Chapter 16
How are epigenetic marks transmitted from one generation to the next?

Epigenetics, intergenerational inheritance, transgenerational inheritance, chromatin, DNA methylation, histone modifications, noncoding RNAs, germ cells, gametes, embryos

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Inheritance can occur in either of two forms – genetic or non-genetic. The latter is termed epigenetic inheritance. Genetic inheritance refers to semi-conservative replication of DNA which faithfully copies the template DNA sequence present in the double helix structure of the chromosomes of a parent cell (the genome) into two double helix copies to be transmitted to daughter cells during mitosis or meiosis I. Epigenetic inheritance refers to transmission from parent cells to daughter cells or generation to generation of reversible modifications to the DNA or associated proteins or of noncoding RNAs associated with the DNA (the epigenome), and is based on different mechanisms depending on which epigenetic modification is being copied or transmitted. All of these mechanisms function to transmit information from one generation to the next, and this information is critical for proper development and function of each subsequent generation, but is also subject to disruption by environmental or lifestyle effects.

Epigenetic marks

Epigenetic marks (also known as – epigenetic programming) are manifest in a variety of forms. In each case, the pattern of these marks is normally heritable through mitosis or meiosis and it contributes to regulation of gene expression by influencing chromatin structure. Methylation of the 5-carbon position of cytosines (Cs) present in 5'-3' CpG dinucleotiedes in mammalian DNA to form 5-methyl cytosine is perhaps the best studied epigenetic mark (Chapter 13). An absence or low level of DNA methylation (hypomethylation) is typically associated with a decondensed chromatin

state and active or primed gene transcription, while presence or a high level of DNA methylation (hypermethylation) is typically associated with a condensed chromatin state and repressed gene transcription. A well-understood mechanism termed "maintenance DNA methylation" normally faithfully propagates the presence of DNA methylation patterns during DNA replication (Fig. 1). A 5'-3' CpG dinucleotide in one DNA strand will normally be complemented by a 3'-5' CpG dinucleotide in the opposite strand of a double helix. A "fully methylated" site will have methylated Cs in the CpG dinucleotides in both strands. Upon semi-conservative DNA replication, an unmethylated C will be inserted in the new CpG dinucleotide complementary to the existing methylated or unmethylated CpG dinucleotide in the parent or template DNA strand. If the C in the template strand was methylated, this will create a transient "hemimethylated" state in which the C in the parent strand is methylated but that in the newly synthesized strand is unmethylated. However, the DNMT1 maintenance DNA methyltransferase which is normally associated with the DNA replication complex recognizes this hemimethylated state and inserts a methyl group onto the unmethylated C, restoring the site to fully methylated status. If the original site was unmethylated (no methylated C on either strand) then it will remain unmethylated following DNA replication since no transient hemimethylated state will be achieved at any point. In addition to maintenance DNA methylation, other DNA methyltransferases can catalyze "de novo" methylation which results in a previously unmethylated CpG site (no methylation on the C in the CpG dinucleotides on either strand) becoming fully methylated (= the Cs in the CpG dinucleotides on both strands becoming methylated). Finally, it is also possible for a fully methylated site (methylated Cs on both strands) to become "actively demethylated" such that neither C remains methylated. Active demethylation is normally catalyzed by the TET enzymes.

Other epigenetic marks include a variety of different post-translational modifications of histones in nucleosomes. These include both methylation or acetylation, as well as several other modifications including phosphorylation, ribosylation, sumolation, and ubiquitination, among others. Like DNA methylation, histone modifications are known to influence chromatin structure – either promoting chromatin condensation which represses transcriptional activity or chromatin decondensation which activates or primes transcriptional activity. They do this either directly by influencing

