DNA Methylation in Epigenetic Control of Gene Expression

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Abstract Over three decades ago DNA methylation had been suggested to play a role in the regulation of gene expression. This chapter reviews the development of this field of research over the last three decades, from the time when this idea was proposed up until now when the molecular mechanisms involved in the effect of DNA methylation on gene expression are becoming common knowledge. The dynamic changes that the DNA methylation pattern undergoes during gametogenesis and embryo development have now been revealed. The three-way connection between DNA methylation, chromatin structure and gene expression has been recently clarified and the interrelationships between DNA methylation and histone modification are currently under investigation. DNA methylation is implicated in developmental processes such as X-chromosome inactivation, genomic imprinting and disease, including tumor development. This chapter discusses all these issues in depth.

1 Introduction

The involvement of DNA methylation in cell differentiation and gene function was suggested more than three decades ago by Scarano (1971) and later by Holliday and Pugh (1975) and Riggs (1975). This suggestion was substantiated by some early data and formulated in a working hypothesis by Razin and Riggs in 1980. A large body of experimental data has been accumulated over the past two decades clearly indicating that epigenetic control of gene expression in mammals involves DNA methylation and that this control of expression is associated with gene-specific methylation patterns (Yeivin and Razin 1993). DNA methylation happens to be a perfect clonally inherited epigenetic feature of the genome. De novo methylation by the de novo methyltrans-

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Progress in Molecular and Subcellular Biology
P. Jeanteur (Ed.)
Epigenetics and Chromatin

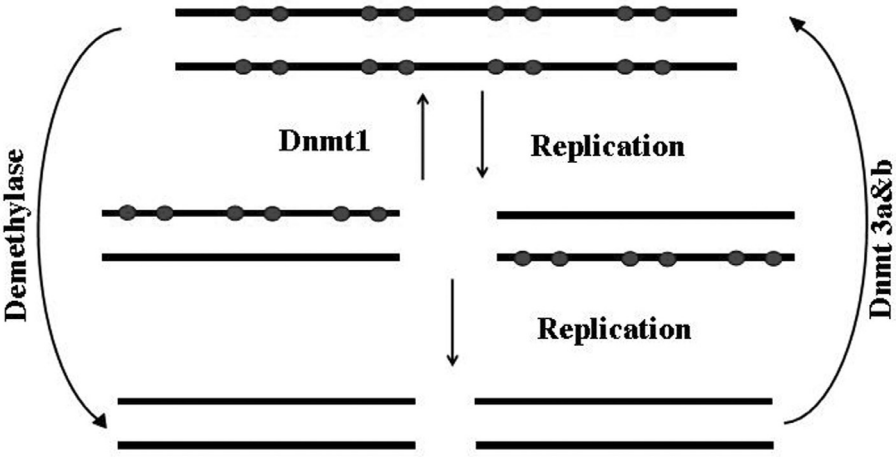

Fig. 1. Metabolism of DNA methylation. *Dnmt1* The DNA methyltransferase-maintenance enzyme; *Dnmt3a&b* de novo methyltransferases; *filled circles* methylated CpG sites

ferases Dnmt3a and Dnmt3b and demethylation by a yet uncharacterized demethylase establish the methylation patterns which are then maintained by a maintenance methyltransferase (Dnmt1) (Fig. 1; Razin and Kafri 1994).

Methylated cytosine residues in mammalian DNA (5 metCyt) are almost always found in the small palindrome CpG thus symmetrically positioned on the two DNA strands (Sinsheimer 1955). Methyltransferase activity, which is present in all cells, acts at the replication fork to restore the fully methylated state of the DNA (Gruenbaum et al. 1982, 1983). In the absence of this maintenance methylation, the DNA may lose its methylation by a so-called passive demethylation mechanism. After two rounds of replication without methylation, 50 % of the DNA molecules will be unmethylated on both strands and the other 50 % will be hemimethylated. However, in most systems studied so far, the demethylation process in the cell involves an active mechanism in which specific methylated sites undergo active demethylation, not necessarily associated with replication (Razin and Kafri 1994). On the other hand, fully unmethylated DNA can undergo de novo methylation by one of several de novo methyltransferases which are present primarily in embryonic cells (Okano et al. 1999; Fig. 1).

