are known as "silent mutations" because they either occur in a region of the genome that does not encode a protein or RNA product, or because the specific change in DNA sequence does not impact the function of the encoded product. A small number of mutations are actually beneficial to cellular function. These are often referred to as "gain-of-function" mutations and when beneficial these mutations have provided the raw material upon which evolution is based. Finally, there are "loss-of-function" or mutations that typically lead to either a reduction or complete loss in one or more cellular functions, and these mutations are therefore directly detrimental to the cell (Eyre-Walker and Keightley, 2007).

Examples of well-documented mutagenic environmental effects include ultraviolet light, nuclear radiation, and various chemical agents including tobacco, alcohol, pesticides, asbestos, lead, mercury and many others. Many of these agents can cause either large- or small-scale mutations. Often a mutagenic agent will induce DNA damage, which, if not repaired (see below), will become a permanent or "fixed" mutation. Importantly, once a mutation becomes permanent it is heritable and will be passed on to all daughter cells emanating from the cell in which the mutation initially occurs. The likelihood of a "reverse mutation" is vanishingly low, which is why these are referred to as "permanent" mutations (Friedberg et al., 2006).

Mutations can occur in either germ cells (the spermatozoa and their precursors in males or the ova [eggs] and their precursors in females) or somatic cells (all other cells of the body other than the germ cells). A permanent mutation in the germ line (known as a germline mutation) has the potential to be transmitted transgenerationally to subsequent generations if the sperm or egg that gives rise to an offspring carries that mutation (Singer and Yauk, 2010). In that case, the mutation will become a "constitutional" mutation in the next generation because if it is present in the original diploid genome of the fertilized egg (zygote), it will be propagated to all cells of the body. A permanent mutation that occurs in any somatic cell (called a somatic mutation) will be transmitted to all daughter cells emanating from that original mutated somatic cell, but will not be transmitted to subsequent generations. Thus germline mutations have the potential to be transgenerational whereas somatic mutations do not.

4. Accumulation of genetic mutations is mitigated by cellular mechanisms that maintain genetic integrity

Potentially mutagenic effects occur at a remarkably high frequency, even when cells are not subjected to a specific mutagenic effect. Thus, normally occurring environmental factors such as UV light and radiation can cause DNA damage resulting in as many as 1 million individual molecular lesions per cell per day (Lodish et al., 2004). Fortunately, the vast majority of these potentially mutagenic effects do not lead to the creation of permanent mutations in cells. This is because cells possess multiple mechanisms to mitigate the effects of mutagenic agents in the form of DNA repair activities. DNA repair pathways in mammalian cells include base excision repair, nucleotide excision repair, mismatch repair, single-strand break repair, double-strand break repair, and nonhomologous end joining, among others (Friedberg et al., 2006). As their names imply, different DNA repair pathways function to repair different types of damage to DNA. Collectively, these pathways are highly efficient in their ability to repair DNA damage such that such damage typically does not lead to permanent mutations. This is critical to the cell's ability to maintain its genetic integrity and, thus, its normal functionality. Interestingly, germ cells have been shown to maintain genetic integrity at a more stringent level than somatic cells, even in the same individual (Walter et al., 1998). This is consistent with the "Disposable Soma Theory" originally proposed by T.B. Kirkwood (1977), which holds that because germ cells normally give rise to the entire next generation, it is particularly important for genetic integrity to be maintained in these cells and therefore relatively beneficial for germ cells to exert additional energy to achieve this. Indeed, it has been shown that several DNA repair pathways are more active in germ cells than in somatic cells (Walter et al., 2003).

In the event that an individual cell does accumulate extensive DNA damage, there is an additional set of mechanisms by which an organism can mitigate potentially harmful effects of this damage by eliminating the individual cells carrying extensive damage from the population. This is achieved by activating cell death pathways including apoptosis, autophagy or necrosis. Typically it is

only large-scale genetic damage that is capable of triggering programmed cell death (Friedberg et al., 2006). Taken together, DNA repair and cell death pathways effectively preclude the accumulation of permanent mutations in cells or the accumulation of cells bearing extensive genetic damage within a tissue or organ. Thus, while environmental or endogenous effects frequently threaten to induce permanent defects in the genome, the actual accumulation of such defects is normally limited.

