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CpG methylation, chromatin structure and gene silencing—a three-way connection

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The three-way connection between DNA methylation, gene activity and chromatin structure has been known for almost two decades. Nevertheless, the molecular link between methyl groups on the DNA and the positioning of nucleosomes to form an inactive chromatin configuration was missing. This review discusses recent experimental data that may, for the first time, shed light on this molecular link. MeCP2, which is a known methylcytosine-binding protein, has been shown to possess a transcriptional repressor domain (TRD) that binds the corepressor mSin3A. This corepressor protein constitutes the core of a multiprotein complex that includes histone deacetylases (HDAC1 and HDAC2). Transfection and injection experiments with methylated constructs have revealed that the silenced state of a methylated gene, which is associated with a deacetylated nucleosomal structure, could be relieved by the deacetylase inhibitor, trichostatin A. Thus, methylation plays a pivotal role in establishing and maintaining an inactive state of a gene by rendering the chromatin structure inaccessible to the transcription machinery.

Keywords: histone deacetylation/MeCP2/mSin3A/transcriptional repression

For many years, evidence has accumulated suggesting that CpG methylation in mammalian DNA is involved in gene silencing. First, gene-specific methylation patterns correlate inversely with gene activity (Yeivin and Razin, 1993). Secondly, artificial demethylation of gene sequences results in activation, whereas *in vitro* methylation of promoter sequences represses gene activity (Razin and Cedar, 1991). Over the past two decades, a large body of data has been produced depicting chromatin structure as a major component in determining the potential for gene activity. In addition, heterochromatic regions in the mammalian genome were shown to be associated with high levels of CpG methylation (Razin and Cedar, 1977).

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 can not overcome repression once chromatin is assembled on the methylated template (Kass *et al*., 1997).

These observations clearly indicated that silencing of a gene by methylation involves the generation of a chromatin structure that limits promoter accessibility. However, the molecular link between the methyl groups on the DNA and the modification of chromatin remained obscure.

Histone acetylation and gene activity

The recent discovery of a number of components of the multiprotein complexes that mediate transcriptional repression via deacetylation of the core histones H3 and H4 (Wolffe, 1997), paved the way to the discovery of the molecular link between the methyl groups on the DNA and chromatin modification.

In yeast and *Drosophila*, where methylation of DNA has not been detected, protein factors such as Ume6A in yeast bind to specific silencer sequences and serve to anchor to the DNA a multiprotein transcriptional repression complex that includes Sin3 corepressor and Rpd3 histone deacetylase (Kadosh and Struhl, 1997). This complex is responsible for the deacetylation of lysine residues on histones H3 and H4, resulting in the repression of a number of genes involved in meiosis.

Such repressor complexes have recently been described in mammals as being assembled in response to physiological changes. In this case, the transcriptional repression complex is anchored to specific DNA sequences, such as the E Box-related signals. This anchorage is mediated by the Mad–Max heterodimer, or by heterodimers of unliganded hormone receptors, such as the thyroid hormone receptor and retinoic acid receptor (TR–RXR). When bound to the DNA, these heterodimers recruit the mSin3 corepressor, which is associated with at least eight different polypeptides, including the histone deacetylases HDAC1 and HDAC2 (Pazin and Kadonaga, 1997). The deacetylases remove acetyl moieties from specific lysine residues on histones H3 and H4, uncovering the positively charged lysine residues. The interaction between the positively charged lysines with the DNA presumably restricts nucleosome mobility on the DNA, rendering the promoter inaccessible to the transcription machinery (Ura *et al*., 1997).

From the methyl moiety on the DNA to the deacetylase through the transcriptional repression complex

Recent studies in mammalian systems, where methylation clearly plays a role in gene silencing, indicate that methylation mediates the formation of a multiprotein repression complex that induces changes in histone acetylation. This

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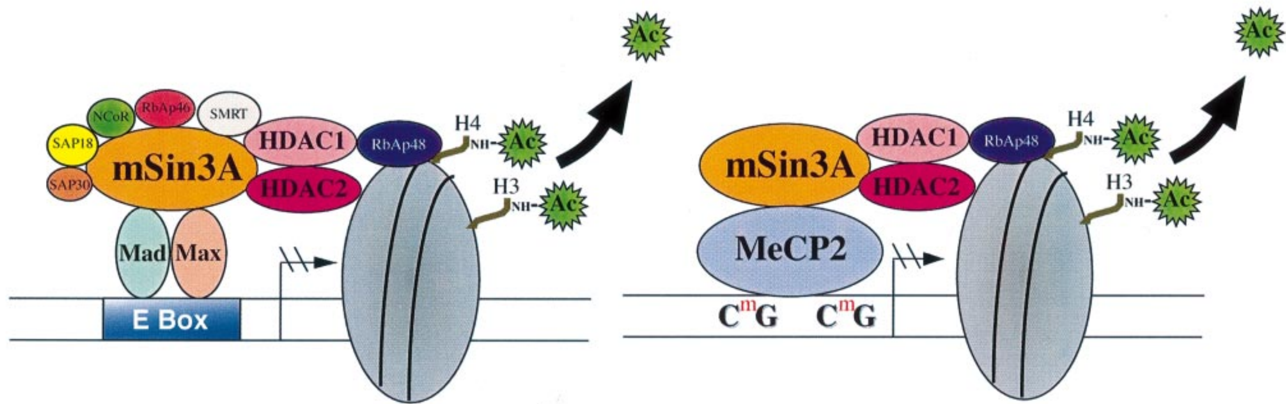