The mammalian genome is characterized by a bimodal pattern of DNA methylation. Seventy to 80 % of all CpG dinucleotides in the genome are methylated (Ehrlich et al. 1982). The remaining CpGs that are constantly unmethylated are clustered in CpG-rich islands. These unmethylated CpG islands are usually found in promoter regions extending to the first exon of housekeeping genes (Bird 1986). Unmethylated CpG islands are found in somatic cells in the gametes and in embryonic cells throughout embryogenesis and gametogenesis (Kafri et al. 1992). The unmethylated state of CpG

islands is rigorously maintained by a mechanism that involves efficient island demethylation activity (Frank et al. 1991; Brandeis et al. 1994). In contrast to housekeeping genes, tissue-specific genes lack CpG islands and are generally methylated in nonexpressing cells, but are unmethylated in their cell type of expression (Yeivin and Razin 1993). The bimodal methylation pattern in the mammalian genome is faithfully maintained by the DNA methyltransferase 1 (Dnmt1) (Bestor and Ingram 1983). This maintenance methyltransferase methylates hemimethylated DNA during replication (Gruenbaum et al. 1983), and therefore propagates the methylation pattern for many generations of actively dividing cells (Razin and Riggs 1980). Gene-specific methylation patterns which are observed in adult tissues reflect the result of dynamic changes in methylation that are known to take place during embryogenesis (Razin and Kafri 1994).

2 Changes in Gene-Specific Methylation Patterns During Early Embryo Development

The gene-specific methylation patterns in oocyte and sperm, which are acquired during differentiation of the germ cells (Kafri et al. 1992), contribute to a combined methylation pattern in the zygote which is erased by an active demethylation mechanism during the first two or three cleavages (Kafri et al. 1993). This undermethylated state of the genome that results from this demethylation process persists through the blastula stage. This had been shown for both embryo genomic DNA (Monk et al. 1987) and specific gene sequences (Kafri et al. 1992). Global de novo methylation takes place post implantation, leaving CpG islands unmethylated. Primordial germ cells (PGCs) emerge from the unmethylated epiblast escaping the global de novo methylation that takes place following implantation. Germ cells that are first seen at day 7.5 post coitum (p.c.) in the base of the allantois (McCarrey 1993) are unmethylated (Monk et al. 1987). The germ cells remain undermethylated until after cells populate the gonads at 11.5–12.5 days p.c. De novo methylation and gene-specific demethylation take place during gametogenesis after differentiation to male and female gonads (Kafri et al. 1992; Fig. 2). This de novo methylation that is followed by gene-specific demethylations is much like what happens in the embryo proper during gastrulation and further development (Kafri et al. 1992).

In the gastrula, a process of gene-specific demethylation starts, concomitant with cell differentiation (Benvenisty et al. 1985; Shemer et al. 1991). This process continues well into adult life and ends in the fully differentiated cell with the final gene-specific methylation patterns that are observed in adult tissue (Yeivin and Razin 1993).