5. Environmental disruptions of the epigenome lead to epimutations

A more recently appreciated concept is that just as defects can occur in the genome of a cell in the form of genetic mutations, defects can also occur in the epigenome of a cell in the form of epimutations (Whitelaw and Whitelaw, 2008; Martin et al., 2011). Two important aspects of the concept of epimutations are that they (1) represent abnormalities in one or more epigenetic mechanisms functioning within a particular cell type and (2) are potentially heritable from parental cell to daughter cells or, if they occur in germ cells, from generation to generation. Two types of epimutations have been described – primary and secondary (Whitelaw and Whitelaw, 2008). A primary epimutation is characterized as an initial defect within an epigenetic parameter (e.g. an aberrant DNA methylation pattern) that is then propagated, as such, to subsequent daughter cells produced from the initial cell in which the primary epimutation occurred. Examples of primary epimutations could include defects in normal DNA methylation patterns, histone modification patterns or protein–DNA interactions regulating the expression of one or more specific genes in a particular cell type. Because states of epigenetic programming are generally assumed to be heritable, primary epimutations are also assumed to be heritable, though because of the fact that there is a well-established mechanism for heritability of DNA methylation patterns, this is particularly true of epimutations affecting this epigenetic parameter. A secondary epimutation is characterized as an initial genetic mutation that impacts the function of a regulator of epigenetic programming (e.g. a mutation in a gene encoding a DNA methyltransferase) such that the epigenome is affected. Because a secondary epimutation results from a primary genetic mutation, it is typically heritable through either mitosis or meiosis.

While the genome is maintained in a relatively constant state, the epigenome is normally much more plastic in nature. Thus, as noted above, the genome remains essentially constant in every cell type of the body whereas each different cell type is characterized by a distinct epigenome. Beyond this, however, the modifications of DNA or chromatin that make up the epigenome are reversible and subject to reprogramming within the germ line during each generation. As a result, epigenetic programming inherited from the previous generation via gametic genomes is typically erased and then re-established during the development and differentiation of germ cells in the subsequent generation (see below). Therefore, abnormalities that are strictly epigenetic in nature, such as primary epimutations, are normally expected to be reversed or "corrected" during gametogenesis. Of course, defects in the epigenome that are caused by an initial genetic mutation (secondary epimutations) will not be subject to correction by germline-specific epigenetic reprogramming, and would only be corrected by a very unlikely reverse genetic mutation. Interestingly, it has also been suggested that epigenetic defects can predispose genetic defects, as would be the case in the event of abnormalities in epigenetic regulation of genes encoding products that normally regulate or mitigate the occurrence of mutations (Toyota and Suzuki, 2010). Although no formal designation of this type of epimutation has been suggested, it would seem that these might be placed in a separate category termed "tertiary epimutations." In this regard, it has also been shown that the relative level of maintenance of genetic integrity in a cell type is subject to epigenetic reprogramming (Murphey et al., 2009).

6. The epigenome is inherently more susceptible to environmental disruption than the genome

Unlike the situation discussed above regarding effects that impact the integrity of the genome (i.e. potentially mutagenic effects), there are no known cellular mechanisms that immediately mitigate the accumulation of epimutations. Thus, any effect emanating from the environment or occurring within the cell itself that may cause a primary epimutation is not subject to immediate correction by any sort of cellular repair mechanism (Fig. 1B). Furthermore, because the epigenome is inherently much more labile in nature than the genome, it stands to reason that it is likely to be much more susceptible to defects induced by environmental effects than is the genome. Indeed, it is likely that the epigenome is ultimately the functional target of a much larger proportion of environment effects than is the genome. For this reason, it has been estimated that the rate of primary epimutations is one–two orders of magnitude greater than that of somatic DNA mutations, and that the contribution of epimutations to human disease has therefore been underestimated (Horsthemke, 2006).

The significance of this observation is that, when considering the impact of the environment on cellular, tissue, organ or organismal function, much greater attention should be paid to the impact of environmental agents on the epigenome than has been accorded in the past. Indeed, it seems that until relatively recently, potential deleterious effects of the environment on the epigenome were largely ignored in favor of a focus on environmental effects impacting the genome (Fig. 1). This was despite the fact that, as argued above, the epigenome warrants significant attention as the most likely target of detrimental effects of environmental disruption on cellular function.

