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DNA Methylation and Histone Deacetylation in the Control of Gene Expression: Basic Biochemistry to Human Development and Disease

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DNA methylation is a major determinant in the epigenetic silencing of genes. The mechanisms underlying the targeting of DNA methylation and the subsequent repression of transcription are relevant to human development and disease, as well as for attempts at somatic gene therapy. The success of transgenic technologies in plants and animals is also compromised by DNA methylation-dependent silencing pathways. Recent biochemical experiments provide a mechanistic foundation for understanding the influence of DNA methylation on transcription. The DNA methyltransferase Dnmt1, and several methyl-CpG binding proteins, MeCP2, MBD2, and MBD3, all associate with histone deacetylase. These observations firmly connect DNA methylation with chromatin modifications. They also provide new pathways for the potential targeting of DNA methylation to repressive chromatin as well as the assembly of repressive chromatin on methylated DNA. Here we discuss the implications of the methylation–acetylation connection for human cancers and the developmental syndromes Fragile X and Rett, which involve a mistargeting of DNA methylation-dependent repression.

Rett syndrome Fragile X syndrome Methyl-CpG binding proteins DNA methyltransferase Histone deacetylase Chromatin remodeling $p16^{INK4}$ 5-aza 2′deoxycytidine

IN considering the relationships between DNA methylation and chromatin, it is useful to recognize that various compartments exist in the genome with respect to methylation status. Chromosomes and chromatin are also compartmentalized with respect to histone modifications. DNA methylation occurs throughout the genome but is enriched in transcriptionally inactive heterochromatin (18,74). In contrast, hypomethylated sequences are enriched in the transcriptionally active fraction of the genome (69,70,100). Histone hyperacetylation is associated with transcriptionally active (or potentially active) chromatin chromosomal compartments (40,100), whereas histone hypoacetylation is associated with the transcriptionally inactive or heterochromatin compartment (50). These observations immediately suggest a reciprocal relationship between DNA methylation and histone acetylation, such that deacetylated histones accumulate on hypermethylated DNA.

In vertebrates, the genomes of somatic cells are globally methylated with the exception of CpG islands (12). These CpG islands represent GC-rich regions of DNA about 1 kb in length, which include the promoters of more than 60% of human RNA polymerase II transcribed genes (3). Normally CpG islands are not methylated and the chromatin of CpG islands is enriched in hyperacetylated histones and deficient in histone H1 (100). Histone hyperacetylation and H1 deficiency are characteristics of active chromatin (59,104). Methylation of CpG islands is associated with the selective silencing of genes on the inactive X-chromosome (88), genes silenced by

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genomic imprinting (75,86), and the repression of tumor suppressor genes in various cancers (43,89). Both methylated and unmethylated DNA are assembled into nucleosomes (27,77), but these nucleosomes may contain differentially modified histones.

CHROMATIN STRUCTURE AND DNA METHYLATION

The key structural element of chromatin is the nucleosome core. Each nucleosome core contains two molecules of each of the four core histones: H2A, H2B, H3, and H4, around which is wrapped 146 bp of DNA (63). Each core histone has two domains: a histone fold domain, which is involved in histone–histone interactions and in wrapping DNA in nucleosomes, and an amino-terminal tail domain that lies on the outside of the nucleosome, where it can interact with other regulatory proteins and with DNA (6). The amino-terminal tail domains are lysine rich and are targets for acetylation. Acetylation greatly reduces the affinity of the histone H4 tail for DNA (46). The physical consequences for nucleosomal integrity of acetylating all of the histone tails in the absence of any other proteins are relatively minor [reviewed in (120)]. However, there is a modest reduction in the wrapping of DNA around the histone octamer and nucleosomes pack together less efficiently in arrays (9,78,103). More dramatic transitions are revealed through the use of other DNA binding proteins as probes of nucleosomal integrity. Transcription factor TFIIIA does not bind efficiently to a 5S rRNA gene within a nucleosome if the core histones are not acetylated, but it does bind following acetylation of the histones (59,105). Likewise, Gal4 binding to nucleosomal DNA is facilitated by acetylation of histone H4 (108). These observations demonstrate that for some transcription factors, acetylation of the amino-terminal tails substantially weakens the constraints on DNA imposed by the core histones. Other in vivo events or forces might further destabilize acetylated chromatin. Histone acetylation provides a molecular mechanism by which DNA can be rendered generally accessible to transacting factors while still maintaining a nucleosomal architecture. Hyperacetylated histones are found on the hypomethylated CpG islands (100) and on chromosomal domains that are preferentially accessible to nuclease (39).

