PATTERNS OF HEREDITY AND GENETIC ALTERATION; EPIGENETICS OF MAMMALS

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Contents

1. Introduction
2. DNA Methyltransferase in Mammals
2.1. Dnmt1
2.2. Dnmt3a and Dnmt3b
3. DNA Methyltransferase Defective Mice
4. Genomic Imprinting and DNA Methylation
5. X-Chromosome Inactivation and DNA Methylation
6. Methyl-CpG-Binding Proteins
6.1. MeCP2
6.2. MBD1
6.3. MBD2
6.4. MBD3
6.5. MBD4
7. Chromatin Formation
8. Remodeling of Nucleosomes
9. Histone Acetylation and Nucleosome Remodeling
10. Histone Deacetylation
11. SIN3 Complex
12. Mi2-NuRD Complex
13. Transition Regulation Under the Control of Chromatin Remodeling Factors
Acknowledgments
Glossary
Bibliography
Biographical Sketch

UNESCO – EOLSS SAMPLE CHAPTERS

Summary

Gene regulation in higher animals, including humans (*Homo sapiens*) and mice (*Mus musculus*), is controlled through complex mechanisms that include epigenetics indicating “heritable changes in gene expression that occur without changes in DNA sequence.” Epigenetics dealt with in this overview describes how the molecular mechanism of gene regulation is controlled by selective activation or inactivation of genes, comprised of an upstream region, promotor region, protein-coding region, and downstream region. The CpG in DNA sequences is a substrate of DNA

methyltransferase, which forms methyl-CpG and signals the methyl-CpG to bind proteins. In the presence of stimulating transcription factors with DNA, the rate of gene transcription by RNA polymerase II is reduced by about 1/25 000 in the case of a gene heavily methylated and bound by methyl-CpG binding protein. Acetylation of histones stimulates the fluidity of chromatin, and after remodeling (reconstruction); the chromatin becomes ready for initiation of transcription. Deacetylation of histones suppresses the ability to be transcribed. The methylation of DNA in the sperm and egg is controlled differently, and the methylated DNA is demethylated during early embryogenesis. During development of the embryo, organized methylation of specific genes may proceed progressively, enabling the normal development of embryo to fetus to adult animals.

