Journal of Cellular Biochemistry 94:257–265 (2005)

DNA Methylation and Chromatin Structure: The Puzzling CpG Islands

Paola Caiafa* and Michele Zampieri

Department of Cellular Biotechnology and Hematology, University of Rome 'La Sapienza,' 00161 Rome, Italy

Abstract DNA methylation is the epigenetic modification, which introduces 5mC as fifth base onto DNA. As for the distribution of 5mCs, it is well known that they distribute themselves in a non-random fashion in genomic DNA so that methylation pattern is characterized by the presence of methylated cytosines on the bulk of DNA while the unmethylated ones are mainly located within particular regions termed CpG islands. These regions represent about 1% of genomic DNA and are generally found in the promoter region of housekeeping genes. Their unmethylated state, which is an essential condition for the correct expression of correlated genes, is paradoxical if one considers that these regions are termed CpG islands because they are particularly rich in this dinucleotide, which is the best substrate for enzymes involved in DNA methylation. Anomalous insertion of methyl groups in these regions generally leads to the lack of transcription of correlated genes. An interesting scientific problem is to clarify the mechanism(s) whereby CpG islands, which remain protected from methylation in normal cells, are susceptible to methylation in tumor cells. How the CpG moieties in CpG islands become vulnerable or resistant to the action of DNA methyltransferases and can thus lose or maintain their characteristic pattern of methylation is still an open question. Our aim is to gather some mechanisms regarding this intriguing enigma, which, despite all energy spent, still remains an unresolved puzzle. J. Cell. Biochem. 94: 257–265, 2005.

Key words: DNA methylation; CpG islands; *Alu* sequences; poly(ADP-ribosyl)ation

DNA METHYLATION MACHINERY

DNA methylation [for reviews see Costello and Plass, 2001; Bird, 2002; Zlatanova et al., 2004] is the post-synthetic modification which, by transferring a methyl group from S-adenosylmetionine to carbon-5 of cytosine ring, introduces 5mC as new base on DNA. Enzymes involved in this reaction are DNA methyltransferases and their preferential target for methylation are cytosines located in CpG dinucleotides. The mCpG dinucleotides are distributed in a non-random fashion in genomic DNA so that methylation pattern is characterized by the presence of methylated cytosines on the bulk of DNA, while the unmethylated ones are mainly located within particular regions termed CpG islands. Specific DNA methylation pattern results from the combination of maintenance and de novo methylation and demethylation processes. Maintenance methylation, which occurs within a minute or two after replication, recognizes and modifies hemimethylated sites generated during DNA replication thus preserving the tissue-specific methylation pattern. DNA methyltransferase 1 (Dnmt1) is considered primarily responsible for maintenance methylation due to its preference for hemimethylated DNA, its maximum level of expression being in S cell-cycle phase [Szyf et al., 1991] and not the least because of its association with proliferating cell nuclear antigen (PCNA) during DNA replication at DNA replication foci [Chuang et al., 1997]. Dnmt3a and Dnmt3b are considered responsible for the de novo methylation process, which explains how the pattern of methylation can be changed by introducing new

Grant sponsor: MIUR (Ateneo, Cofin, and FIRB); Grant sponsor: AIRC (Associazione Italiana Ricerca sul Cancro); Grant sponsor: Istituto Pasteur, Fondazione Cenci Bolognetti.

*Correspondence to: Dr. Paola Caiafa, Dipartimento di Biotecnologie Cellulari ed Ematologia, Sezione di Biochimica Clinica, Università di Roma "La Sapienza," Viale Regina Elena 324 (Policlinico), 00161 Roma, Italia.
E-mail: caiafa@bce.uniroma1.it

Received 9 September 2004; Accepted 13 September 2004
DOI 10.1002/jcb.20325

methyl groups onto DNA at sites in which neither strand was previously methylated. These enzymes play an important role during early stages of embryonic development when a bimodal pattern of methylation is defined [Kafri et al., 1992]. It is important to say that it is too simplistic to consider that only these enzymes are involved in DNA methylation as for most of them there are several isoforms and new Dnmts have been identified [for review see Robertson, 2002]. A great deal of research is being carried out to establish their roles. The original distribution of roles mentioned above for the main Dnmts may have to be reconsidered in light of the fact that some of them have been seen cooperating in maintaining DNA methylation and gene silencing in human cancer cells during DNA replication [for review see El-Osta, 2003].

The thermodynamic problem, concerning the removal of methyl groups from cytosine makes the understanding of DNA demethylation mechanism somewhat complicated. A DNA demethylase activity, capable of catalyzing replacement of the methyl group with a hydrogen derived from water and of releasing the methyl group in the form of methanol, has been proposed as well as the alternative possibility of the removal of 5mC or 5mCpG dinucleotide by glycosylase activity and their substitution with the unmethylated base or dinucleotide. Whatever the enzymatic mechanism(s) may be, DNA demethylation is an active process in cells and plays a role not only in the definition of the methylation pattern in early stages of development but also in the passage towards pathological events as tumorigenesis [for reviews see Cedar and Verdine, 1999; Bird, 2003].