A role for specialized chromatin structures in mediating transcriptional silencing by methylated DNA has been suggested by several investigators. High levels of methyl-CpG correlate with transcriptional inactivity and nuclease resistance in endogenous chromosomes (5). Methylated DNA transfected into mammalian cells is also assembled into a nuclease-resistant structure containing unusual nucleosomal particles (4,57). These unusual nucleosomes migrate as large nucleoprotein complexes on agarose gels. These complexes are held together by higher order protein–DNA interactions despite the presence of abundant micrococcal nuclease cleavage points within the DNA. Individual nucleosomes assembled on methylated DNA appear to interact together more stably than on unmethylated templates (57). The inclusion of methyl-CpG binding proteins and their associated proteins provides a possible explanation for the assembly of a distinct chromatin structure on methylated DNA (52,71,73).

The accessibility of chromatin to nucleases could also be affected directly by the stability with which the histones interact with DNA within the nucleosome. DNA methylation does not influence the association of core histones with the vast majority of DNA sequences in the genome (26,27,77). However, for certain specific sequences, such as those found in the Fragile X mental retardation gene 1 promoter, methylation of CpG dinucleotides can alter the positioning of histone–DNA contacts and the affinity with which these histones bind to DNA (33).

The exact chromatin structure found in vivo can also be a consequence of gene activity. Linker histones, such as H1, are relatively deficient on the transcribed region of genes (53). So it is not surprising that transcriptionally inactive chromatin containing methyl-CpG should show an increase in the abundance of histone H1, whereas DNA sequences lacking methyl-CpG are deficient in H1 (7,100). In vitro studies indicate that histone H1 can interact preferentially with methylated naked DNA under some conditions but not others (44,61,68). Importantly, there is no measurable preference for the assembly of H1 into a nucleosomal architecture containing methylated DNA (22,23,77). In *Ascobolus immersus* elimination of histone H1 is without effect on methylation-dependent gene silencing (8). Moreover, recent in vivo studies indicate that rather than functioning as a general transcriptional repressor, histone H1 is highly specific with respect to the genes whose activity it regulates (14,97). It seems probable that the major differences between chromatin assembled on methylated versus unmethylated DNA will be determined by the inclusion of methylation-specific DNA binding proteins and their associated corepressor complexes.

The assembly of higher order chromatin structures relies on interactions between adjacent nucleosome cores (28). The final chromatin fiber is an irregular structure that can contain both histones and nonhistone proteins. The chromatin fiber is partitioned into

topologically separated loop domains (11). Such loop domains have boundaries defined by attachment to a scaffold or matrix (32,81). Scaffold or matrix attachment regions (SARS/MARS) associate with specific types of proteins including some that selectively recognize methylated DNA like MeCP2 (19,116). DNA methylation may therefore influence the structure and function of chromosomes at the loop level as well as through the more local modification of chromatin structure.

CHROMATIN FUNCTION AND DNA METHYLATION

There are features of transcriptional repression dependent on methylated DNA that may be explained by methylation-specific repressors operating more effectively within a chromatin environment. Transcriptional repression is strongly related to the density of DNA methylation (15,48,55). There is a nonlinear relationship between the lack of repression observed at low densities of methyl-CpG and repression at higher densities. These results led to the demonstration that local domains of high methyl-CpG density could confer transcriptional repression on unmethylated promoters in *cis* (54). Chromatin assembly itself might promote this "action at a distance" by juxtaposing co-repressor complexes recruited by methyl-CpG binding proteins with the regulatory elements under control through the compaction of intervening DNA.