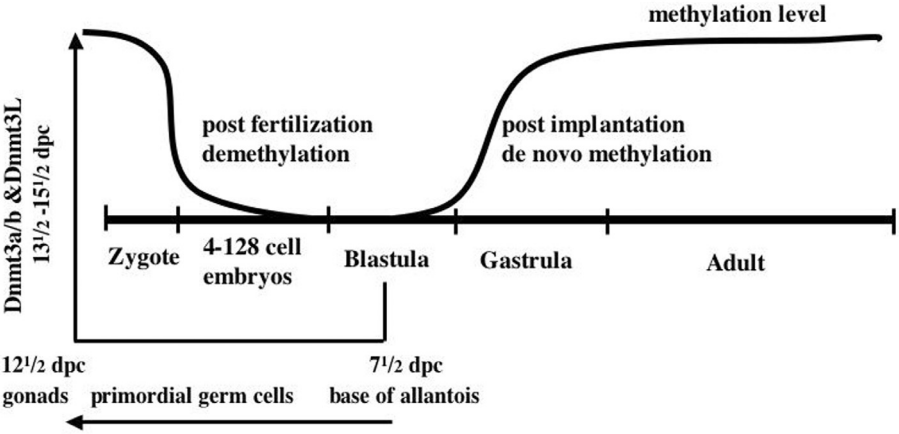

Fig. 2. DNA methylation changes during gametogenesis and early embryogenesis

3
Effect of Methylation on Gene Expression

DNA methylation has been implicated as playing a role in the multi-level hierarchy of control mechanisms that govern gene expression in mammals (Razin and Cedar 1984). Three major lines of evidence led to this conclusion: (1) genes tend to be undermethylated in the tissue of expression and stably methylated in all other tissues (Yeivin and Razin 1993); (2) genes that are inactive in vivo can be activated by treatment with the potent demethylating agent, 5-azacytidine (Jones 1984); and (3) in vitro methylated genes introduced into fibroblasts in culture remain methylated and suppressed (Stein et al. 1982; Yisraeli et al. 1988). The inverse correlation between DNA methylation and gene activity that was demonstrated for a large number of genes suggested that DNA methylation acts to repress gene activity. The transcription suppression by CpG methylation can be accomplished by two basic mechanisms: (1) *direct* interference with the binding of transcription factors and (2) *binding of multiprotein repressory complexes* resulting in the formation of an inactive chromatin structure.

3.1
Direct Transcription Inhibition

Early experiments established that local cytosine methylation of particular sequences could directly interfere with binding of a transcription factor (Tate and Bird 1993). In an in vivo footprinting experiment, methyl groups interfered with the binding of liver-specific factors to the Tat gene (Becker et al.

1987). Methylation at the downstream region of the late E2A promoter of adenovirus type 2 prevents protein binding (Hermann et al. 1989), and methylation of a site in the promoter region of the human proenkephalin gene inhibits expression and binding of the transcription factor AP2 (Comb and Goodman 1990). Cytosine methylation also prevents binding of a HeLa cell transcription factor required for optimal expression of the adenovirus major late promoter (MLP) (Watt and Molloy 1988). Maybe the most prominent example is that of a chromatin boundary element binding protein (CTCF) that binds only to its unmethylated site and can block the interaction between the enhancer and its promoter when placed between the two elements (Bell et al. 1999). The effects demonstrated with the examples described above suggest that methylation at the 5′ end of the gene is frequently sufficient to suppress gene activity (Keshet et al. 1985). However, it should be noted that a direct effect of DNA methylation on binding of specific factors has not always been observed. For example, the transcription factor, Sp1, binds and facilitates transcription even when Sp1 sites are fully methylated (Holler et al. 1988). Another example of a transcription factor that binds efficiently to its recognition site, even in its methylated state, is the chloramphenicol acetyltransferase transcription factor (CTF) (Ben-Hattar et al. 1989). In spite of the lack of effect of methylation on the binding of CTF in vitro, methylation of the site reduced

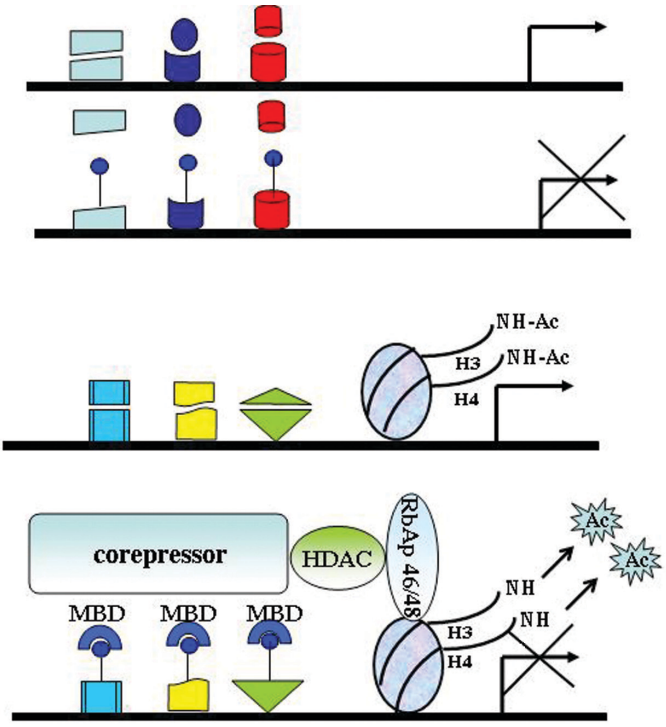

Fig. 3. a,b (*Legend see page 156*)