In recent years, however, there has been a dramatic change in the frequency of reports of environmental effects impacting the epigenome (Skinner, 2011). Thus, exposure to a wide variety of environmental toxicants, particularly during either in utero development or early postnatal life, but also at other times during the lifetime of an individual, has been associated with the occurrence of epigenetic abnormalities that have, in turn, been associated with various deleterious effects in humans (Jablonka, 2004; Jiang et al., 2004). Epimutations can lead to the aberrant activation or repression of certain genes leading to disease states that can be unique or can phenocopy genetic diseases (Martin et al., 2011). In addition, epimutations have been implicated in the etiology of cancer, and a portion of these have been ascribed to environmental effects (Dobrovic et al., 2009). Finally, very recent studies have shown that epimutations can result from defects in a cell's ability to maintain proper epigenetic programming as well as from the initial acquisition of abnormal programming (Denomme et al., 2011). Taken together, this has led to the suggestion that epigenetics holds substantial potential for furthering our understanding of the molecular mechanisms of environmental toxicants, as well as for predicting health-related risks due to conditions of environmental exposure and individual susceptibility (Bollati and Baccarelli, 2010).

7. Heritability of epimutations within a generation

Because epigenetic modifications are, by definition, heritable through DNA replication and cell division, an epimutation will typically be propagated in a clonal manner to all cells that are

produced from any cell in which an initial epimutation occurs. Thus, following the initial spontaneous occurrence of an epimutation in any somatic cell type, all daughter cells emanating from that original cell should also possess the same epimutation. There are no known mechanisms functioning in somatic cell types to specifically correct, reverse or mitigate epimutations. However, some epigenetic modifications are not completely stable and are therefore termed ‘‘metastable’’ (Rakyan et al., 2002). These modifications can be lost in a stochastic manner, and this could lead to the random disappearance of either an epimutation or a normal epigenetic modification in any particular somatic cell. Therefore, spontaneous primary epimutations occurring in somatic tissues tend to be stochastic, mosaic and occasionally reversible, and on this basis, the somatic and germline (see below) transmission of epimutations, and, hence, any phenotypes these epimutations influence, may be propagated in patterns that do not follow the standard rules of Mendelian genetic inheritance (Martin et al., 2005).

8. Germline-specific correction or reprogramming of epimutations diminishes transmission between generations

Although epimuations are commonly somatic events, they can also occur in the germ line (Cropley et al., 2008). The extent to which the epigenome is more likely to be the target of environmental (or endogenous) disruption than the genome is offset by the fact that the epigenome is normally subject to reprogramming during development and differentiation of the germ line (Allegrucci et al., 2005) (Fig. 2). Germline-specific epigenetic reprogramming has been most extensively characterized on the basis of changes in genome-wide DNA methylation patterns during development. A majority, though not all, of the methylation present in gametic (sperm or egg) genomes is removed in the early embryo shortly after fertilization (Kafri et al., 1992; Morgan et al., 2005). Allele-specific methylation associated with imprinted genes as well as methylation associated with certain repeat sequences and transposons does not undergo this early demethylation step in the preimplantation embryo. This is followed by a genome-wide de novo methylation event that occurs coincident with, or shortly after implantation and gastrulation in the mouse embryo. All cells of the early embryo proper, including those that will contribute to both the somatic and germ lineages, undergo this de novo methylation step. No further genome-wide changes in DNA methylation occur in cells contributing to somatic lineages, though there are many examples of locus-specific changes in DNA methylation patterns in these cells that contribute to activation or repression of specific genes in a tissue- or cell-type-specific manner. Additional genome-wide changes in DNA methylation patterns do occur in germline cells however. Thus, there is another global demethylation event that occurs as primordial germ cells (PGCs) migrate to and colonize the genital ridges. This demethylation event is more extensive than the demethylation event that occurs in the preimplantation embryo in that allele-specific methylation is erased from imprinted genes. In addition, at least some of the methylation associated with repeat sequences is also lost. Finally, there is another genome-wide de novo methylation step that occurs in germ cells during the late fetal (especially in male germ cells) and/or postnatal (especially in female germ cells) stages. Subsequent to this final global de novo methylation event, there are no further genome-wide changes in DNA methylation patterns in germline cells, though, as with somatic cells, there are numerous gene-specific changes in DNA methylation patterns associated with transcriptional regulation of specific genes that are either expressed or repressed during gametogenesis in each sex. In addition, genomic imprints are reset in differentiating germ cells such that paternally imprinted genes become methylated on both alleles during spermatogenesis and maternally imprinted genes become methylated on both alleles during oogenesis (Bartolomei and Tilghman, 1997; Reik and Walter, 2001).

Therefore it appears that while the epigenome is initially the more likely target of disruption by environmental effects, there is a unique opportunity to reverse or correct such defects during germline-specific epigenetic reprogramming that should normally preclude transgenerational transmission of primary epimutations.