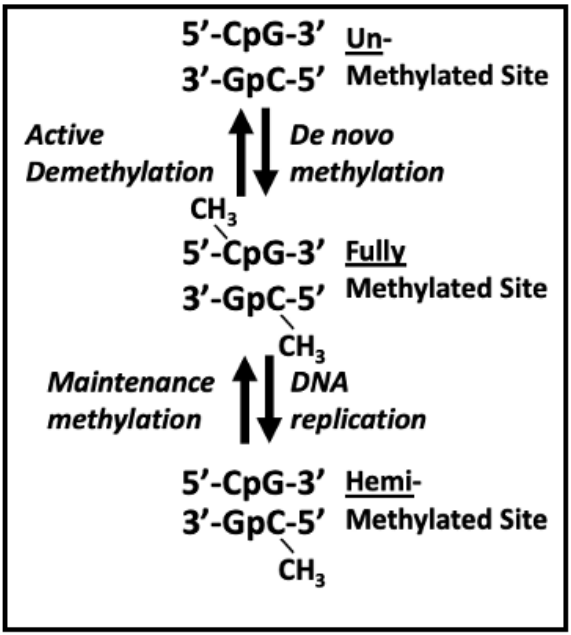

Figure 1. Epigenetic inheritance of DNA methylation states. In mammalian genomes, DNA methylation normally occurs at 5'-CpG-3' dinucleotides. A CpG dinucleotide in one strand of double-stranded DNA will be paired with a CpG dinucleotide in the opposite strand. If neither CpG is methylated the site is "unmethylated." If both CpGs are methylated, the site is "fully methylated." During semi-conservative DNA replication of a fully methylated site, the newly synthesized strand will initially carry an unmethylated CpG creating a transient "hemimethylated site." The hemimethylated site is recognized by the DNMT1 maintenance DNA methyltransferase which adds a methyl group to return the site to fully methylated status, thereby facilitating epigenetic inheritance of a fully methylated site. An unmethylated site can become directly methylated on both CpGs by a process called "de novo methylation." A fully methylated site can become directly unmethylated by a process called "active demethylation."

charge and, hence, affinity among neighboring nucleosomes, or indirectly by attracting chromatin remodeling complexes in a non-sequence-specific but a histone modification-specific manner (Chapter 14). Finally, non-coding RNAs, including both long and

small ncRNAs, also contribute to regulation of chromatin structure, although the mechanism(s) by which this is accomplished are less well understood. ncRNAs have the potential to serve as "sequence guides" when their sequence is complementary to that in the target DNA (Chapter 15). Empirical evidence indicates that patterns of histone modifications and/or ncRNAs are heritable. However, the molecular mechanisms responsible for faithful propagation of these patterns are not well understood. Importantly, the epigenetic programming that regulates cell-type specific gene expression patterns is manifest on the basis of the cumulative patterns of all of these epigenetic marks. Ultimately, cell-type specific gene expression patterns are regulated by transcription factor networks operating within 2-dimensional chromatin landscapes that function within a 3-dimensional interactome within the cell.

Epigenetic programming/reprogramming

Epigenetic marks are, by definition, reversible. Their patterns differ in each cell type, and further vary as a function of development, aging, influences from the environment, and/or disease states. Importantly, epigenetic programming undergoes major periods of erasure and resetting during each generation. This is termed "epigenetic reprogramming." Epigenetic programming contributes to regulation of cell-type specific gene expression which, in turn, directs cell-type specific differentiation. The epigenome serves as a liaison between the environment and the genome. This includes sensing position within the developing embryo and fetus to direct proper development of different cell types within the body, as well as mediating subsequent changes during development, aging, environmental or lifestyle effects, or disease states. Intergenerational transmission of epigenetic programming is mediated primarily by the germ line and early embryo. Germline development and gametogenesis are regulated by mechanisms similar to those that control tissue- or cell-type specific gene expression in any somatic cell type, resulting in production of spermatozoa in males and ova in females. However, unlike somatic cell differentiation, germline differentiation is not a terminal process given the function of the gametes which give rise to the next generation. Thus, germline cells must undergo extensive cell-type specific differentiation to form the gametes, but the resulting gametes must retain the potential to form, upon unification via fertilization, an entire new embryo and individual. This mandates the need for major phases of epigenetic

reprogramming in the early embryo and germ line (Fig. 2). Thus, in the early embryo, epigenetic programming that was associated with production of the gametes is largely, and rapidly, erased following fertilization. Subsequently, a new wave of epigenetic programming is established by the time of implantation which predisposes specification of the germ layers and the germ line. Certain inherited,

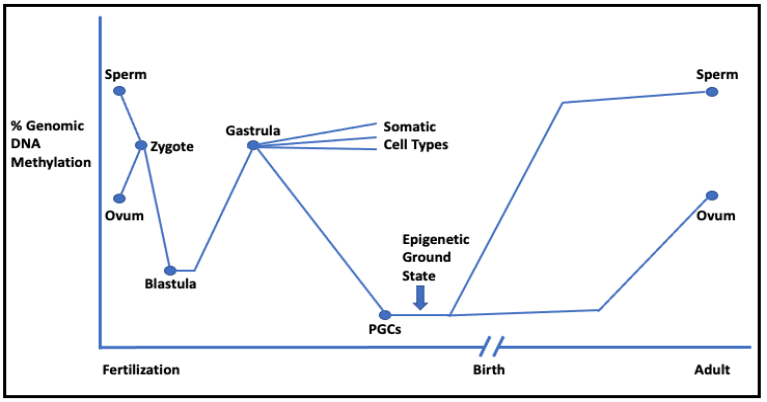

Figure 2. Dynamic, genome-wide changes in DNA methylation throughout the mammalian lifespan exemplified in the mouse. Genome-wide levels of DNA methylation undergo dynamic transitions throughout the lifespan of an individual. These include embryonic reprogramming in the preimplantation embryo in which DNA methylation inherited from the sperm and ovum is largely erased by the time the blastula is formed. This is followed by re-establishment of genome-wide DNA methylation at about the time of gastrulation in precursors of both the soma and germ line. Locus-specific epigenetic programming in precursors of specific somatic cell lineages then contributes to development of each somatic cell type, with no further genome-wide reprogramming of DNA methylation. A second major wave of genome-wide demethylation occurs during the unique process of germline reprogramming which is even more extensive than that which occurs during embryonic reprogramming, leading to the epigenetic ground state. This is followed by resetting of DNA methylation patterns in the developing germ cells to produce gametes carrying epigenetic programming needed for development of the subsequent generation. Figure modeled after Lee et al. (2014).