Early experiments using the microinjection of templates into the nuclei of mammalian cells suggested that the prior assembly of methylated, but not unmethylated, DNA into chromatin represses transcription (20). The importance of a nucleosomal infrastructure for transcriptional repression dependent on DNA methylation was reinforced by the observation that immediately after injection into *Xenopus* oocyte nuclei, methylated and unmethylated templates both have equivalent activity (55) (Fig. 1). However, as chromatin is assembled, the methylated DNA is repressed with the loss of DNase I hypersensitivity and the loss of engaged RNA polymerase. The requirement for nucleosomes to exert efficient repression can be explained in several ways. Methyl-CpG binding proteins might recruit a co-repressor complex that directs the modification of the chromatin template into a more stable and transcriptionally inert state. Methyl-CpG binding proteins might also bind more efficiently to nucleosomal rather than to naked DNA. Any cooperative interactions between molecules could propagate their association along the nucleosomal array even into unmethylated DNA segments. This latter mechanism is analogous to the nucleation of heterochromatin assembly at the yeast telomeres by the DNA binding protein RAP1, which then recruits the repressors SIR3p and SIR4p that organize chromatin into a repressive structure (35,41). All of these potential mechanisms could individually or together contribute to the assembly of a repressive chromatin domain.

If methylated DNA directs the assembly of a specialized repressive chromatin structure, it might be anticipated that the transcriptional machinery will have less access to such a structure than the orthodox chromatin assembled on unmethylated promoters and genes. Activators such as Gal4-VP16 can normally penetrate a preassembled chromatin template to activate transcription, even in the presence of histone H1 (1,58). However, once chromatin has been assembled on methylated DNA, Gal4-VP16 can no longer gain access to its binding sites and activate transcription (55) (Fig. 1). This suggests that the specialized features of chromatin assembly on methylated DNA provide a molecular lock to silence the transcription process permanently (93). This capacity of DNA methylation to strengthen transcriptional silencing in a chromatin context could be an important contributor to the separation of the genome into active and inactive compartments in a differentiated cell. This hypothesis is strengthened by the observation that regulatory mechanisms exist within chromosomes to prevent the silencing of genes directed by DNA methylation and histone deacetylation (29,83). Insulator and matrix attachment elements protect against the hypermethylation and hypoacetylation of transgenes and the extinction of their expression (29,83).

METHYL-CpG BINDING PROTEINS, CO-REPRESSORS, AND CHROMATIN

The definition of methyl-CpG binding proteins has led to several recent insights into how DNA methylation modifies chromatin structure. MeCP2 is the archetypical methyl-CpG binding protein defined in vertebrates (62) and is representative of a family of proteins containing similar methyl-CpG binding domains (MBDs) (42,79,113). Within MeCP2, the amino-terminal 85 amino acids comprise the MBD. This domain consists of a wedge-shaped structure with four antiparallel β-strands constituting one face of the wedge of which the two longer β-strands are proposed to interact with the major groove of DNA (113). The MBD of MeCP2 can recognize a single symmetrically methylated CpG either as naked DNA (72) or when exposed in the major groove of nucleosomal DNA (23). MeCP2 can bind stably to methylated DNA in nucleosomes (23) and chromatin (71).