Histone modifications, DNA methylation, and proteins able to bind methylated DNA play a dynamic role in determining chromatin structure suitable for gene expression or silencing. At present, much information is available as to how several epigenetic modifications work together in determining regions of chromatin that have to be more or less suitable for transcription [for review see Jaenisch and Bird, 2003]. An unresolved problem is the timing with which the various epigenetic events occur in establishing gene expression. On this subject at least two scenarios can be suggested. One foresees DNA methylation as the first event, as it was observed that histone deacetylase can reach chromatin through its association with proteins able to recognize and bind methylated DNA [Jones et al., 1998; Nan et al., 1998]. The second scenario considers DNA methylation to be the final stabilizing moment, which occurs only after epigenetic events associated with histones (i.e., K9H3 methylation) have already shut down gene expression [for review see Bird, 2001].

DISTRIBUTION OF mCpG AND CpG DINUCLEOTIDES ON GENOMIC DNA

The frequency with which CpG dinucleotides are found on genome is much lower than expected, except for CpG island regions where their number is nearer to the expected one. This happened during evolution due to the presence of spontaneous deaminase activity in nuclei [for review see Jones and Baylin, 2002]. This enzymatic reaction transforms methylated cytosine into thymine while the unmethylated cytosine is transformed into uracil. Subsequent control and repair mechanisms recognize uracil as an extraneous base on DNA and thus substitute it, while this substitution does not happen where thymine is concerned as it is a common base on DNA.

Going back to CpG islands being enriched in CpG dinucleotides, the explanation lies in the non methylation of their normal state, which preserves their regions from deamination-dependent mutations.

Summarizing some characteristics of CpG islands [Antequera and Bird, 1993; Antequera and Bird, 1994; Antequera, 2003] there are about 30,000 generally located in the 5′ promoter region of housekeeping genes, sometimes overlapping the coding region to variable extents (usually the first exon). Although their sequence is enriched in CpG dinucleotides, which are the best substrates for DNA methyltransferase, the CpG islands are unmethylated. There is evidence that the transcription of genes associated with them is active when these regions are in unmethylated state, while it is inhibited when these regions undergo methylation [for reviews see Jones and Baylin, 2002; Robertson, 2002]. It is to note that CpG islands have recently been found in several tissue-specific and imprinted genes within the genes themselves without their methylation blocking gene expression [for review see Jones, 1999].

Along DNA other discrete regions, enriched in CpG dinucleotides, must be considered. They are repetitive elements termed B elements [for

review see Turker 2002] in mice and *Alu* elements in humans [Takai and Jones, 2002]. Analysis of sequence of human chromosomes 21 and 22 has led to the definition of more stringent parameters, which allow the distinguishing of CpG islands and *Alu* sequences [Takai and Jones, 2002]. The new parameters for definition of CpG islands are fixed in this way: minimum size $\geq$0.5 kbp, C + G content $\geq$55% and observed CpG/expected CpG $\geq$0.65. The important difference between CpG islands and *Alu* repeats lies in the fact that the latter are a good substrate for the DNA methylation that occurs in these regions that are generally methylated. B and *Alu* sequences are defined *cis*-acting methylation centers both as suspected of signalling de novo DNA methylation and as—in absence of a boundary—they may be able to spread methylation to the adjacent DNA regions.

A strong association is shown between CpG islands and *Alu* repeats. A detailed analysis of the sequence of human chromosomes 21 and 22 showed that 54.4% of CpG islands occur between *Alu* regions and the number of CpGs that are found in 5′ regions of genes, is about the same as the number of *Alu*-associated CpGs [Takai and Jones, 2002].

JIGSAW OF UNMETHYLATED STATE OF CpG ISLANDS

Concerning the regulation of DNA methylation process, two unresolved questions are:

a) to clarify the mechanism by which CpG islands are protected from methylation during replication and in chromatin;
b) to understand how CpG islands, located in the promoter regions of tumor suppressor genes, become methylated during tumorigenesis while they are unmethylated in normal cells.

There has been a lot of research to discover the mechanism that normally keeps CpG islands unmethylated, some of which has tried to pinpoint *cis*-acting sequences able to conduct such an important mechanism. As CpG island sequences have been seen to be methylatable in in vitro experiments the hypothesis that they are intrinsically unmethylatable has fallen through [Bestor et al., 1992].

Attention of many researchers has been diverted to consensus binding sites for transcription factor Sp1 [Brandeis et al., 1994; Macleod et al., 1994; Mummaneni et al., 1998]. The mouse *aprt* housekeeping gene — whose CpG island includes the promoter, the first and second exons, and the first intron — has been chosen for the study of this mechanism as Sp1 consensus sites are present in its promoter region. Their connection to the mechanism that keeps the CpG islands unmethylated is being researched with interest as these Sp1 sites often occupy a strategic position both in the mouse B1 [for review see Turker, 2002] and in the human *Alu* repetitive elements that often flank CpG islands [Takai and Jones, 2002]. B1 and *Alu* sequences—because of their high methylation level—are considered methylation centers and Sp1 sites in these patterns are seen as boundaries able to stop the spreading of methylation from B1 and *Alu* elements to unmethylated CpG islands (Fig. 1).

