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CpG Islands in Chromatin Organization and Gene Expression[1]

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CpG islands are stretches of DNA sequence that are enriched in the $(CpG)_n$ repeat and are present in close association with all housekeeping genes as well as some tissue-specific genes in the mammalian genome. Methylation of CpG islands strongly influences both structural organization and function of chromatin. The presence of a CpG island in a given chromosomal domain can, by itself, give rise to relatively open and active chromatin. Recently, several histone acetyltransferases, histone deacetylases, and chromatin remodeling factors have been found to be part of the transcription machinery. It is becoming increasingly clear that CpG islands and their methylation status may influence the function or recruitment of these newly discovered chromatin remodeling factors, especially the histone deacetylases. In addition, CpG islands may also play a significant role in the reorganization of chromatin during mammalian spermiogenesis.

Key words: chromatin remodeling, DNA methylation, histone acetylation and deacetylation, spermiogenesis, transition proteins.

The G+C content of the mammalian genome is approximately 40%, a significant proportion being present as CpG dinucleotide. CpG islands, by definition, are sequences in the size range of 0.5-2 kb, characterized by higher GC levels than average DNA. DNA sequences with CpG islands typically contain above 50% G+C, with CpG sequences clustered in an otherwise CpG-depleted bulk DNA. CpG islands contain frequent *Hpa*II restriction sites (CCGG) and can therefore be detected as *Hpa*II tiny fragments or HTF. They are found associated with all housekeeping genes and some of the tissue-specific genes (*1, 2*). In the evolutionary context, CpG islands are highly conserved and well maintained (*3*). During the last decade, considerable progress has been made in our understanding of the functional significance of CpG islands. It is also becoming increasingly clear that CpG islands are the hot spots for chromatin remodeling, which in turn can regulate gene expression. A global picture is emerging of how a CpG island can govern the chromatin organization and consequent gene expression. Methylation of CpG islands, in addition to its role in modulating gene expression, has also been implicated as one of the mechanisms underlying genomic imprinting in mammals. Despite the rapid progress made in these areas, there are still several questions unanswered which we would like to highlight in this minireview. We have also discussed the possible, intimate relationship between the influence of CpG islands on chromatin organization and the histone acetylation pattern of CpG island-containing chromatin domain.

Origin, maintenance, and distribution of CpG islands

CpG islands are very scarce in the genomes of cold-blooded vertebrates (*4*). A comparison of homologous genes from cold-blooded and warm-blooded vertebrates has revealed that the cold-blooded vertebrates possess "primitive CpG islands" which do not have higher GC levels, *Hpa*II sites and G/C boxes. Since CpG doublets increase with increasing GC content of exons, introns, and intergenic sequences, it is suggested that the origin and evolution of CpG islands in the vertebrate genome are associated with compositional transition in genes and isochores (*5*). It is likely that the GC-rich coding sequences present in H3 isochores and related sequences have undergone directional changes (specifically in third codon position) leading to G and C enrichment. Antequera and Bird (*6*) have observed that the mouse genome has 16% less CpG islands than the human genome. This could be due to the creation of new islands in human or due to the loss of ancestral lineage. CpG dinucleotides in the human genome are most often replaced in mouse by TpG or its complement, CpA, as a consequence of mutation of the CpG to TpG. It is generally believed that the genes of an invertebrate ancestor were implanted in entirely nonmethylated DNA (*7*). During the course of evolution, DNA methylation spread through the genome as vertebrates evolved. However, by some unknown mechanism the promoters as well as the *cis*-acting regulatory elements were kept free from methylation (*8*). The nonmethylated CpG nucleotides along with an increased G+C content might have generated the present CpG islands (*3*). A question that is often encountered in this context is how these CpG islands have been kept methylation-free in a heavily methylated mammalian genome. Three mechanisms have been proposed to address this question. Firstly, GC-rich DNA may be poorly methylated (*9*). Secondly,

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there may be a CpG island-specific demethylation activity (*10*). Indirect evidence in favor of this mechanism is the observation that when partially methylated CpG islands are transfected into fertilized eggs or embryonic cells, the methylation is lost (*11*). Thirdly, some factors, like Sp1, are constantly bound to the CpG island sequence, which prevents the DNA methyltransferase from acting on CpG islands (*12*).