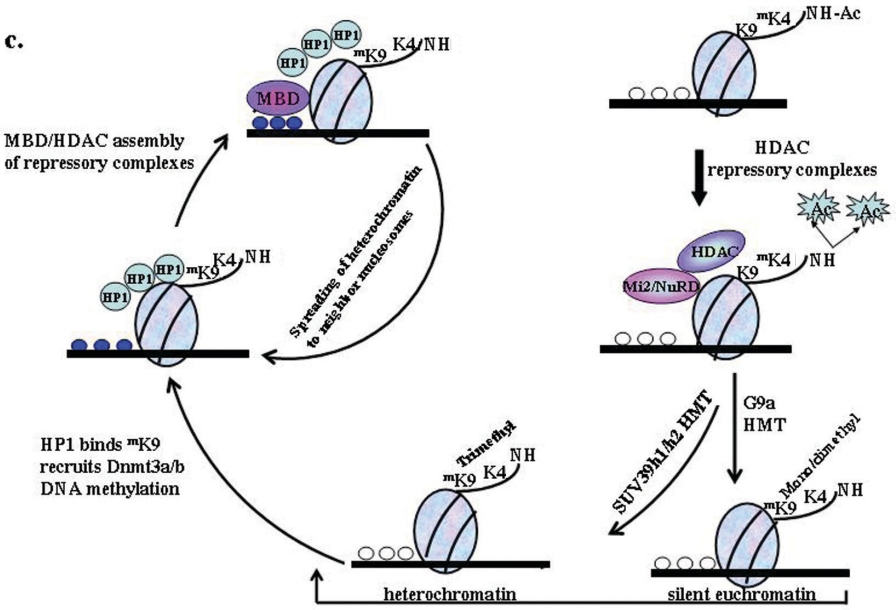

Fig. 3. Effect of methylation on gene expression. **a** Direct gene repression. Methylated transcription factor binding sites (*blue lollipops*) prevent binding of transcription factors to the corresponding sites, thus inhibiting transcription initiation (*horizontal arrow*). **b** Methyl binding domain proteins (*MBD*s) bind to methylated CpG sites (*blue lollipops*), recruit corepressory proteins that bind to histone deacetylases (*HDAC*s), which in turn are bridged to nucleosomal histone (*NH*) H3 and H4 by RbAp46/48, resulting in deacetylation of acetylated lysine residues, causing repression of transcription initiation (*horizontal arrow*). **c** Epigenetic modifications of nucleosomal histones and DNA form silent euchromatin or heterochromatin depending on the histone methyltransferase (*HMT*) that methylated lysine K9. *mK4* Methylated lysine K4; *Ac* acetyl; *NH* amino tail of histone H3; *K9* lysine K9; *open circles* unmethylated CpG sites; *closed blue circles* methylated CpG sites; *HDAC* histone deacetylase; *MBD* methyl binding domain protein

the activity of the gene in vivo. The discrepancy between the in vitro and in vivo results suggests that although methylation of *cis* elements may affect promoter activity, other mechanisms may be involved in promoter repression as well (Fig. 3a).

3.2 Indirect Transcription Inhibition

For a long time studies on DNA methylation and gene expression did not address the question of how chromatin structure might affect gene function. This is in spite of the fact that nucleosomal DNA had already been known to be richer in 5-methylcytosine than internucleosomal DNA (Razin and Cedar

1977; Ball et al. 1983) and that DNaseI sensitivity is a property of transcriptionally active regions of chromatin and correlated with undermethylation of transcribed genes (Sweet et al. 1982). Almost a decade later microinjection and transfection experiments using in vitro methylated gene sequences revealed that DNA methylation results in the formation of inactive chromatin (Keshet et al. 1986), and that the silencing effect exerted by CpG methylation is observed only after the methylated DNA acquired its appropriate chromatin structure (Buschhausen et al. 1987). Moreover, whereas the repressed state of the gene, which is exerted by DNA methylation alone, can be alleviated by a strong activator such as GAL4-VP16, the activator cannot overcome repression once chromatin is assembled on the methylated template (Kass et al. 1997).