The diffusion of methylation from methylated CpG dinucleotides toward the adjacent unmethylated ones has previously been amply shown [for review see Lindsay and Adams, 1996], but without explaining how CpG islands are protected from the spreading of methylation. As concerns the expression of mouse *aprt* gene, it has been shown that among the Sp1 consensus sites, sites three and four are required for gene transcription while site two is the specific one in the blocking of methylation spreading and is reinforced in this role by sites three and four [Mummaneni et al., 1998]. Their silencing is a gradual process in differentiated cells [Yates et al., 2003]. If it is the boundary caused by Sp1, which isolates the islands from a general state of methylation, it can be presumed that in tumorigenesis this mechanism is, in some way, lost (Fig. 1).

However, data in literature are not unanimous as while some experiments show that the gene-correlated CpG island is methylated when Sp1 binding sites, in correspondence with gene, are disrupted by direct mutagenesis [Brandeis et al.,1994; Macleod et al., 1994; Mummaneni et al., 1998], no anomalous methylation of CpG islands is found in mice in which Sp1 is not expressed following homozygous deletion [Marin et al., 1997]. Anyway this mechanism cannot be considered universal as Sp1 consensus sites are not always present in promoter regions of housekeeping genes. It is reasonable to think that some other transcription factor(s), through their ties with active promoters

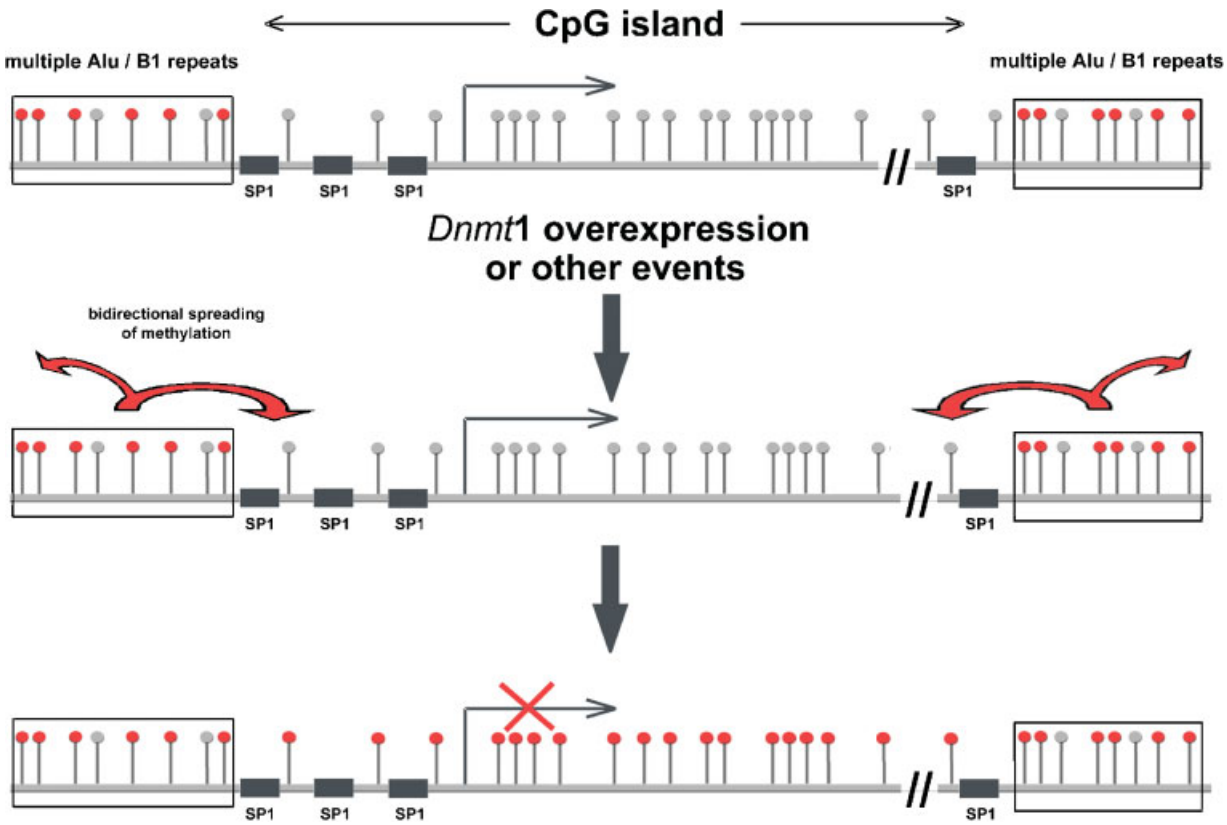

Fig. 1. *Alu* repeats and CpG islands are physically and functionally correlated on genomic DNA: a role for Sp1 elements. In the scheme CpG island is juxtaposed to multiple *Alu* elements, which have been proposed as "de novo methylation centers." Sp1 sites, located upstream and downstream of transcription start in the island, play a role in preventing methylation. Cluster of Sp1 elements is suggested as boundary region capable of protecting CpG island from the spreading of methylation arising from *Alu* elements. Grey and red lollipops represent non-methylated and methylated CpGs, respectively.