9. Examples of epimutations that do not appear to be corrected by germline-specific epigenetic reprogramming

Surprisingly, there does appear to be transgenerational transmission of certain primary epimutations. As with genetic mutations, an epimutation that is inherited from a parent will normally be propagated to all cells in the body and is therefore termed a ‘‘constitutional’’ epimutation (Hitchins, 2010). However, as noted above, if an epimutation is unstable, it may be lost in certain cells leading to mosaicism. Epimutations associated with aberrant repression of certain tumor suppressor and/or DNA repair genes are known to increase cancer susceptibility and have been shown to be transmitted transgenerationally (Hitchins, 2010; Martin et al., 2005). Transgenerational epigenetic inheritance is not limited to mammals, but has also been observed in several other taxa and is believed to serve as one driving force for evolution (Jablonka and Raz, 2009).

Certain epimutations known to be caused by environmental effects such as exposure in utero to endocrine disruptors have also been shown to be transgenerational. Thus fetal exposure to agents such as the fungicide, vinclozolin, or others (Anway et al., 2005; Skinner et al., 2011) has been shown to lead to the occurrence of a variety of adult-onset diseases or phenotypes including fertility defects, as well as cancer of the prostate, liver, colon, breast, stomach and lung, among others in ensuing offspring (Skinner et al., 2010). While the occurrence of abnormalities in F1 offspring might be ascribed to a direct toxic effect of the fetal exposure to an environmental disruptor, that explanation cannot account for the striking observation that following a single exposure period during fetal development of the F1 generation, defects were then seen in subsequent generations extending to at least the F4 generation (Anway et al., 2005). In this case, the possibility that the phenotypic effects are due to induction of genetic mutations in the exposed fetuses could be ruled out by the high incidence of occurrence of defects in offspring (up to 90%) and by the fact that the occurrence of affected individuals in subsequent generations did not follow standard Mendelian inheritance patterns (Anway et al., 2005). Therefore this effect appears to represent the induction of primary epimutations in the exposed fetuses with at least some of those occurring in the germ line and being transmitted to subsequent generations. This observation is profound because it suggests that a single exposure in utero to an environmental disruptor can induce defects that will be transmitted to multiple subsequent generations. Interestingly, the timing of the effective exposure to the disruptor coincides with the germline-specific wave of genome-wide demethylation as PGCs enter the genital ridges and prior to the following stage of global de novo methylation that reestablishes DNA methylation in the germline genome (Fig. 2). This suggests that the transient period of hypomethylation in the germline genome represents a period of particular susceptibility to the induction of epimutations, some of which appear to escape subsequent germline-specific epigenetic reprogramming during gametogenesis thus facilitating ongoing transgenerational inheritance. Whether or not the other period of genome-wide hypomethylation that occurs during preimplantation embryonic development is also a window of susceptibility to environmentally induced epimutations is yet