These observations clearly indicate that silencing of a gene by methylation frequently involves the generation of a condensed chromatin structure that may, among other things, limit promoter accessibility to the transcription machinery. Although it has become increasingly clear that DNA methylation and chromatin structure correlate, these two epigenetic marks have only recently been connected mechanistically (Razin 1998). The discovery of the two methyl binding proteins, MeCP1 (Meehan et al. 1989) and MeCP2 (Lewis et al. 1992), helped us to realize that DNA methylation is connected with chromatin structure and gene expression. MeCP1 was later shown to be a multiprotein repressory complex (Feng and Zhang 2001). However, MeCP2, being a purifiable protein, had been cloned and characterized (Meehan et al. 1992; Nan et al. 1993). MeCP2 that is known to bind to methylated CpG-rich heterochromatin (Nan et al. 1996) contains a methyl binding domain (MBD) and a transcriptional repressory domain (TRD) (Nan et al. 1997). MeCP2 binds to the methylated DNA by its MBD and recruits the corepressor Sin3A through its TRD (Jones et al. 1998; Nan et al. 1998). MeCP2 anchors to the DNA a multiprotein repressory complex that causes histone deacetylation and chromatin remodeling by two histone deacetylase activities, HDAC1 and HDAC2. Transcriptional inactivation caused by this deacetylation could be alleviated by the HDAC inhibitor trichostatin A (TSA) (Eden et al. 1998; Jones et al. 1998; Nan et al. 1998). These observations finally solved the long suspected three-way connection between DNA methylation, condensed chromatin structure and gene silencing (Razin 1998). A search of EST databases with the methyl-binding domain (MBD) sequence of MeCP2 revealed four additional MBD proteins, MBD1–MBD4 (Hendrich and Bird 1998).

Recent studies in mammalian systems, where DNA methylation clearly plays a role in gene silencing, have shown that methyl binding proteins when bound to methylated CpG residues recruit corepressory proteins that in turn interact with two histone deacetylases HDAC1 and HDAC2. These histone deacetylases act on specific acetylated lysine residues within histones H3 and H4. This process is mediated by the RbAp46/48 proteins that serve as a bridge between the repressory complexes and the nucleosomal histones (Razin 1998; Kantor and Razin 2001). Histone deacetylation appears to be required for stable gene

silencing (Eden et al. 1998; Jones et al. 1998; Nan et al. 1998) perhaps by shaping an inheritable 'closed' chromatin structure (Kantor et al. 2003; Fig. 3b).

It is now clear that MeCP2 is not the sole multiprotein repressory complex involved in transcriptional repression. MeCP1, which was discovered over a decade ago (Meehan et al. 1989), turns out to be a histone deacetylase multiprotein complex composed of ten components that include two MBD proteins, MBD2 and MBD3 (Ng et al. 1999; Feng and Zhang 2001). MeCP1 was found to share most of its components with a third multiprotein repressory complex Mi2/NuRD. This histone deacetylase-chromatin remodeling complex contains MBD3 (Zhang et al. 1999) which had been shown to play a distinctive role in mouse development (Hendrich et al. 2001).

The most studied multiprotein histone deacetylase repressory complexes to date are Mi2/NuRD, MeCP1 and MeCP2/Sin3A. The MBD proteins associated with these complexes are MBD3, MBD2 and MeCP2, respectively (Kantor and Razin 2001). Yet, many other histone deacetylase repressory complexes may exist.

Interestingly, DNA methyltransferases were also found to be components of histone deacetylase repressory complexes. The de novo DNA methyltransferase Dnmt3a binds deacetylases and is recruited by the sequence-specific repressory DNA binding protein RP58 to silence transcription (Fuks et al. 2001). The repressory activity of this complex is independent of Dnmt3a methyltransferase activity. The human maintenance DNA methyltransferase (DNMT1) forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters (Robertson et al. 2000). In parallel, DNMT1 binds HDAC2 and the corepressory DMAP1 to form a complex at replication foci (Rountree et al. 2000). The interaction between DNMT1 and histone deacetylase suggests that histone deacetylases or histone acetylation patterns can play a role in targeting DNA methylation, as well. Alternatively, DNMT1 may directly target deacetylation to regions that should become methylated. In any event, apparently methylation and deacetylation could act in concert to potentiate the repressed state. Furthermore, methyltransferases possess two activities, DNA methylation and transcriptional repression.