Recent studies have used fluorescent *in situ* hybridization (FISH) technique to analyze the chromosomal distribution of CpG islands. These sequences are shown to be located predominantly at the early replicating regions (G bands) and highly concentrated in the T band (a subset of the R band) (*13*). In humans, chromosomes 19 and 22 are highly enriched in CpG islands, while very few islands are present in chromosome 18. This heterogeneous distribution of CpG islands may be directly correlated with the density of functional genes in a given chromosome. Therefore, it is generally believed that the mammalian chromosomes are organized into domains with characteristic CpG island density. CpG islands have also evolved in chicken. Interestingly, they are highly concentrated on the micro-chromosomes rather than the macro-chromosomes in the chicken genome (*14*). Contrary to the clustering of CpG islands in a selective manner in chicken and human, there is no such preference for CpG island localization in the mouse genome (*15*).

Regulation of chromatin organization and gene expression by the methylation of CpG islands

Cytosine in CpG dinucleotides is frequently (60-90%) methylated in vertebrate genomes. Methylation of DNA at cytosine in the CpG dinucleotide residues is attractive as a candidate to explain genomic imprinting. Imprinted genes are those genes whose expression is determined by their parental origin. Recent reviews have discussed extensively the role of DNA methylation in genome imprinting (*16, 17*). Some recent examples showing a correlation between CpG island methylation and genome imprinting are *Tfg2r* gene of mouse (*18*), human *PET1/MEST* gene (*19*), human *SNRPN* gene (*20, 21*), and *U2af1-rs1* gene of mouse (*22*). The largest cluster of imprinted genes is the X-chromosome itself (*23*). The methylation patterns of human and mouse inactive X-chromosome are not similar. However, in both cases, the CpG islands of the active X-chromosome are devoid of methylation (*24*). In humans, 60 out of 61 CpG islands in the *PGK1* gene are methylated in the inactive X-chromosome. Methylation has also been linked to transcription silencing of Alu elements (*25*).

The CpG island-associated genes also get hypermethylated in tumor cell lines and solid tumors. These changes in methylation pattern may cause significant alteration in the control of gene expression during tumorigenesis. An attractive testable hypothesis is that accidental methylation of the CpG islands associated with tumor suppression genes may inactivate their expression, leading to tumorigenesis. Such epigenetic suppression was first reported for the retinoblastoma susceptibility gene (*Rb-1*) (*26*). More recently, CpG methylation-induced epigenetic suppression has been reported for the inactivation of *VHL* tumor suppressor gene (*27*) and the *bcr-abl* locus in chronic myelogenic leukemia (*28*). Jones and colleagues (*29*) have shown that the CpG islands of muscle determination gene, *MyoD*, which are not methylated during the establishment of cell lines, are methylated immediately after transformation with a chemical carcinogen. Many sites in the CpG islands that become methylated during this transformation were also correlated with heterochromatinization of *MyoD*, as evidenced by a decreased sensitivity to cleavage of DNA in nuclei by *Msp*I (*30*). In fact, methylation of CpG islands provides a putative mechanism for turning off those genes whose activities are not required during development or tumorigenesis. It could also work as a host defense mechanism to protect the genome against transposable elements.

Although the molecular mechanism of CpG methylation-mediated transcriptional repression is not clearly understood, substantial progress has been made in recent years. Two possible mechanisms have been proposed. One model, the so-called "direct mechanism," suggests that methylation of CpG islands may prevent the binding of transcriptional machinaries. However, several lines of experimental data seem to contradict this model. For example, some promoters are transcribed as naked DNA independent of methylation (*31*). Similarly, binding affinities of most transcription factors to their cognate sites do not change substantially upon methylation (*32, 33*). In addition, this mechanism can not explain the CpG methylation-mediated global transcription regulation. The alternate, and more probable, model suggests that transcriptional regulation could opercate *via* specific binding of repressor(s) which can recognize methyl CpG dinucleotides. This model is presented schematically in Fig. 1. There are two known methyl CpG-binding repressors, MeCP1 and MeCP2 (*34*). MeCP2 is present in all cell types except the germ cells and is found localized to the pericentromeric heterochromatin in mouse (which is also the region of highest 5-methyl cytosine concentration) (*35*). Although MeCP2 is dispensable for the viability of embryonic stem cells, it is essential for normal embryonic cell development (*36*). MeCP2 has been recently shown to repress transcription from methylated promoters but not from unmethylated promoters (*37*). This study also indicates that, in fact, MeCP2 is a chromosomal protein which can displace H1 from the nucleosome. It was proposed that MeCP2 mostly interacts with corepressor/histone deacetylase (corepressor/HDAC) complex and thereby recruits it onto the methylated CpG chromatin (*38*). Eventually, deacetylation of histones may induce chromatin condensation and repress transcription. It is possible that an unstable transcription initiation complex is assembled onto the methylated DNA, but after chromatin assembly and MeCP2 binding, this complex eventually falls apart (Fig. 1, B, C, and D), giving rise to an abortive transcription initiation. Recent reports show that MeCP2 does in fact interact with a corepressor (Sin3A)/deacetylase complex (*39, 40*). Furthermore, these studies also demonstrate that methylation-dependent transcriptional silencing can be reversed by Trichostatin A (TSA), a potent deacetylase inhibitor. Thus, on this ground, "methylation meets acetylation" (*41*).