containing CpG islands, can act as *trans*-acting elements able to protect the unmethylated state of these DNA regions. Experiments, carried out on T24 bladder carcinoma cell line, in which the remethylation kinetics was examined for *p16* gene after it had been demethylated by treatment with 5-aza 2′-deoxycytidine, have shown that the timing of remethylation varied, being longer for promoter region than for coding regions of gene, i.e. exon 2. This could be due to the presence of transcription factor(s) on promoter region capable of impeding Dnmt1 from entering DNA [Bender et al., 1999].

An important point in determining variation of methylation patterns is the nuclear level of Dnmt1 as it has been often seen to be high in tumor cells [Baylin et al., 2001]. Thus anomalous increased level of the enzyme could be involved in determining aberrant introduction of new methyl groups onto DNA. Housekeeping gene promoters are hypermethylated, with the silencing of correlated genes, in human cell lines expressing 50-fold increased level of Dnmt1 (HMT) [Vertino et al., 1996] and further research, performed on the same cells, has shown that not all *loci* containing CpG rich regions are equally affected by methylation in cells overexpressing Dnmt1 but about 70% of them were resistant to de novo methylation and only a few of them (3.8%) were methylation-prone [Feltus et al., 2003]. Recently, it has been shown that RNA interference (RNAi)-mediated silencing of Dnmt1 leads to promoter demethylation and re-expression of some tumor suppressor genes in several cancer cells [Robert et al., 2003; Suzuki et al., 2004]. This confirms the important role played by Dnmt1 in tumorigenesis although additional data have shown that CpG island hypermethylation is maintained in human cancer cells after homozygous deletion [Rhee et al., 2000] or RNAi-mediated depletion of Dnmt1 [Ting et al., 2004].

As, not all tumor cells in which there is hypermethylation of tumor suppressor genes show an

overexpression of Dnmt1 [Eads et al., 1999], and as the expression of Dnmt1 is cell-cycle dependent [Szyf et al., 1991], attention has been diverted to the idea that the expression of Dnmt1 can occur in an anomalous cell-cycle phase—i.e., in G_1/early S phase—when CpG rich regions and active genes replicate [Delgado et al., 1998]. It has been observed that remethylation process can also occur in cells blocked in G_1 [Bender et al., 1999].

In this scenario the competition existing between p21 and Dnmt1 for the same binding domain on PCNA [Chuang et al., 1997] may play an important role. PCNA is the protein to which Dnmt1 binds when, immediately following replication, the maintenance methylation intervenes to guarantee that methylation pattern of the parent strand is conserved on the newly synthesized one. In early S phase, when CpG island regions replicate [Delgado et al., 1998], the level of Dnmt1 is too low to compete with p21 for the binding site on PCNA and so replication occurs without subsequent methylation. It has been suggested that, as yet, an unidentified biological event, able to induce Dnmt1 expression in early S phase, could make the binding to PCNA easier thus allowing the methylation of early replicating genes in tumorigenesis [Baylin, 1997]. Moreover, Dnmt1 seems to be a cofactor capable of regulating negative expression of p21 through a direct or indirect link with Sp1 [Milutinovic et al., 2004]. Thus, an anomalous overexpression of Dnmt1 in the early S phase could further facilitate the formation of PCNA–Dnmt1 complex.

The biological event able to induce Dnmt1 overexpression in the early S phase could be poly(ADP-ribosyl)ation as it has been found that competitive inhibition of PARPs leads to introduction of anomalous methyl groups onto DNA [Zardo et al., 1997, 1999; Zardo and Caiafa, 1998; de Capoa et al., 1999], hyperexpression of Dnmt1 in G_1/early S phase and increase of the amount of Dnmt1 that co-immunoprecipitates with PCNA in this phase [Zardo et al., 2002], (Fig. 2, Panel A). How poly(ADP-ribosyl)ation is involved in the control of Dnmt1 expression is still unclear and it is to define whether it is involved in the regulation of *Dnmt1* gene or in the regulation of another gene or protein involved in the regulation of Dnmt1 expression.