Although MBD1 has been shown to cause methylation-mediated transcription silencing in euchromatin (Fujita et al. 1999; Ng et al. 2000), it is not known to participate in any of the known histone deacetylase multiprotein repressory complexes. Nevertheless, its repressory effect can be alleviated by TSA, suggesting that MBD1 is part of a yet unknown histone deacetylase repressory complex. For a comprehensive discussion of all methylation-associated repressory complexes, see the review by Kantor and Razin (2001).

It has recently become clear that the flow of epigenetic information may be bidirectional. DNA methylation affects histone modification which in turn can affect DNA methylation. The interaction between these covalent modifications of chromatin may shed light on the yet unsolved mechanisms concerning the establishment of heterochromatin, its spreading along large domains of the genome and its stable inheritance. The DNA methylation–het-

erochromatin interrelationship including the three epigenetic marks, DNA methylation, histone methylation and acetylation, is described in Fig. 3 c.

The DNA in an active chromatin domain is unmethylated and histone H3 is acetylated, methylated on lysine at position K4 but unmethylated at lysine k9. Binding of a histone deacetylase repressory complex to the promoter region results in histone deacetylation and K4 demethylation. Two different histone methyl transferases methylate K9 depending on the position of the gene. The G9a HMT methylates K9 to the monomethyl and dimethyl level if the gene is in euchromatin, and the other methylase, Suv39h1 and h2, methylates K9 to the trimethyl level if positioned in heterochromatin. The heterochromatic protein HP1 binds to the dimethyl and trimethyl K9 and recruits the de novo methyltransferases Dnmt3a and b. This methylated structure recruits more HP1 to neighboring nucleosomes, thereby leading to a continuous process of spreading of the heterochromatic structure.

4
DNA Methylation and Genomic Imprinting

DNA methylation was implicated in several developmental processes such as X-chromosome inactivation and genomic imprinting. DNA methylation in X-inactivation is discussed in Chapter 4 (this Vol.). Genomic imprinting is perhaps the best studied developmental process in which DNA methylation plays a pivotal role. The fact that the parental genomes contribute unequally to the development of a mammalian fetus was first demonstrated by nuclear transfer studies (McGrath and Solter 1984; Surani et al. 1984). While androgenetic embryos that contain two paternal copies of the genome show very poor embryonic development with normally developed extraembryonic tissues, parthenogenetic embryos, which contain two maternal genomes, show normal embryo development and underdeveloped extraembryonic tissues. These observations suggested that both sets of parental genomes are required for proper development. This conclusion gained further support by genetic experiments in which mice containing uniparental duplications of subchromosomal regions showed phenotypes that were either embryonic lethal or developmentally retarded (Cattanach and Kirk 1985).

This phenomenon suggested that expression of a subset of genes depends on their parental origin and that such differential expression of genes must be controlled by epigenetic modifications that take place during gametogenesis when the parental alleles are in separate compartments. The epigenetic mark could then serve as a signal to discriminate between the two parental alleles post fertilization and help to maintain this discrimination during embryonic development and adult life. The mark should then be erased and created anew during gametogenesis according to the gender of the offspring.

What epigenetic modification could best fulfill these requirements? DNA methylation is a good candidate since it can be erased by demethylation,

established through de novo methylation and propagated by the maintenance methyltransferase (see Fig. 1). In fact, differentially methylated regions (DMRs) were found in all imprinted genes (Razin and Cedar 1994). Interestingly, DMRs are usually located in CpG islands. This is in contrast to the general rule that characterizes CpG islands being invariably unmethylated. The monoallelic methylation of DMRs is established in the gametes by Dnmt3a, Dnmt3b and Dnmt3L. Dnmt3L which in itself lacks methyltransferase activity is believed to recruit Dnmt3a and Dnmt3b to methylate the maternal allele in imprinted genes (Bourc'his et al. 2001; Hata et al. 2002). The differential methylation that is established in the gametes or early in embryo development is maintained throughout development (Fig. 4). Therefore methylation of the DMRs must escape the genome-wide demethylation that takes place in the early embryo and also the global de novo methylation that takes place post implantation (Kafri et al. 1992; see Fig. 2). However, the imprint must be erased by an as yet unknown demethylase that should work at the time period when the primordial germ cells migrate to the gonads (Fig. 4).