Histone acetylation and deacetylation have been previously shown to play an important role in transcription (for review Ref. *42*). Hyperacetylated histones appear to accumulate in actively transcribed chromatin, whereas hypoacetylated histones are the diagnostic features of repressed chromatin. Recently, proteins that were initially identified as transcriptional regulators have been shown to

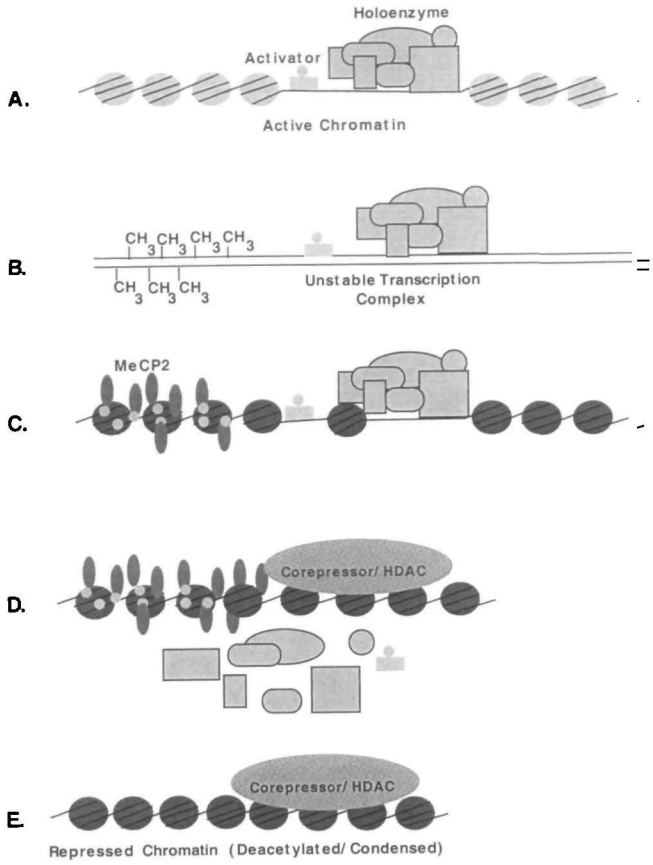

Fig. 1. **A model for CpG methylation-dependent chromatin remodeling leading to inactivation of transcription.** (A) Unmethylated CpG island containing active chromatin: activator and RNA polymerase holoenzyme are bound to the relatively nucleosome-free region of the chromatin. (B) Partially methylated promoter with unstable transcription complex. (C) Upon assembly, the methylated sites of the promoter are recognized by MeCP2. (D) Binding of MeCP2 leads to the recruitment of co-repressor and histone deacetylase complex (corepressor/HDAC), displacing the activator and holoenzyme components. (E) Deacetylation of histones helps in chromatin condensation and transcription repression.

possess histone acetyltransferase (HAT) activity or to be associated with histone deacetylase (HDAC) (for review Ref. *43*). Although these factors have been shown to be involved in transcription regulation at some level, direct roles for intrinsic HAT or HDAC activities are yet to be established in most cases. The most notable exceptions are yeast GCN5 (*44, 45*) and human CBP (*46*), where the direct involvement of both activities has been demonstrated. Furthermore, whether and how acetylation or deacetylation alter the nucleosome structure is yet to be understood completely. It has been suggested that deacetylation of lysine ε-amino groups could facilitate the interaction between the positively charged N-terminal histone tail and the negatively charged phosphate backbone of DNA. In contrast to this view, however, recent crystal structure study of nucleosomes (*47*) suggests that deacetylation of the histone tail might lead to compaction of the chromatin by favoring inter-nucleosomal interactions.

Methylation of CpG islands has been reported to result in a compact, nuclease-resistant nucleosomal structure (*48*). These nucleosomes migrate as a large nucleoprotein complex in agarose gel. Furthermore, nucleosomes assembled on methylated DNA appear to interact with each other more strongly than nucleosomes on unmethylated DNA (*49*). It is not clear whether binding of MeCP2 and histone deacetylation are involved in this methylation-dependent alteration of chromatin structure. However, binding of MeCP2 to methylated DNA has been reported to result in a loss of histone H1 (*37*) from this DNA. Such replacement of histone H1 with MeCP2 in the methylated CpG clusters may imply the formation of an altered local conformation.