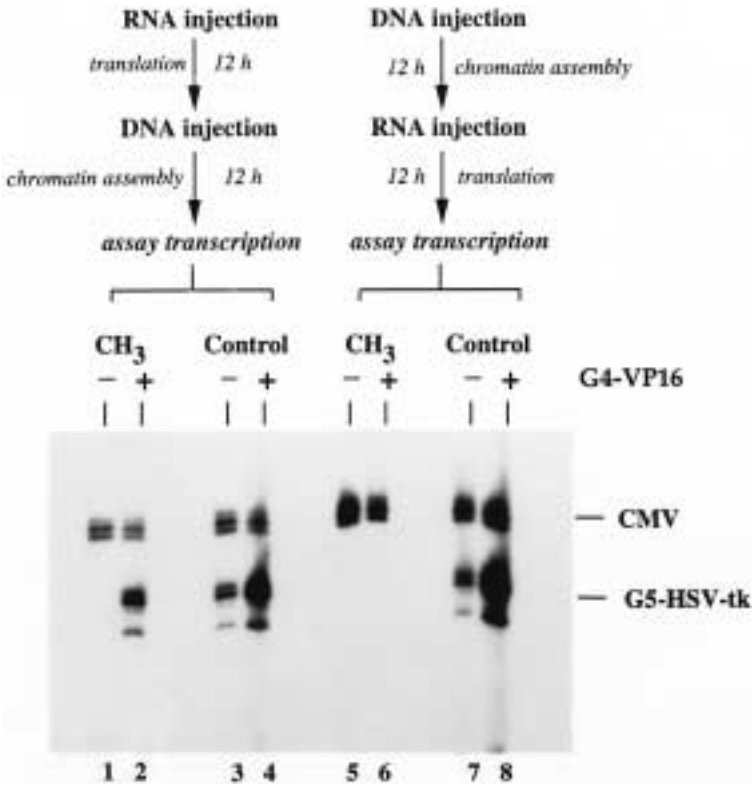

Figure 1. GAL4-VP16 fails to activate transcription from the methylated and chromatinized HSV tk promoter. The effect of overexpressed GAL4-VP16 on transcription was assayed as indicated. RNA encoding GAL4-VP16 was injected either before (lanes 1–4) or after (lanes 5–8) chromatin assembly of methylated (lanes 1, 2, 5, 6) or unmethylated (lanes 3, 4, 7, 8) plasmid pG5-HSVtk, as indicated. Transcriptional activity was assayed by primer extension as before. Co-injection of pCMVCAT (0.25 ng per oocyte) serves as an internal standard [see (55) for details of a similar experiment].

In vivo MeCP2 is an abundant chromosomal protein that is concentrated in the centromeric heterochromatin of mouse cells (62,74). This localization requires both DNA methylation and the methyl-CpG binding domain of MeCP2 (72,74). The available evidence indicates that MeCP2 has a high selectivity for association with methylated DNA within chromatin and chromosomes. Within the C-terminal portion of MeCP2 is a transcriptional repression domain (TRD). The TRD confers repression when tethered to a Gal4 DNA binding domain. Immunoprecipitation experiments established that the TRD interacts with the SIN3–histone deacetylase complex; an inhibition of histone deacetylation can reverse some of the transcriptional repression conferred by the TRD (52). These observations provided the first compelling evidence for a direct linkage between the covalent modification of DNA by CpG methylation and the targeted deacetylation of the core histones as a major component of transcriptional repression in vertebrate cells (13,119). The methylation–deacetylation connection has been further substantiated by the characterization of other MBDs and the co-repressor complexes within which they function.

Four other MBD proteins have been characterized having substantial similarity to the MBD of MeCP2 (42). Two of these proteins, MBD2 and MBD3, are components of co-repressor complexes that also contain histone deacetylase (76,109). MBD2 is a component of a complex described as MeCP1 (76). Aside from the presence of histone deacetylase and an associated protein RbAp48, relatively little is known about the composition of MeCP1. In contrast, MBD3 is a component of the well-characterized Mi-2 complex (109–111). The Mi-2 complex represents the active biochemically defined entity within a crude coimmunoprecipitated fraction known as NuRD or NRD (123–125). The Mi-2 complex consists of six polypeptides: MBD3, which is a methyl-CpG binding protein; the histone binding protein RbAp48; histone deacetylase; a 66-kDa GATA zinc finger protein; a DNA binding protein Mta-1-like; and the Mi-2 nucleosomal ATPase (109,111). These diverse polypeptides have an interesting set of properties that, taken together as a complex, provide significant insight into how DNA methylation can be associated with histone deacetylation (Fig. 2). The presence of the Mta-1-like and MBD3 proteins will stabilize the