1. Introduction

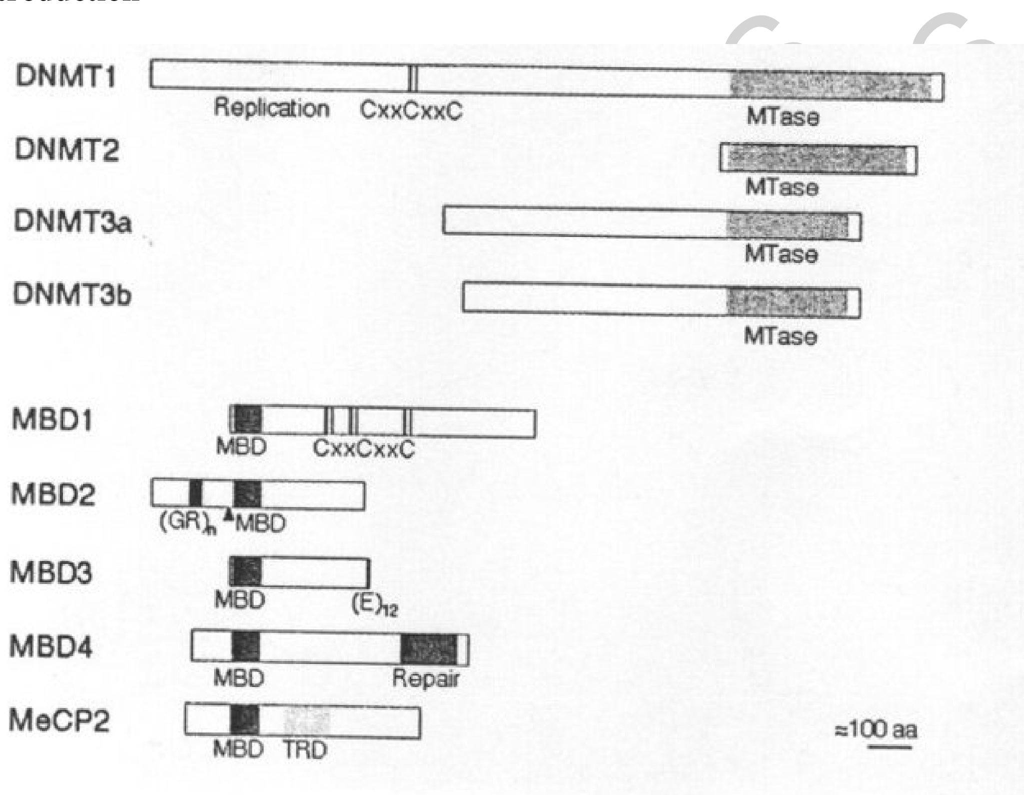

Figure 1. Proteins of the mammalian DNA methylation system (Adapted from Bird and Wolffe, 1999)
DNMT proteins each possess a region of strong similarity to cytosine DNA methyltransferases (MTase). MBD proteins share a well-conserved methyl-CpG-binding domain (MBD). The COOH-terminal "replication" box in DNMT1 is required for localization to replication complexes. CxxCxxC domains occur in both MBD1 and DNMT1. (GR)n and (E)12 refer to glycine-arginine and glutamic acid repeats, respectively. The "repair" domain of MBD4 is a T-G mismatch glycosylase. TRD refers to the transcriptional repression domain of MeCP2. The arrowhead on MBD2 marks an AUG at the NH2-terminus of a potential translation product called MBD2b, which is a candidate demethylase. Splice variant forms occur, but for simplicity are not shown.

In the process of gene regulation in mammals, numerous transcription factors, DNA methylation, histone acetylation and deacetylation, the formation and remodeling of chromatin all occur co-operatively to support normal development of the embryo from the fetal stage to adulthood. In relation to inactive heterochromatin, the methylation of cytosine in CpG suppresses the expression of genes. The methylation of CpG, the methyl-CpG (mCpG) in the promoter region and also around the initiation point for transcription of a gene, effectively inhibits gene expression.
The methyl-CpG constitutes 60–90% of CpG in the genome, and non-methylated CpG is found as a cluster, frequently observed in the promoter region of a gene. Such non-methylated regions of about 1 Kbp constitute 15% of the human genome, and are called "CpG islands." From the pattern of bulk DNA methylation, heterochromatin and retrotransposon DNA repeats are heavily methylated.

Complex proteins designated methyl-CpG binding protein are known, which control the activity of histone deacetylase and chromatin remodeling in response to methyl-CpG. Five groups of methyl-CpG binding proteins, MBD1, MBD2, MBD3, MBD4, and MeCP2 have been identified in humans.