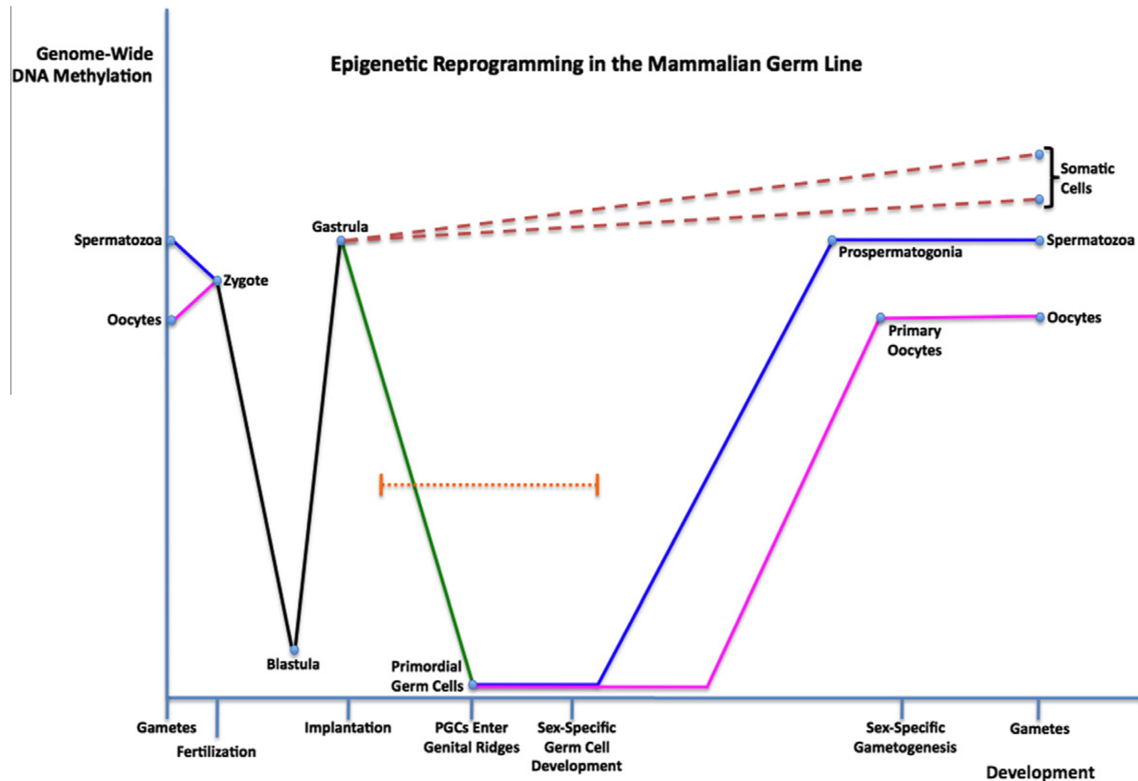

Fig. 2. Epigenetic reprogramming in the mammalian germ line based on studies from the mouse. Epigenetic reprogramming is depicted on the basis of changes in relative levels of genome-wide DNA methylation (specifically CpG methylation). Relative levels of genome-wide DNA methylation are represented on the *Y* axis and different key stages during fertilization, embryonic and fetal development and postnatal gametogenesis are represented in chronological order on the *X* axis. Methylation levels unique to the male germ line are shown in blue and those unique to the female germ line are shown in pink. Methylation at embryonic stages prior to specification of the germ line are shown in black and those following allocation of primordial germ cells (PGCs) but common to both sexes are shown in green. DNA methylation is present in both the male (spermatozoa) and female (oocytes) gametes, though there is slightly more in the male gamete. Following fertilization to form the zygote, there is a rapid loss of much, but not all inherited DNA methylation from both the paternal and maternal genomes. Notably, parent-of-origin, allele-specific methylation associated with imprinted genes is not lost at this stage, nor is methylation associated with various transposon and repeat sequences. Thus, at the blastula stage the genome is largely, but not completely demethylated. This is followed by a genome-wide de novo methylation event that replaces a significant level of methylation by the time development proceeds to the gastrula stage, and this methylation is present in the precursors of both the somatic and germ cell lineages. No subsequent genome-wide changes in DNA methylation occur in the somatic cell lineages and each somatic lineage adopts its own final characteristic of DNA methylation in the adult (dashed brown lines). However, shortly after allocation of PGCs in the epiblast, there is another major demethylation event as these cells migrate to the genital ridges. This demethylation includes erasure of methylation at imprinted genes and at many repeat sequences as well. Thus, early fetal germ cells reach a level of hypomethylation that is not found in any other cell type at any other stage during the life cycle of the organism. PGCs and then early prospermatogonia in the male, or primary oocytes in the female, retain this hypomethylated state for several days before undergoing another de novo methylation event which occurs earlier in the male germ line than in the female germ line. This ultimately gives rise to the methylation levels found in the mature gametes so that this cycle can be repeated during each generation. These changes in DNA methylation levels facilitate erasure of inherited epigenetic programming and resetting of new programming in the germ line during each generation. The unique window of susceptibility to disruption of epigenetic programming caused by certain environmental disruptors that leads to transgenerational inheritance of epimutations (Anway et al., 2005) coincides with the unique period of maximum hypomethylation in the germline genomes and is represented here by a dashed orange line.

to be investigated. Similarly, the mechanism(s) by which certain primary epimutations occurring in the germ line escape correction by germline-specific epigenetic reprogramming also remains to be determined.

10. Summary

Both the genome and the epigenome are susceptible to disruption by the environment. While studies of interactions between the environment and the genome have been ongoing for decades, the concept of the epigenome as a target for environmental disruption has only become a focus of research in recent years. This is ironic, as environmental disruption of the epigenome is likely to be a much more frequent event than environmental disruption of the genome. These disruptions lead to two types of effects – (1) disruptions of the genome which are manifest as genetic mutations, and (2) disruptions of the epigenome which are manifest as epimutations. Both types of disruptions lead to potentially heritable changes that can affect not only the original cells in which they initially occur, but subsequent cells resulting from clonal development of the originally affected cell or even transgenerational inheritance if the original defect occurs in a germline cell. Thus, future studies of the effects of environmental toxicants on the function of cells should take into account potential effects impacting either the genome or the epigenome, or both.

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