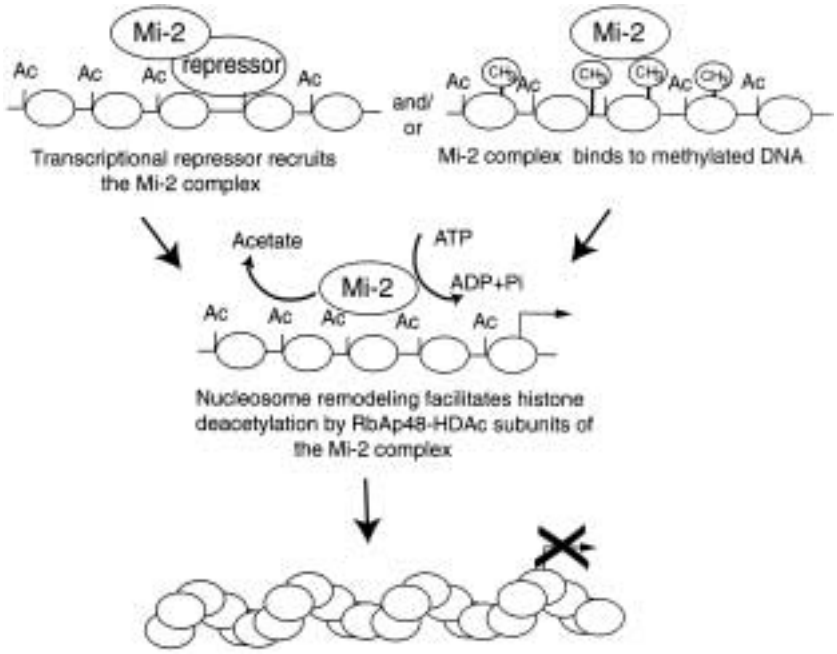

Figure 2. The Mi-2 complex can be targeted by DNA bound repressors, or by DNA methylation, or by the two in combinantion. Once recruited the Mi-2 complex uses the energy of ATP hydrolysis to disrupt chromatin and facilitate histone deacetylation.

interaction of the Mi-2 deacetylase complex with methylated nucleosomal DNA. This will allow the Mi-2 complex to become stably embedded within the chromatin infrastructure that it is both remodeling and deacetylating.

The Mi-2 complex components are conserved to the invertebrates lacking DNA methylation such as *Drosophila melangaster* and *Caenorhadbditis elegans*. The *Drosophila* Mi-2 homolog is targeted by the Hunchback transcriptional repressor involved in embryonic patterning (56). In human cells the E7 transcriptional repressor of human papillomavirus targets Mi-2, indicating that other regulatory pathways aside from DNA methylation contribute to gene control (17). DNA methylation may serve an auxiliary role in these events, which is to amplify and stabilize a state of repression targeted by other proteins. The *Xenopus* Mta-1-like protein selectively interacts with methylated DNA (109) yet also has homologs in *C. elegans* that have key roles in establishing the body plan during embryogenesis (95). *Drosophila* also contains a protein homologous in Mta-1-like and a MBD homolog remarkably similar to MBD2 and 3 (109). Although the DNA binding capacity of these proteins remains to be determined, the *Drosophila* MBD protein binds methylated DNA (Ballestar and Wolffe, unpublished observations). Although *Drosophila* lacks methylation, if the genome is artifically methylated then developmental phenotypes occur, leading eventually to death (65). The retention of such proteins in *Drosophila* and *C. elegans* leads to the suggestion that these organisms with small genomes, short life times, and limited cell division cycles to reproductive competence lost DNA methylation as a regulatory pathway rather than never acquiring it. This is most probably because they do not need to maintain a high degree of epigenetic memory (87).

The enzymatic properties of the Mi-2 complex offer some insight into how chromatin remodeling is essential for histone deacetylation. The catalytic histone deacetylase subunit itself, found in the Mi-2 complex or in association with SIN3, will deacetylate free histone but not nucleosomal histone. A key element in understanding this aspect of chromatin biology is in the properties of another subunit of the Mi-2 and SIN3 complexes RbAp48. RbAp48 is a WD-40 repeat protein that is predicted to form a β-propeller structure similar to that of β-transducin (96,114). The seven-bladed structure provides ample possibilities for interactions with multiple proteins. RbAp48 was originally characterized as a Rb-binding protein (84,85), which cofractionates with HDAC1 (99). Subsequent work has established that RbAp48 and the related protein RbAp46 interact with core histones H2A and H4 (106,107). The capacity of RbAp48 to associate with core histones provides a means of tethering histone deacetylase next to the histone tails that are deacetylated. RbAp48 also fractionates with the *Xenopus* HAT1 histone acetyltrans-

ferase (49). Earlier work had shown that the mixing of the mammalian histone acetyltransferase subunit Hat1p with the related protein RbAp46 greatly stimulates acetyltransferase activity (107). RbAp48 is also found associated with the chromatin assembly factor CAF-1 (107). However, CAF-1 lacking RbAp48 will still associate with histones, whereas Hat1p lacking RbAp46 does not show such a stable association. It has therefore been proposed that the sole function of the RbAp in CAF-1 is to attract the RPD3 histone deacetylase to sites of newly synthesized histone deposition (107).