Many observations, however, suggest that the CpG methylation-mediated deacetylation cannot be the only way to regulate the gene expression in eukaryotes. For example, many genes in yeast and fruitflies are regulated by histone acetylation, but there is no detectable methyl-CpG in these organisms (*42*). Furthermore, it has been shown that although the active X-chromosome of a marsupial species, *Macropus eugenii*, is hyperacetylated relative to its inactive counterpart, no concomitant increase in methylation is observed (*50*). These observations suggest that the global gene regulation by histone acetylation may operate in both methylation-dependent and -independent manners.

Altered chromatin structure at CpG islands and its significance

As discussed in the previous section, methylation of CpG islands, in most cases, insures that the associated gene would be assembled into inactive chromatin, thereby preventing its expression. However, 5′ domains of all housekeeping genes and some tissue-specific genes are within the CpG islands that have high GC content and high frequency of unmethylated CpGs. These unmethylated CpG islands (associated with promoters) are highly endonuclease-sensitive. By employing specific restriction endonuclease digestion, this fraction of chromatin can be isolated (*51*). Biochemical analysis showed that this chromatin fraction differs from the bulk chromatin in three important ways. First, it contains very low amounts of histone H1. Second, histones H3 and H4 in its nucleosome core particles are hyperacetylated. And finally, a significant fraction of its DNA is nucleosome-free. Although it is not clear how these different features are related, overall they are diagnostic of active chromatin. Recent progress in our understanding of histone acetylation and its role in transcription suggests that hyperacetylation of histones in the CpG island-associated chromatin is a reflection of the active state of chromatin (for reference see the previous section). The presence of histone H1 in a relatively low amount is also a feature of active chromatin, as is the absence of higher order chromatin structure in the CpG island chromatin.

The central question regarding the altered (active) structure of the unmethylated CpG island-associated chromatin is that of its origin. Two possibilities suggest themselves. In one case, the DNA sequence itself could give rise to the active conformation. Alternatively, it could form as a result of the transcription event. According to the present data, the DNA sequence alone does not appear to be sufficient. For example, although the promoter of human α globin gene contains CpG islands, it is inactive and stably unmethylated in thyroid tissue. If the active structure were a direct consequence of the primary DNA sequence, then it