A second mechanism has been suggested for PARP1 in the control of DNA methylation. In this hypothesis PARP1 in its poly(ADP-ribosyl)ated isoform makes Dnmt1 catalytically inactive (Fig. 2, panel B). To understand this suggested mechanism it must be said that modified isoform of PARP1 presents in its central domain many (about 28) ADP-ribose chains that can even be up to 200 units in length and be branched [D'Amours et al., 1999]. Modified PARP1 can be seen as a molecular adaptor characterized by a clear negative charge onto which chromatin proteins can be attracted and hosted. Several proteins show a greater affinity for these polymers than for DNA so that ADP-ribose polymers compete with DNA for the bond with them. This non-covalent link, which is very strong [Panzeter et al., 1992], is not specifically guided by an attraction between charges, but proteins showing high affinity for polymers have an aminoacid domain, which is responsible for the interaction with ADP-ribose polymers [Pleschke et al., 2000]. Dnmt1 has two possible consensus aminoacid domains for binding with ADP-ribose polymers [Reale et al., 2004]. dsDNA 30 times more concentrated does not remove link between Dnmt1 and ADP-ribose polymers and in vitro experiments have shown how ADP-ribose polymers present on modified PARP1 almost completely inhibit the catalytic activity of Dnmt1, while unmodified PARP1 is not able to inhibit the enzyme. These data, added to the fact that the two proteins co-immunoprecipitate in vivo and that PARP1 is in its modified form in this complex, suggest that modified PARP1 trapping Dnmt1 through ADP-ribose polymers, is responsible for the catalytic inactivation of the enzyme in chromatin [Reale et al., 2004]. Through this mechanism the non-methylated state of CpG islands could be protected and a functional anomaly in the poly(ADP-ribosyl)ation process could therefore be responsible for new aberrant methylation (Fig. 2, panel B).

Thus, inhibition of PARP activity could allow new methyl groups to be inserted onto DNA both during replication and in chromatin: during replication because inhibition of PARP activity induces Dnmt1 overexpression in G_1/S phase and increases the formation of the active complex PCNA-Dnmt1 in this anomalous phase [Zardo et al., 2002] and in chromatin because unmodified PARP1 is unable to inhibit Dnmt1 [Reale et al., 2004].

The existence of a protein, which links Dnmt1 inhibits its catalytic activity, thus preventing the introduction of new methyl groups onto