parent-of-origin, allele-specific differences in epigenetic programming (at imprinted loci) are retained throughout this embryonic reprogramming process. However, uniquely in the developing germ line, there is a second wave of even more extensive epigenetic reprogramming that includes erasure of unique programming inherited from each parent resulting in the "epigenetic ground state" . This is followed by yet another wave of de novo epigenetic programming that is similar on both copies of the genome in each developing germ cell such that each haploid gamete in any individual ultimately carries similar programming that will be transmitted to the next generation.

Transgenerational epigenetic inheritance of epimutations

Whereas the genome is a highly stable entity which is protected by DNA repair mechanisms that significantly mitigate the occurrence of genetic mutations, the epigenome is a highly labile entity that is, by definition, reversible and responsive to changes in the environment or the lifestyle or health of the individual and lacks the benefit of any sort of repair mechanism other than reprogramming. As a result, there is a significant potential for disruption of normal epigenetic programming due to aberrant environmental, lifestyle or health conditions, resulting in abnormalities known as "epimutations." Abundant evidence now exists in support of the potential for exposure to disruptive substances such as endocrine disrupting chemicals (EDCs), or aberrant lifestyle choices or imposed circumstances such as famine or dietary deficiencies to induce epimutations in both the soma and germ line. Because epigenetic progamming is heritable, epimutations induced in one generation can potentially be transmitted to subsequent generations. This has now been clearly demonstrated by multiple studies. Theoretically, exposure to a disruptive environmental influecnce could induce an epimutation via a direct toxic effect that might not be heritable. However, studies have now demonstrated that a single exposure of an F0 generation pregnant female and the F1 fetuses she is carrying can result in defective phenotypes in not only the directly exposed F1 offspring, but also in subsequent generations, even in the absence of any subsequent exposure to the disruptive influences. Actually, development of epimutant phenotypes in F2 generation offspring of an exposed pregnant female could also represent a direct, non-heritable, toxic disruption of the epigenome in the primordial germ

cells (PGCs) present in the F1 fetuses carried by the F0 pregnant female, since those PGCs will give rise to the F2 generation. However, studies have shown that a single exposure of an F0 pregnant female can induce and predispose multigenerational transmission of epimutations beyond the F2 generation – to the F3, F4 or even F5 generations – with no further exposure to the initial causative effect. Additional studies have shown that these exposures are not inducing genetic mutations. Thus, this represents initial disruption of normal epigenetic programming to generate epimutations that are subsequently transmitted to multiple descendant generations via epigenetic inheritance. This is termed transgenerational epigenetic inheritance of environmentally-induced epimutations.

Initial disruptions of the epigenome that are subsequently transmitted to daughter cells via mitosis or generations via meiosis via epigenetic inheritance are termed "primary epimutations." An alternative mechanism by which similar, heritable phenotypic effects can be induced involves an initial genetic mutation that disrupts a normal mechanism by which epigenetic programming is established, maintained and/or propagated. An example would be a defect in a DNA methyltransferase or a histone modifying enzyme which, when defective, fails to establish proper epigenetic programming. Epimutations of this sort are termed "secondary epimutations" and can be transmitted by either genetic or epigenetic inheritance, or both. A third type of epimutations are those that arise as a secondary effect following induction of primary epimutations that, in turn, induce defects in mechanisms that normally maintain genetic integrity (e.g. DNA repair and/or cell death mechanisms), and therefore predispose a subsequent abnormal increase in the accumulation of genetic mutations. These are termed "tertiary epimutations."

Summary

Epigenetic programming is critical to normal cell, tissue and organ function because it regulates unique gene expression patterns required for each differentiated cell type. Epigenetic programming is also heritable and so is normally transmitted during DNA replication, cell division and intergenerational or transgenerational reproduction. It is reversible, and, unlike the genome, the epigenome is different in every cell type and undergoes dynamic changes during development and aging. A unique feature of normal function of the

epigenome is its ability to undergo modifications in response to cues from the environment. This is critical to the normal function of epigenetic programming, but also renders the epigenome susceptible to abnormal disruptions which, given the heritability of epigenetic programming, can be transmitted from generation to generation. Interestingly, past commonly accepted attitudes toward proper parental health held that while a future mother should be cognizant of her lifestyle choices, even prior to conception of her offspring, a future father did not need to worry about any pre-conception exposures or lifestyle choices as long as they did not threaten the genetic integrity of his gametes. We now know that this perception was naive and that a father transmits both his genetic and epigenetic information to his offspring and must therefore be cognizant of his potential pre-conception exposures or lifestyle choices to the same extent as a future mother.

Suggested reading

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