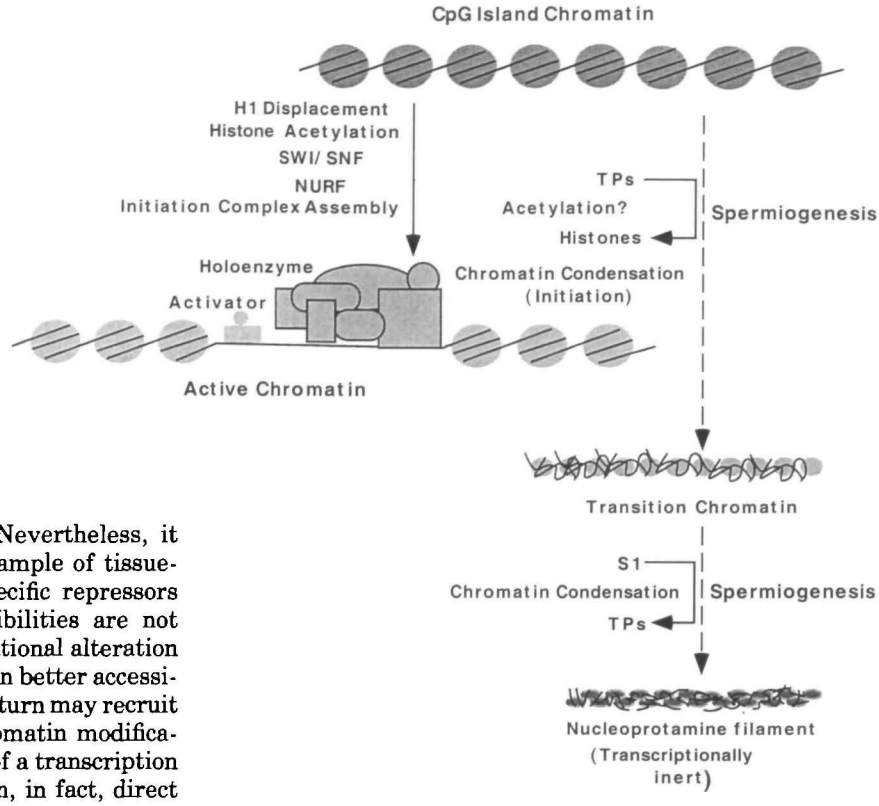

Fig. 2. **Modifications at CpG island-associated chromatin domains.** The CpG island may serve as target for the initiation of histone acetylation and/or recruitment of the different chromatin remodeling factors like SWI/SNF, NURF, and other transcription factors and associated cofactors, which are also facilitated by the displacement of histone H1. In an another physiological event, namely, mammalian spermiogenesis, the CpG island domains may also serve as the target for initiation of chromatin condensation mediated by the transition protein TP2. Transition proteins (TP1, TP2, and TP4) replace the nucleosomal histones and later are themselves replaced by the protamine S1, leading to a highly condensed and transcriptionally inert nucleoprotamine fiber.

should have been constitutively active. Nevertheless, it should be kept in mind that this is an example of tissue-specific gene expression where tissue-specific repressors may indeed be involved. The two possibilities are not mutually exclusive, and a minor conformational alteration in the region of CpG clusters might result in better accessibility to the transcription factors, which in turn may recruit histone acetyltransferases to initiate chromatin modification. Recent reports suggest that binding of a transcription activator and its interaction with HAT can, in fact, direct the HAT recruitment (*52*). Acetylation of histones also seems to be able to facilitate NURF (nucleosome remodeling factor) function (*53*) (see Fig. 2). However, these observations and speculations are yet to be confirmed.

CpG islands may have a role in chromatin organization during spermatogenesis

CpG islands may also be necessary for chromatin organization in different cellular process such as spermatogenesis. Extensive changes in chromatin structure occur during the differentiation of a stem cell into mature spermatozoa. In the spermiogenesis phase (development of sperm after meiosis), nucleosomal chromatin structure is converted into a fiber structure and ultimately into a highly condensed nucleoprotamine filament. A set of transition proteins, namely, TP1, TP2, and TP4, appear during stages 12-15 of spermiogenesis (*54, 55*). These transition proteins replace the nucleosomal histones, package the DNA into transcriptionally inert units, and are themselves replaced finally by the protamine S1, which is the only basic protein present in the final mature epididymal spermatozoa (*56*) (Fig. 2). TP2, being a zinc metalloprotein (*57, 58*), preferentially interacts with GC-rich sequences and condenses the DNA in a zinc-dependent manner (*59*). Recently we have shown that TP2 binds to a human CpG island sequence. We have further demonstrated that TP2 cannot bind to the methylated CpG island sequence (*60*). Our observations suggest that TP2 may initiate condensation using the unmethylated CpG islands as the target sequence, and since CpG islands are present in the promoter domains of several genes, binding of TP2 may also result in the repression of transcriptional activity. Our preliminary results suggest that TP2 can indeed repress transcription *in vitro* (Kundu and Rao, unpublished observations). The genomic locus of TP2 also contains a CpG island whose methylation status varies from tissue to tissue (*61*). The significance of the TP2-CpG island in relation to the ability of TP2 to bind to the CpG island sequence needs to be addressed in a future study.

The appearance of transition proteins TP1, TP2, and TP4 during spermiogenesis is unique to mammals. In most other species, there is a direct conversion from nucleosomal type of chromatin to nucleoprotein fiber (*46*). Why only mammals have evolved an intermediate stage during spermiogenesis involving transition proteins has been a long-standing puzzle. In this connection, it is also interesting to note that the CpG islands are far more abundant in mammals (*5*). It remains to be seen if there is any correlation between the evolution of CpG islands and TP2 in mammals.

Conclusion

The role of the CpG island as a sequence motif in chromatin organization and, consequently, in gene regulation is not yet clearly understood. The possible involvement of CpG island chromatin as a target site to initiate the chromatin modification by several chromatin-modulating activities like HAT/HDAC, SWI/SNF complex, NURF complex *etc.* is yet to be established. Nothing is known yet about the mechanism of gene silencing in the unmethylated CpG island promoter. The involvement of some nonhistone proteins in this modification cannot be ruled out. However, CpG methylation-mediated chromatin remodeling (deacetylation) and transcription regulation (repression) seem to be evident. It has not yet been shown that histone deacetylation, *per se*, is involved in this CpG methylation-mediated gene silencing. Some proteins like TP2 can also use the CpG island sequence to reorganize the chromatin under special

physiological circumstances, like spermatogenesis. The role of histone acetylation and CpG island chromatin in chromatin organization and gene regulation during spermatogenesis is yet to be explored. However, growing interest in this exciting field of molecular biology will undoubtedly elucidate most of the questions we have raised and, consequently, clarify the role of this unique sequence motif, the CpG island, in chromatin organization and gene regulation.

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