In fact, several imprinted genes (Igf2r, H19, Snrpn and Xist) among others have been shown to obey these rules (Brandeis et al. 1993; Stoger et al. 1993; Ariel et al. 1995; Tremblay et al. 1995; Shemer et al. 1997). DNA methylation may therefore play a dual role in the imprinting process. The differential methylation may mark the parental alleles, allowing the transcriptional machinery of the cells to distinguish between the parental alleles. In addition, DNA methylation can directly affect promoter activity of the imprinted genes.

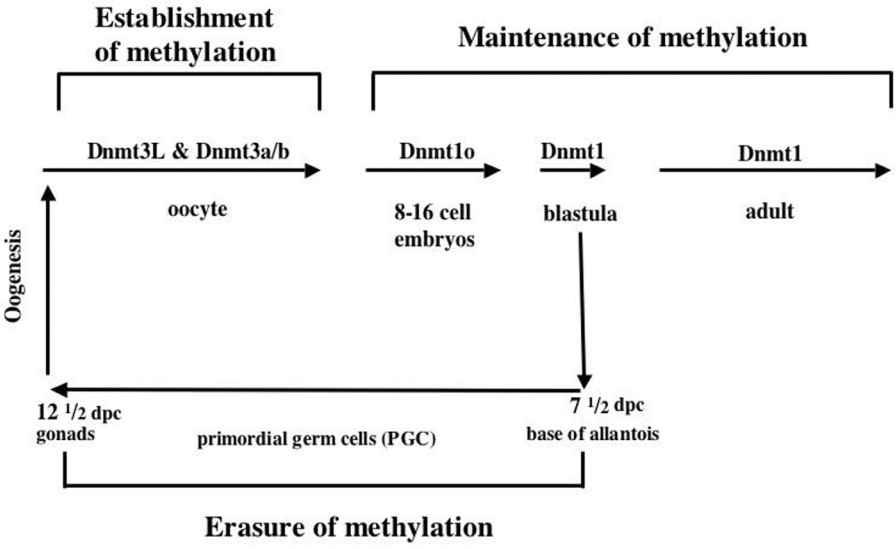

Fig. 4. DNA methylation changes of imprinted genes during gametogenesis and embryo development

Alternatively, DNA methylation can indirectly affect monoallelic expression of a gene by silencing a promoter of an antisense gene, thus allowing production of antisense RNA only from the allele on which the imprinted gene is repressed (Rougeulle et al. 1998; Lyle et al. 2000).

Loss of function of DMRs, or defects in their methylation, frequently cause loss of imprinting associated with neurobehavioral disorders. Such epigenetic defects within a 2-Mb domain on human chromosome 15q11–q13 cause two different syndromes. Prader-Willi syndrome is caused by loss of function of a large number of paternally expressed genes (Buiting et al. 1995), while silencing of the maternally expressed genes within the domain causes Angelman's syndrome (Reis et al. 1994). The imprinting of this entire domain is regulated by an imprinting center that constitutes a DMR within the 5′ region of the imprinted SNRPN gene. Individuals with deletions of this region on the paternal allele have Prader-Willi while another sequence located 35 kb upstream of SNRPN confers methylation of the SNRPN DMR on the maternal allele, thereby inactivating the paternally expressed genes on the maternal allele. When this upstream region is deleted on the maternal allele, the SNRPN DMR does not become methylated (Perk et al. 2002). Consequently, the entire domain on the maternal allele remains unmethylated and all paternally expressed genes on the maternal allele are activated while maternally expressed genes are silenced, thus causing Angelman's syndrome. A model was proposed suggesting that the upstream sequence together with the SNRPN DMR constitute a complex imprinting box responsible for both the establishment and maintenance of the imprinting state at PWS/AS domain on both alleles (Shemer et al. 2000).