Fig. 1. Transcriptional repression multiprotein complexes. The transient repression complex is assembled in response to changes in physiological conditions. Heterodimers such as Mad–Max or unliganded hormone receptors recognize repressor elements on the DNA such as the E Box. The DNA-bound heterodimer interacts with the corepressor mSin3A which in turn interacts with at least eight different polypeptides. This long list of polypeptides includes SMRT that interacts with mSin3A and HDAC1, RbAp48 that serves as a molecular bridge between HDAC1 and histone H4 (Taunton *et al*., 1996; Verreault *et al*., 1996), and the two deacetylases HDAC1 and HDAC2. The deacetylases remove acetyl moieties from the ε amino acetyl groups (NHAc) of lysine residues on histones H3 and H4. These two histone molecules constitute, together with H2A and H2B, the core particle of the nucleosome. The stable repression complex is formed initially by the interaction of MeCP2 with the DNA at methylated CpG residues (C^mG) which then recruits and interacts with mSin3A to which HDAC1 and HDAC2 are associated. The final repression complex may include all other protein factors as in the transient repression complex, but, as yet, no evidence exists to support it. The stable repression complex is presumably assembled at early stages of embryo development on methylated genes that should be silenced permanently.

complex is based on the methyl-binding protein MeCP2 which has been shown to contain, in addition to its methyl binding domain (MBD), a transcriptional repressor domain (TRD) (Nan *et al*., 1997). This TRD has been shown recently to overlap with a region that interacts directly with the corepressor mSin3A. Immunoprecipitation experiments show that antibodies raised against MeCP2 co-precipitate MeCP2, mSin3A, HDAC1, and HDAC2 (Nan *et al*., 1998).

Experiments using the deacetylase inhibitor trichostatin A relieved the TRD-mediated repression induced by MeCP2 (Nan *et al*., 1998). Although these important experiments revealed the link between MeCP2-induced repression and histone deacetylase activity, they failed to show directly that methyl groups present at the 5′ end of the gene induce the changes in histone acetylation that lead to gene repression. However, this missing link is demonstrated in recently published results obtained by two different sets of experiments. In one experimental system, *in vitro* methylated and unmethylated constructs, based on the hsp70 promoter, were injected into *Xenopus laevis* oocytes. As shown before (Kass *et al*., 1997), following the assembly of chromatin on the transfected DNA, the methylated gene is shut off whereas the gene in the unmethylated construct remains active. The methylation-directed repression could be alleviated, and DNase I hypersensitivity restored, by treatment of the *X.laevis* oocytes with the deacetylase inhibitor trichostatin A. It could, therefore, be concluded that inhibition of histone deacetylase activity allows remodeling of the chromatin to a transcriptional competent state. In the same study MeCP2 was identified in *X.laevis* oocytes and shown to biochemically cofractionate with mSin3A and histone deacetylases. Moreover, antibodies against MeCP2 co-immunoprecipitated MeCP2, mSin3A (but not mSin3B) and deacetylase activity. These observations established a causal link between methylation-dependent silencing and chromatin modification (Jones *et al*., 1998).

The other set of experiments used cells which were stably transfected with *in vitro* methylated and unmethylated *tk* gene constructs. To determine whether methylation can induce changes in histone acetylation of either the unmethylated or methylated gene, the presence of the gene in the highly acetylated nucleosomes was analyzed by PCR. This analysis revealed that the highly acetylated nucleosomal fractions that contain the gene are found preferentially in the cells transfected with the unmethylated construct (Eden *et al*., 1998). This finding allowed the conclusion that methylation influences local histone acetylation. To determine whether the relatively deacetylated histones associated with the methylated *tk* gene cause transcriptional repression directly, the cells transfected with the methylated *tk* gene were treated with trichostatin A. This treatment elevated the nucleosomal acetylation level at the gene-containing sequences and rendered this region DNase I sensitive. Above all, this treatment caused a significant increase in transcription rate from the methylated *tk* gene (Eden *et al*., 1998).