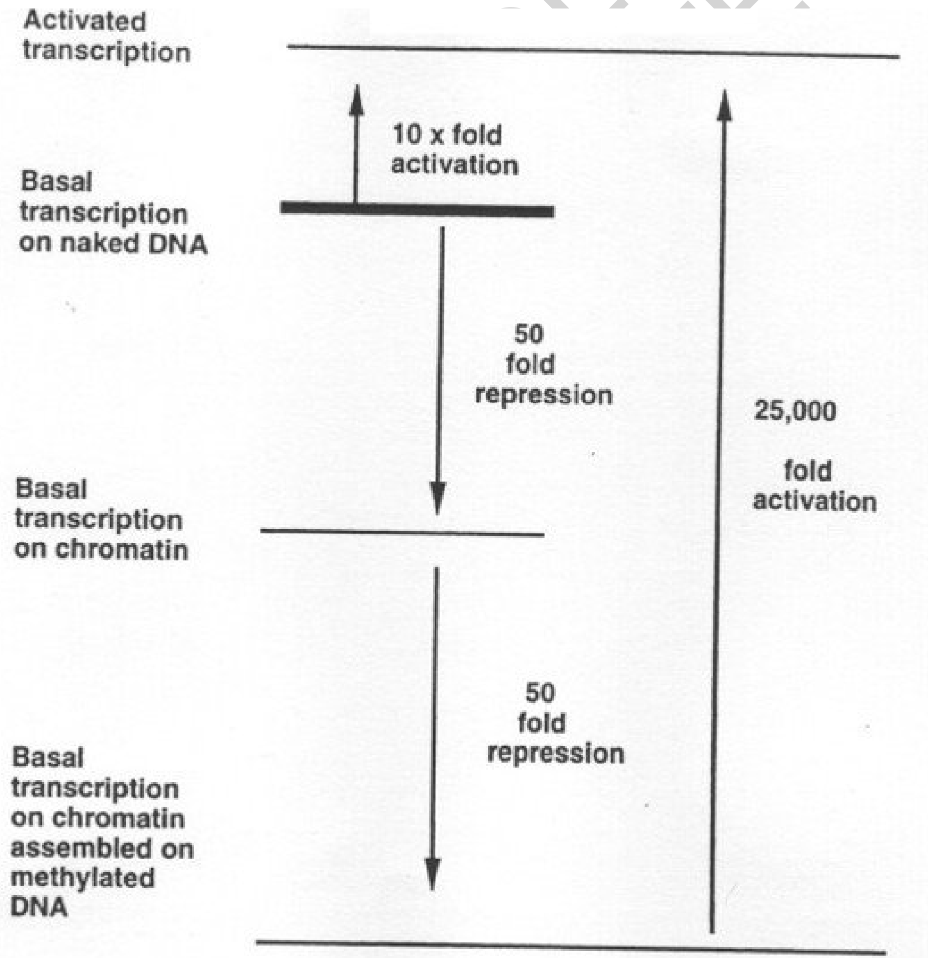

Figure 2. DNA methylation and the dynamic range of transcription rate (Adapted from Bird and Wolffe, 1999)
DNA methylation may expand the range of transcriptional regulation beyond that

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achievable by chromatin alone. Transcriptional activators can raise transcriptional activity above basal levels. It is proposed that these activators can achieve the same efficiency of transcription from chromatin, and chromatin templates assembled on methylated DNA; however, the range of transcriptional regulation is increased greatly under these latter conditions through the contribution of chromatin and DNA methylation to the reduction of basal transcriptional efficiency.

DNA methyltransferase recognizes CpG sequences and catalyses 5-methylcytosine formation. The methyl residue is donated by S-adenosyl methionine. The DNA methyltransferases consist of two groups: Maintenance methylase and *de novo* methylase. Four species of DNA methyltransferases—*Dnmt1, Dnmt2, Dnmt3a and Dnmt3b*—are known in mammals. Dnmt1 is essential for the maintenance of mCpG. Dnmt3a and Dnmt3b are known to function as *de novo* DNA methyltransferases. These are summarized in Figure 1.

On the basis of the transcriptional activity of naked DNA, the protein complex of transcription factors (transcriptosome) stimulates the rate of gene transcription tenfold. The formation of chromatin with DNA incorporating histone octamers reduces the rate of transcription to one-fiftieth. Further methylation of DNA forming complexes with methyl-CpG binding proteins reduces the rate of transcription to one-fiftieth. Thus, methylated DNAs in chromatin forming complexes with methyl-CpG binding proteins have the transcriptional activity of approximately 1/2500 compared with naked DNA. Therefore, the rate of gene transcription with transcription factors is about 25 000-fold higher than that of a gene with methylated chromatin and methyl-CpG binding proteins, as summarized in Figure 2.