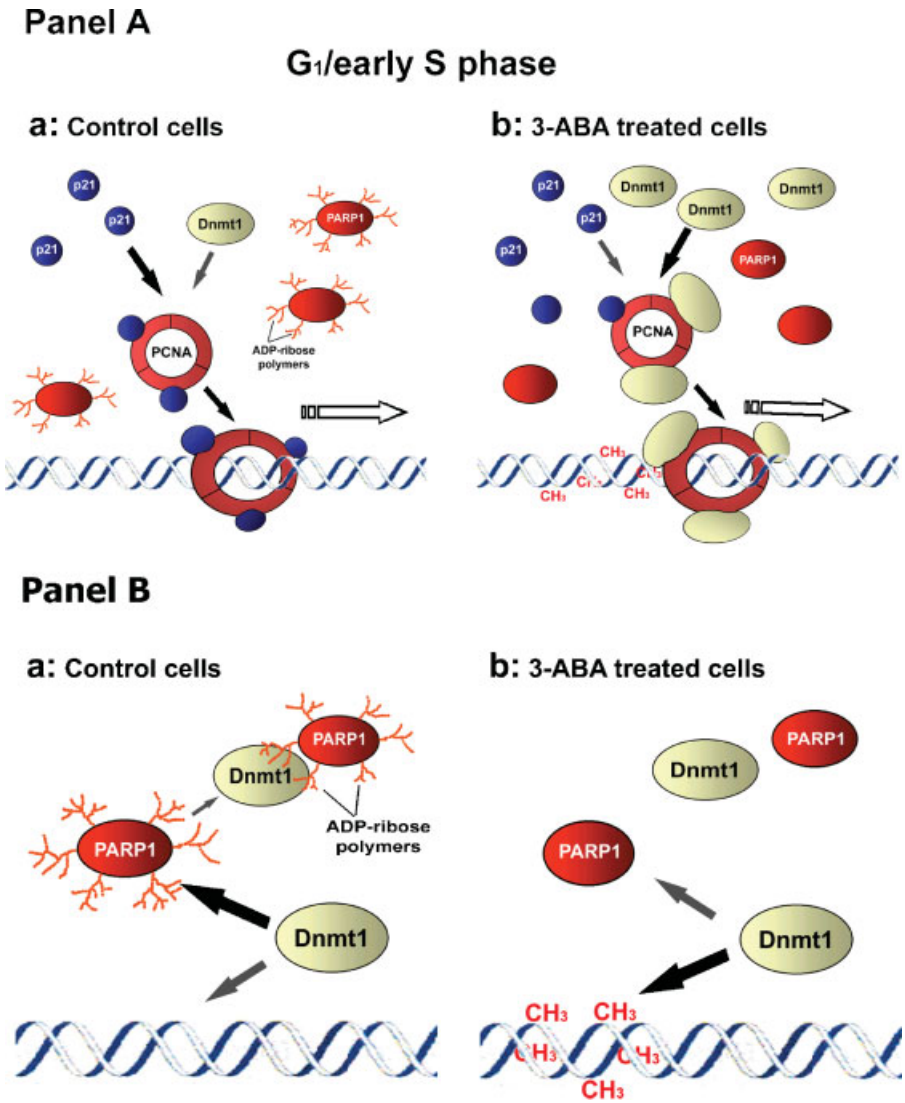

Fig. 2. Inhibition of PARP activity induces DNA hypermethylation. **Panel A** reports the scheme proposed to explain DNA hypermethylation dependent on PARP inhibition during DNA replication. **a**: In normal cells, modified PARP1 plays a role in controlling the expression of Dnmt1 in the appropriate cell-cycle phase, and thus the level of Dnmt1-PCNA active complex is low in G_1/S phase. **b**: Inhibition of PARP activity induces overexpression of Dnmt1 in G_1/S phase, causing anomalously high level of the active Dnmt1-PCNA complex in this phase. **Panel B** reports the scheme proposed to explain DNA hypermethylation dependent on PARP inhibition in chromatin. **a**: In normal cells, ADPR-polymers, present on PARP1, link in uncovalent way Dnmt1. Affinity of Dnmt1 for ADPR-polymers is so high that they compete with dsDNA 30 times more concentrated for the binding with the enzyme and the presence of ADPR-polymers almost completely inhibits the Dnmt1 activity in vitro. **b**: Inhibition of poly(ADP-ribosyl)ation frees Dnmt1 from its tie with PARP1 and in absence of ADPR-polymers Dnmt1 activity is restored. Thus, following inhibition of poly(ADP-ribosyl)ation, the two mechanisms could cooperate in inducing anomalous DNA hypermethylation.

DNA has been suggested. This role is played by Rb [Pradhan and Kim, 2002], a protein involved in cell-cycle control and therefore having an important role as tumor suppressor gene. It has been shown that Rb links through its pockets B and C the regulatory domain of Dnmt1 and that following this association the bond existing between Dnmt1 and DNA is destabilized. Overexpression of Rb leads to DNA hypomethylation. All this suggests that this mechanism plays a role in the hypermethylation of oncosuppressor genes in tumors where often Rb is absent or mutated [Hanahan and Weinberg, 2000].

The outcome that ORIs map within or immediately adjacent to CpG islands has been seen as a mechanism probably involved in maintaining the unmethylated state of CpG islands.

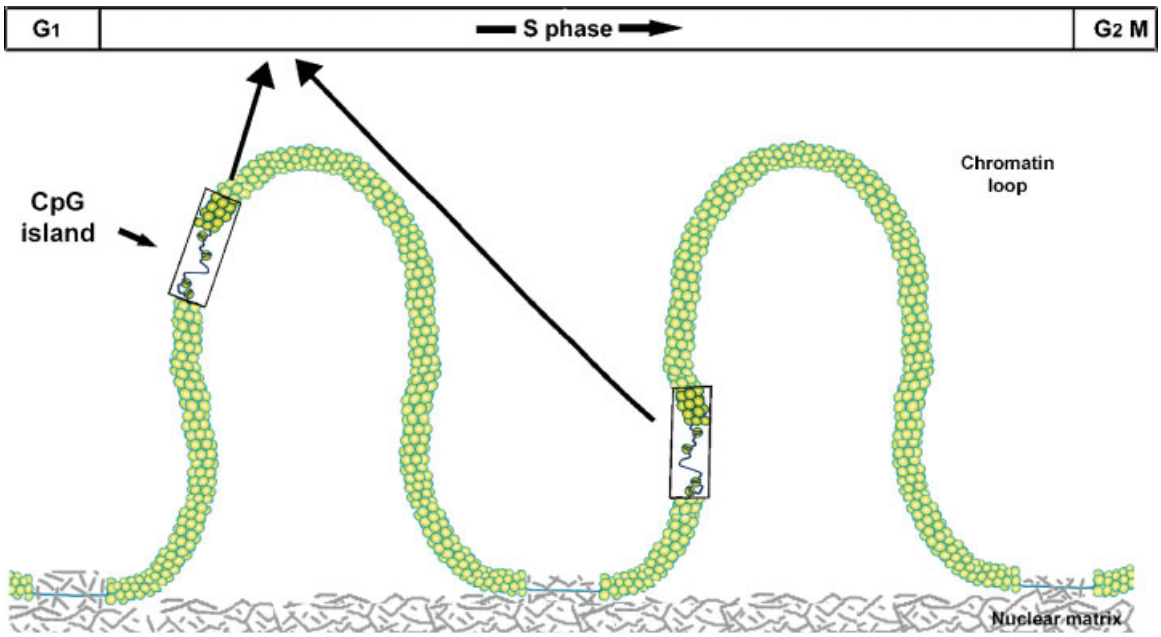

Fig. 3. ORIs are often located at CpG island regions in vivo. The fact that ORIs map within or immediately adjacent to CpG islands has been seen as a mechanism probably involved in maintaining the unmethylated state of CpG islands. CpG islands, because of their localization in decondensed chromatin regions, could offer an easier replication starting point than condensed chromatin. Thus CpG islands and correlated active genes replicate in early S phase while silent genes in more advanced S phase. The lower level of Dnmt1 in early S phase than during the S phase favours the maintaining of the unmethylated state of CpG islands.

