

WHAT IS THE RELATIONSHIP BETWEEN DNA METHYLATION AND TRANSCRIPTION?

One of the main functions of DNA methylation in vertebrates is **NEGATIVELY** controlling gene expression. This is probably responsible for the essential role that DNA methylation plays in embryogenesis. In transgenic mice it was shown that knocking out most of the DNA methylation inhibits embryogenesis midway, therefore normal DNA methylation is essential for mammalian development. Regions that regulate the expression of genes (promoters and enhancers) are often CpG-rich and hypomethylated (low level of methylation or no methylation) in all tissues. CpG-rich regions of vertebrate DNA are called CpG islands. Some genes have their transcription regulatory sequences hypomethylated in a tissue-specific manner that corresponds to the tissue-specificity of their expression. Not only does DNA methylation help down-regulate expression of some normal vertebrate genes, but also, it is involved in negatively regulating the expression of foreign DNAs or “parasitic” DNA sequences that could disrupt normal cellular function. These sequences, which are generally highly methylated and concomitantly turned off, include the following:

foreign DNAs taken up by mammalian cells or transgenes introduced into zygotes (important complication in gene therapy, which can lead to methylation-induced gene silencing; sometimes this methylation occurs because the newly introduced gene is a repeat of an endogenous gene)

transposable elements (DNA sequences that replicate themselves independently of the rest of the genome and then reinsert into random positions of the genome giving multiple copies; a very large percentage of human DNA consists of such potentially harmful sequences)

some viral DNAs (e.g., in latent Epstein Barr viral genomes)

proviruses (DNA sequences from oncogenic RNA viruses which integrate into the host genome).

WHY IS DNA METHYLATION ESPECIALLY IMPORTANT TO FEMALE MAMMALS?

Besides DNA methylation being necessary for the completion of embryogenesis, it is also required for inactivation of a single X chromosome in female mammalian cells. Methylation of the XIST gene on the long arm of the X chromosome represses expression of this *cis*-acting gene so that its RNA product cannot coat this X chromosome thereby causing the chromosome's inactivation. This phenomenon illustrates that some exceptional genes code for RNA products that dynamically control cell function without encoding a protein and that some DNA sequences only exert their effects on the chromosome on which they reside or on DNA sequences near them (i.e., they act in *cis*, not in *trans*). XIST gene expression is necessary to inactivate the X chromosome expressing this gene and cannot inactivate the homologue in female mammalian cells that is not expressing this gene.

X_{active} has a methylated XIST gene, which is turned off & so cannot inactivate other X-linked genes in *cis*.

X_{inactive} has an unmethylated XIST gene, which is turned on & so can inactivate other X-linked genes in *cis*.

WHAT IS THE RELATIONSHIP BETWEEN DNA METHYLATION AND IMPRINTING DISEASES OR CERTAIN TRIPLET REPEAT DISEASES, ESPECIALLY THE FRAGILE X SYNDROME?

DNA methylation also plays a critical role in gene imprinting. This imprinting refers to the small percentage of mammalian genes that are expressed only if they are transmitted by the father (in the case of some imprinted genes) or by the mother (in the case of other imprinted genes). The initial marks for gene imprinting are introduced into the chromosomes during male gametogenesis and female gametogenesis. Much evidence suggests that differential DNA methylation in the male and female germline is responsible for imprinting. Some genes that are subject to imprinting play a role in a class of diseases called triplet repeat diseases. The most common of these is the fragile X syndrome. All triplet repeat diseases involve a dramatic increase in the number of copies of a trinucleotide sequence in the region of the affected gene. The fragile X syndrome, but not all triplet repeat diseases, involves abnormal DNA methylation. Increased methylation of a CpG island accompanies the increase in the number of copies of the triplet in the 5' untranslated region of the *FMRI* gene in affected patients. This increased methylation causes the inactivation of expression of the gene, which results in this common form of inherited mental retardation. This increased methylation causes the inactivation of expression of the gene, which results in this common form of inherited mental retardation. Another newly understood phenomenon in mammalian genetics is illustrated by recent studies on imprinting, which indicate that DNA methylation may control expression of a gene not only by directly regulating transcription initiation from the promoter or enhancer of the gene, but also, by affecting transcription of an oppositely oriented DNA sequence overlapping the imprinted gene (antisense regulation).

WHAT HAPPENS IF A PERSON IS MISSING A SMALL PERCENTAGE OF DNA METHYLATION?

The only genetic disease known thus far to involve Mendelian inheritance of abnormal DNA methylation is the ICF syndrome (immunodeficiency, centromeric region instability, facial anomalies). ICF is a very rare recessive disease with variable symptoms except for the invariant immunodeficiency of unknown origin and the diagnostic rearrangements (including whole-arm deletions and multibranching with up to 12 arms) in the vicinity of the centromere of chromosome 1 or 16 in mitogen-stimulated lymphocytes. ICF patients have abnormal hypomethylation in the juxtacentromeric heterochromatin of chromosomes 1 and 16. ICF is caused by mutations inactivating both alleles of one of the three known DNA methyltransferase genes. This indicates that the lack of only a small fraction methylation of cytosine residues in the genome (e.g., 7% of the methylation of brain DNA) results in human disease.

WHAT MAJOR SOURCE OF HUMAN DISEASE VERY OFTEN INVOLVES ABNORMAL DNA METHYLATION?

Although most DNA methylation changes occur in embryogenesis, postnatal alterations in DNA methylation patterns are also found. One important source of alterations in postnatal DNA methylation is decreases in methylation (**hypomethylation**) and increases in methylation (**hypermethylation**) of other DNA sequences during carcinogenesis. This abnormal DNA methylation is often observed in cancers. Cancers, as a group, have significantly lower levels of m⁵C in their DNA than do normal human tissues.

There is not only correlative evidence that DNA methylation plays a role in cancer formation but also experimental results supporting a cause-and-effect relationship between abnormal alterations in DNA methylation and carcinogenesis. In carcinogenesis, in addition to overall genomic **hypomethylation**, **hypermethylation** of many tumor suppressor genes, is common and is implicated in tumor formation.