Altered allelic methylation and expression patterns of the imprinted gene IGF2 have been found in Beckwith-Wiedemann syndrome (BWS) patients. BWS is a pre- and post-natal growth syndrome associated with predisposition for childhood tumors. Translocation breakpoints in a number of BWS patients map to the imprinted gene KCNQ1 which is located in the center of the 800-kb BWS region on human chromosome 11p15.5. The translocations in BWS are associated with loss of imprinting of IGF2 but not H19 (Brown et al. 1996). It appears that this impairment in imprinting involves the differentially methylated intronic CpG island in KCNQ1. In a small number of BWS patients, hypomethylation of the KCNQ1 CpG island correlated with biallelic expression of IGF2 (Smilinich et al. 1999; Paulsen et al. 2000). Deletion of this CpG island on the paternal chromosome 11 leads to silencing of KCNQ1 antisense transcript and activation of KCNQ1, $p57^{KIP2}$ and SMS4 which are located downstream on the normally repressed paternal allele. It is therefore possible that this CpG island is at least part of an imprinting center on human chromosome 11p15.5 and its orthologous region on mouse chromosome 7.

5
DNA Methylation and Disease

Many examples exist where DNA methylation in non-imprinted genes goes awry, causing neurodevelopmental disorders, such as ATR-X, ICF, Rett and Fragile-X syndromes, or the imprinting disorders Prader-Willi, Angelman's and Beckwith-Wiedemann syndromes described above. This clearly suggests that control of gene expression that is associated with DNA methylation is particularly important in brain development and function. ATR-X (α-thalassemia, mental retardation, X-linked) patients are known to have methylation defects that result from high methyltransferase activity in neurons (Goto et al. 1994). Such high activity of methyltransferase in mice results in delayed ischemic brain damage (Endres et al. 2000) associated with hypo- or hypermethylation of repetitive sequences (Gibbons et al. 2000).

ICF (immunodeficiency, centromeric instability and facial anomaly) syndrome is linked to mutations in the de novo methyltransferase gene DNMT3B (mapped to chromosome 20q) affecting its carboxy terminal catalytic domain (Hansen et al. 1999), resulting in hypomethylation of the normally heavy methylated repetitive sequences (Kondo et al. 2000) and single copy sequences on the inactive X-chromosomes (Miniou et al. 1994; Bourc'his et al. 1999). Mice knocked out in Dnmt3b show similar demethylation and could therefore serve as an experimental ICF model (Okano et al. 1999). How DNMT3B deficiency affects brain development remains to be elucidated.

Another syndrome that is manifested in mental retardation and is associated with methylation-dependent gene silencing is the X-linked Rett syndrome that results from mutations in the MeCP2 gene (Amir et al. 1999). Mutations that cause the disease disrupt the integrity of the methyl binding domain (MBD) or the transcription repressory domain (TRD) of MeCP2, whose function in gene repression has been discussed above. How MeCP2 mutations lead to developmental defects in the brain is currently a matter under investigation.

The most common form of inherited mental retardation after Down syndrome is Fragile-X syndrome. The X-linked gene that is associated with the disease, Fragile-X mental retardation 1 (FMR1), contains highly polymorphic CGG repeats with an average length of 29 repeats in normal individuals and 200–600 repeats in Fragile-X patients. In addition, the CpG island at the 5′ end of the gene in patients is abnormally methylated and histone deacetylated, causing silencing of the gene (Oberle et al. 1991; Coffee et al. 1999). The reasons for this de novo methylation and the mechanisms driving this de novo methylation are, as yet, unclear.

6 Concluding Remarks

DNA methylation may have evolved as a luxury device for setting up central biological processes. Central to all processes that involve DNA methylation is the control of gene expression. The high complexity of the mammalian genome required a multilevel hierarchy of mechanisms that control gene expression. One of these levels of regulation involves DNA methylation. DNA methylation is a flexible epigenetic feature of the genome that can be established, maintained and erased. Being flexible, this feature had been successfully employed to serve the dynamic changes the cells undergo during gametogenesis and development of the embryo. One striking example of how DNA methylation functions in development is its being an epigenetic mark in discrimination between the alleles in phenomena such as X-chromosome inactivation and genomic imprinting. The importance of DNA methylation in the well-being of the cell is clearly reflected in the large number of genetic diseases, including cancer, that occur when DNA methylation goes awry. Being conserved in evolution, DNA methylation must have had an evolutionary advantage that outweighs the price paid by the cell in the form of genetic disorders. The recent discoveries in the field of epigenetic modifications of nucleosomal histones revealed the role played by DNA methylation in shaping chromatin structure, thereby affecting formation of silent domains in the genome.

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