Analysis of very short DNA fragments (<1.5 kbp), which are formed at the beginning of S phase, allowed the observation that their sequence is similar to that of unmethylated CpG island regions. Replication time of CpG islands is about 2–2.5 h while the bulk of DNA needs more time to replicate. Thus origins of replication colocalize frequently with CpG islands so that the great number of proteic factors involved in these processes could be enough to make access difficult to Dnmt1 that, as mentioned above, is less expressed in early S-phase.

Experiments performed on human X-linked hypoxantine phosphoribosyltransferase gene (*HPRT*) have shown that colocalization of CpG islands and ORIs is dependent on the methylation state of CpG island. In fact the CpG island, which is methylated in the inactive allele, replicates in late S phase [Schmidt and Mignon, 1990]. Thus, unmethylated CpG island regions should be particularly suitable for starting DNA replication because of their uncondensed chromatin structure [Tazi and Bird, 1990] and this colocalization has been suggested as a mechanism by which CpG islands are protected from methylation during replication [Antequera and Bird, 1999], (Fig. 3).

Finally, there are proteins that target Dnmts on specific DNA regions and mediate transcriptional silencing [for reviews see Robertson, 2002; El-Osta, 2003].

At present much information is available but not enough to allow the elaboration of a final model(s) explaining how CpG islands are protected from methylation. In spite of the great effort put into solving this scientific problem, still much has to be done to provide definite proof, which solves this fascinating puzzle.

REFERENCES

Antequera F. 2003. Structure, function and evolution of CpG island promoters. Cell Mol Life Sci 60:1647–1658.

Antequera F, Bird A. 1993. Number of CpG islands and genes in human and mouse. Proc Natl Acad Sci USA 90: 11995–118859.

Antequera F, Bird A. 1994. Predicting the total number of human genes. Nat Genet 8:114.

Antequera F, Bird A. 1999. CpG islands as genomic footprints of promoters that are associated with replication origins. Curr Biol 9:R661–R667.

Baylin SB. 1997. Tying it all together: Epigenetics, genetics, cell cycle, and cancer. Science 277:1948–1949.

Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG. 2001. Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet 10:687–692.

Bender CM, Mark LG, Gonzales FA, Carvell T, Robertson KD, Jones PA. 1999. Roles of cell division and gene transcription in the methylation of CpG islands. Mol Cell Biol 19:6690–6698.

Bestor TH, Gundersen G, Kolsto AB, Prydz H. 1992. CpG islands in mammalian gene promoters are inherently resistant to de novo methylation. Genet Anal Tech Appl 9:48–53.

Bird A. 2001. Methylation talk between histones and DNA. Science 294:2113–2115.

Bird A. 2002. DNA methylation patterns and epigenetic memory. Genes Dev 16:6–21.

Bird A. 2003. Il2 transcription unleashed by active DNA demethylation. Nat Immunol 4:208–209.

Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, Temper V, Razin A, Cedar H. 1994. Sp1 elements protect a CpG island from de novo methylation. Nature 371:435–438.

Cedar H, Verdine GL. 1999. Gene expression. The amazing demethylase. Nature 397:568–569.

Chuang LS, Ian HI, Koh TW, Ng HH, Xu G, Li BF. 1997. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. Science 277:1996–2000.

Costello JF, Plass C. 2001. Methylation matters. J Med Genet 38:285–303.

D'Amours D, Desnoyers S, D'Silva I, Poirier GG. 1999. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J. 342:249–268.

de Capoa A, Febbo FR, Giovannelli F, Niveleau A, Zardo G, Marenzi S, Caiafa P. 1999. Reduced levels of poly(ADP-ribosyl)ation result in chromatin compaction and hypermethylation as shown by cell-by-cell computer-assisted quantitative analysis. FASEB J 13:89–93.

Delgado S, Gomez M, Bird A, Antequera F. 1998. Initiation of DNA replication at CpG islands in mammalian chromosomes. EMBO J 17:2426–2435.

Eads CA, Danenberg KD, Kawakami K, Saltz LB, Danenberg PV, Laird PW. 1999. CpG island hypermethylation in human colorectal tumors is not associated with DNA methyltransferase overexpression. Cancer Res 59: 2302–2306.

El-Osta A. 2003. DNMT cooperativity—the developing links between methylation, chromatin structure and cancer. Bioessays 25:1071–1084.

Feltus FA, Lee EK, Costello JF, Plass C, Vertino PM. 2003. Predicting aberrant CpG island methylation. Proc Natl Acad Sci USA 100:12253–12258.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57–70.

Jaenisch R, Bird A. 2003. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. Nature Genet supp 33:245–254.

Jones PA. 1999. The DNA methylation paradox. Trends Genet 15:34–37.

Jones PA, Baylin SB. 2002. The fundamental role of epigenetic events in cancer. Nature 3:415–428.

Jones PL, Veenstra GJC, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP. 1998. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 19:187–191.

Kafri T, Ariel M, Brandeis M, Shemer R, Urven I, McCarrey J, Cedar H, Razin A. 1992. Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. Genes Dev 6:705–714.

Lindsay H, Adams RL. 1996. Spreading of methylation along DNA. Biochem J 320:473–478.