Taken together, the results of the three studies (Eden *et al*., 1998; Jones *et al*., 1998; Nan *et al*., 1998) clearly demonstrate that the methylation of gene sequences induces transcriptional repression through its capacity to bind MeCP2. This methyl-binding protein tethers a repression multiprotein complex that includes the corepressor protein, mSin3A and the histone deacetylases HDAC1 and HDAC2 (Figure 1). The deacetylase activity, which accompanies the MeCP2-bound mSin3A, can render the promoter of the gene inaccessible to transcription factors by deacetylating histone H3 and H4 (Pazin and Kadonaga, 1997). To reverse such a silenced status of a gene,

demethylation must take place and the repression complex be replaced by an activating complex, which carries the capacity of acetylating histones H3 and H4. This modification of core histones should result in a chromatin structure which is accessible to transcription factors (Wolffe, 1997). Alternatively, the methyl-directed repression can be alleviated by a methylation-overriding effect that can be exerted by a strong activation complex which results in effective acetylation of histones H3 and H4. Several examples exist to suggest that such an overriding effect is possible. The γ globin gene can be activated by butyrate, a deacetylase inhibitor (Zitnik *et al*., 1995). The methylation-mediated transcriptional block of HIV has been shown to be overcome by the presence of the tat protein (Bednarik *et al*., 1990), or by the deacetylase inhibitor trichostatin A (Sheridan *et al*., 1997). Other viruses and some liver proteins, such as PEPCK and vitellogenin, were found to be highly methylated, yet transcriptionally active (Cedar and Razin, 1990).

It should be emphasized that the transcriptional repression complex model does not exclude other mechanisms by which CpG methylation may affect gene expression. Non-histone components of chromatin and transcription factors that bind to the DNA may be sensitive to methylation at the promoter or other regions that participate in the control of the gene (Boyes and Bird, 1992; Levine *et al*., 1992).

Histone H1 is known to play a role in sealing the nucleosome, thereby stabilizing a higher order of chromatin structure (Thoma *et al*., 1979). Histone H1 is also known to be abundant in 5-methylcytosine-rich nucleosomes (Ball *et al*., 1983), absent in unmethylated CpG islands (Tazi and Bird, 1990), and to inhibit *in vitro* transcription (Wolffe, 1989; Croston *et al*., 1991). The extent of this inhibition was also found to depend on the density of methyl groups in the promoter region (Levine *et al*., 1993; Johnson *et al*., 1995). In this regard, it should be mentioned that whereas linker histone binding causes a reduction in nucleosome mobility and a repression of transcription, these effects are independent of core histone acetylation. These observations indicate that core histone acetylation does not prevent linker histone binding and concomitant transcriptional repression (Ura *et al*., 1997).

Stable inheritance of chromatin structure

How cell-specific nucleosomal patterns are inherited during many cycles of DNA replication remains an unresolved problem. DNA methylation patterns were shown to be clonally inherited almost two decades ago (Pollack *et al*., 1980; Wigler, 1981). Since then, the guidance of nucleosomal positioning by the stably inherited methylation patterns has been considered to be an attractive possibility. However, this hypothesis has not been supported by experimental data as yet. The model described here, which establishes a causal link between DNA methylation and deacetylation of core histones, may also shed light on the mechanism by which chromatin structure is maintained through many cell cycles. It has recently been shown that newly assembled nucleosomes are acetylated (Sobel *et al*., 1995; Kuo *et al*., 1996); it is therefore possible that the default state of chromatin structure is based on acetylated nucleosomes. Since the maintenance methyltransferase methylates the nascent DNA strand shortly after replication within the replication fork (Gruenbaum *et al*., 1983), it is possible that MeCP2 binds to the DNA promptly after methylation, thereby recruiting deacetylases as described above. Consequently, inactive regions covered by deacetylated nucleosomes are generated. This newly assembled chromatin must be identical to the parental chromatin structure.

If that is indeed the case, the methylation pattern of the mammalian genome, which is established during development and faithfully maintained through consecutive cell divisions (Razin and Shemer, 1995), could provide the template for the nucleosomal structure of chromatin. According to this scheme, active undermethylated genes will be wrapped around highly acetylated nucleosomes whereas methylated genes will be recognized by MeCP2, the multiprotein repressor complex formed, and histones H3 and H4 deacetylated. Strong support for this idea can be found in the inactive X-chromosome of eutherian female cells. This chromosome is heavily methylated and the nucleosomal histones are strikingly deacetylated (Jeppesen and Turner, 1993).

It is, therefore, possible that the silenced state of the vast majority of the mammalian genomic sequences is established and maintained by a simple mechanism by which the clonally inherited methylation pattern of the genome determines the structure of chromatin by inducing histone deacetylation.

Conclusions

Recent studies clearly demonstrate that CpG methylation induces histone deacetylation, chromatin remodeling and gene silencing through a transcription repressor complex that includes the histone deacetylases (HDAC1 and HDAC2) and is formed around mSin3A. This complex is assembled by interaction of mSin3A with the methyl-binding protein MeCP2 (Figure 1).

The fact that methylation patterns are established in the early embryo and faithfully maintained thereafter, in combination with the recent discovery that methylation determines the status of chromatin acetylation, suggest that the maintenance of chromatin structure through many cell generations is achieved by using, as a template, the clonally inherited methylation patterns. This scheme suggests that the methylation-associated repression is stable and can be alleviated either by demethylation of the DNA or a strong activator that can override the methylation effect.

The stable nature of the methylation-dependent repression suggests that this type of repression is used as a global silencing device to shut off all sequences in the genome, with the exception of housekeeping genes and tissue- and stage-specific genes that are destined to be expressed at the right time and in the right place.

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