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2. DNA Methyltransferase in Mammals

2.1. Dnmt1

The enzyme is highly specific to hemimethyl-CpG, and functions as a maintenance DNA methyltransferase. The cDNA was first cloned in 1998 and designated as mammalian DNA methyltransferase, Dnmt. However, in 1998 the cDNAs for several other DNA methyltransferases were also cloned, and Dnmt was redesignated as Dnmt1. Dnmt1 knockout mice die during development of the early embryo, and the level of methylation of DNA is reduced significantly. Dnmt1 forms a complex with DNA replication coupling factor, and localizes in the nucleus during the DNA synthesis (S) phase of the cell cycle. Dnmt1 is expressed in all cells, and the level of expression is very high in rapidly growing cells. During cell division to eight cells after parthenogenesis, Dmnt1 stays in the cytosol, and then Dnmt1 localizes to the nucleus. In the NH2-terminal region, there is a signal domain supporting localization in the cytosol. Dnmt1 forms complexes with histone deacetylases, HDAC1 (Histone deacetylase 1) and HDAC2, and the tumor suppressor, Rb.

2.2. Dnmt3a and Dnmt3b

Using mouse embryonic stem (ES) cells, *Dnmt1* mutants were obtained by consecutive gene disruption of both wild type alleles. The homozygous mutant cells were viable

with no obvious abnormalities, and had trace levels of DNA methyltransferase. Based on this work, new DNA methyltransferases, Dnmt3a and Dnmt3b were detected, that had the ability to catalyze *de novo* methylation of CpG in DNA. These two enzymes showed substrate specificity in transferring a methyl group to CpG in DNA, and showed no preference for hemi-methyl CpG in DNA. ES cells with no Dnmt3a and Dnmt3b activities by double knockout were established, and the cells were infected with retrovirus. The *de novo* methylation of integrated retroviral DNA was completely lacking in the double knockout cells. Dnmt1 could not complement the deficiency. Dnmt3b is specifically required for the methylation of centromeric minor satellite repeats. Human mutations of DNMT3B are detected in ICF syndrome, which causes a developmental defect with hypomethylation of pericentromeric repeats.

Dnmt2 is known to be very similar to the COOH-terminus region of Dnmt1, but its enzymatic activity is not yet known.

The pattern of DNA methylation by DNA methyltransferase suggests that the process contains a method of selecting the CpG and hemimethyl-CpG in specific regions of chromatin. Such a selection mechanism for DNA methylation may lead to selection of cell lineages during development of the embryo.

3. DNA Methyltransferase Defective Mice

DNA methyltransferase 1 knockout mice (*Dnmt1*) cease development during the middle stage of embryogenesis, corresponding to eight days in the wild type, and the mice die at nine to ten days. ES cells of *Dnmt1* show very low levels of DNA methylation. ES cells divide normally, as in wild type ES cells. However, when they were treated to induce embryonic development, the cells died. The genes for *de novo* DNA methyltransferase, *Dnmt3a* and *Dnmt3b* were knocked out, and the *Dnmt3a*$^{-/-}$ amutant mice were almost normal at birth. However, they died at three to four weeks of age. Embryos with *Dnmt3b*$^{-/-}$ mutant genes die at various developmental stages. The double mutants *Dnmt3a*$^{-/-}$, *Dnmt3b*$^{-/-}$ showed more severe defects, and the embryos died at earlier stages than *Dnmt1*$^{-/-}$ mutant mice.

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Biographical Sketch

Kohji Hasunuma graduated from Tokyo University, Faculty of Science, Department of Biology (Plant Science) in 1966, and from the Graduate School of Biology (Plant Science) in 1971. He served as Research Associate at Tokyo University, Faculty of Arts and Culture 1971~1979 and was Associate Professor at the National Institute for Basic Biology, 1979–1990, and visiting researcher at Carnegie Institution of Washington at Stanford at 1990. Since 1990, he has been professor at Yokohama City University, Kihara Institute for Biological Research. The Hirase Prize was awarded to Professor Hasunuma by the Japanese Society of Plant Morphology in 2000 for his identification of the molecular mechanism of light signal transduction in *Neurospora crassa* and *Pisum sativum*.

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SAMPLE CHAPTERS