Macleod D, Charlton J, Mullins J, Bird AP. 1994. Sp1 sites in the mouse *aprt* gene promoter are required to prevent methylation of the CpG island. Genes Dev 8:2282–2292.

Marin M, Karis A, Visser P, Grosveld F, Philipsen S. 1997. Transcription factor Sp1 is essential for early embryonic development but dispensable for cell growth and differentiation. Cell 89:619–628.

Milutinovic S, Brown SE, Zhuang Q, Szyf M. 2004. DNA methyltransferase 1 knock down induces gene expression by a mechanism independent of DNA methylation and histone deacetylation. J Biol Chem 279:27915–27927.

Mummaneni P, Yates P, Simpson J, Rose J, Turker MS. 1998. The primary function of a redundant Sp1 binding site in the mouse aprt gene promoter is to block epigenetic gene inactivation. Nucleic Acids Res 26:5163–5169.

Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. 1998. Transcriptional repression by methyl-CpG binding protein MeCP2 involves a histone deacetylase complex. Nature 393:386–389.

Panzeter PL, Realini CA, Althaus FR. 1992. Noncovalent interactions of poly(adenosine diphosphate ribose) with histones. Biochemistry 31:1379–1385.

Pleschke JM, Kleczkowska HE, Strohm M, Althaus FR. 2000. Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins. J Biol Chem 275:40974–40980.

Pradhan S, Kim GD. 2002. The retinoblastoma gene product interacts with maintenance human DNA (cytosine-5) methyltransferase and modulates its activity. EMBO J 21:779–788.

Reale A, De Matteis G, Galleazzi G, Zampieri M, Caiafa P. 2004. Modulation of DNMT1 activity by ADP-ribose polymers. Oncogene (in press).

Rhee I, Jair KW, Yen RWC, Lengauer C, Herman JG, Baylin SB, Schuebel KE. 2000. CpG methylation is maintained in human cancer cells lacking DNMT1. Nature 404:1003–1007.

Robert MF, Morin S, Beaulieu N, Gauthier F, Chute IC, Barsalou A, MacLeod AR. 2003. DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. Nat Genet 33:61–65.

Robertson KD. 2002. DNA methylation and chromatin—unraveling the tangled web. Oncogene 21:5361–5379.

Schmidt and Mignon BR. 1990. Asynchronous replication of homologous loci on human active and inactive X chromosomes. Proc Natl Acad Sci USA 87:3685–3689.

Suzuki M, Sunaga N, Shames DS, Toyooka S, Gazdar AF, Minna JD. 2004. RNA interference-mediated knockdown of DNA methyltransferase 1 leads to promoter demethylation and gene re-expression in human lung and breast cancer cells. Cancer Res 64:3137–3143.

Szyf M, Bozovic V, Tanigawa G. 1991. Growth regulation of mouse DNA methyltransferase gene expression. J Biol Chem 266:10027–10030.

Takai D, Jones PA. 2002. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. Proc Natl Acad Sci USA 99:3740–3745.

Tazi J, Bird A. 1990. Alternative chromatin structure at CpG islands. Cell 60:909–920.

Ting AH, Jair KW, Suzuki H, Yen RW, Baylin SB, Schuebel KE. 2004. CpG island hypermethylation is maintained in

human colorectal cancer cells after RNAi-mediated depletion of DNMT1. Nat Genet 36:582–584.

Turker MS. 2002. Gene silencing in mammalian cells and the spread of DNA methylation. Oncogene 21:5388–5393.

Vertino PM, Yen RW, Gao J, Baylin SB. 1996. De novo methylation of CpG island sequences in human fibroblasts overexpressing DNA (cytosine-5-)-methyltransferase. Mol Cell Biol 16:4555–4565.

Yates PA, Burman R, Simpson J, Ponomoreva ON, Thayer MJ, Turker MS. 2003. Silencing of mouse *Aprt* is a gradual process in differentiated cells. Mol Cell Biol 23:4461–4470.

Zardo G, Caiafa P. 1998. The unmethylated state of CpG islands in mouse fibroblasts depends on the poly(ADP-ribosyl)ation process. J Biol Chem 273:16517–16520.

Zardo G, D'Erme M, Reale A, Strom R, Perilli M, Caiafa P. 1997. Does poly(ADP-ribosyl)ation regulate the DNA methylation pattern? Biochemistry 36:7937–7943.

Zardo G, Marenzi S, Perilli M, Caiafa P. 1999. Inhibition of poly(ADP-ribosyl)ation introduces an anomalous methylation pattern in transfected foreign DNA. FASEB J 13: 1518–1522.

Zardo G, Reale A, Passananti C, Pradhan S, Buontempo S, De Matteis G, Adams RL, Caiafa P. 2002. Inhibition of poly(ADP-ribosyl)ation induces DNA hypermethylation: A possible molecular mechanism. FASEB J 16:1319–1321.

Zlatanova J, Stancheva I, Caiafa P, 2004. DNA methylation and chromatin structure. In: Zlatanova J, Leuba SH, editors. Chromatin structure and Dynamics: State-of-the-Art. Amsterdam: Elsevier. pp 309–342.