The importance of the connections between histone deacetylase and histone deposition is that the catalytic subunit does not target free histones efficiently in vitro and, although RbAp48 might facilitate that targeting, the RbAp48-HDAC complex does not interact effectively with nucleosomal histones. Thus, a subnucleosomal particle such as that which occurs during chromatin assembly, or following the activity of SWI2/SNF2 family members (37,118), would represent a favored substrate for deacetylation. The structural foundation for this argument lies with the recognition interface between RbAp48 and histone H4 (106). RbAp48 fails to bind to histone H4 if the N-terminal tail is deleted past aa 28; similarly, the assembly of histone H4 into chromatin is severely reduced if N-terminal deletions extend past aa 32 (30). RbAp48 requires aa 28 through 32 in the context of an intact C-terminus for association with H4. Helix 1 of histone H4, comprising aa 31 to 41 relative to the N-terminus, contributes to heterodimerization with H3 (6) and makes contact with nucleosomal DNA (64). Removal of this helix through aa 36 prevents the assembly of H4 into chromatin entirely (30). Thus, it appears that the determinants of chromatin assembly are very similar to those for association with RbAp48.

RbAp48 does not appear capable of gaining access to its interaction site within H4 when assembled into nucleosomes (64,106,107). Other endogenous proteins or chromatin remodeling events may be required to allow access of RPD3-RbAp48 to chromatin in vivo. The ability of RbAp48 to bind to H4 in the absence of H3 (106) indicates that an RPD3-RbAp48 complex may recognize and deacetylate histones during their assembly into chromatin in vivo. An attractive model is that RPD3-RbAp48 may modify histones during nucleosome assembly onto DNA templates (Fig. 3). Successive replacement of the histone acetyltransferase Hat1p by CAF-1 followed by the histone deacetylase, all of which contain RbAp46, RbAp48, or homologs and interact with core histone H4, may facilitate this process (112). The Mi-2 protein is a nucleosomal ATPase of the SWI2/SNF2 family. The connection between chromatin remodeling and histone deacetylation again offers insight into how corepressors might function.

In this context, Mi-2 presumeably has to disrupt the nucleosome to allow access of RbAp48 to the histone-fold domain of histone H4. As discussed above, this domain normally lies sequestered inside

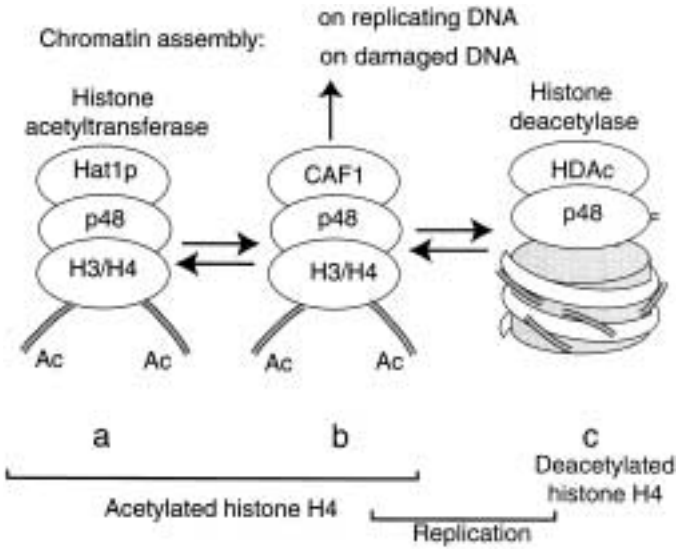

Figure 3. A model for roles of RbAp48-associated proteins during chromatin assembly. Rbp48 is a component of (a) a cytoplasmic histone acetyltransferase with hat1p; (b) a chromatin assembly factor with CAF1; and (c) a histone deacetylase HDAC. Depending on the subunit composition, this protein will be variously equipped to contribute to all these functions in which the modification state of H4, its cellular localization and deposition in a nucleosome, will change as indicated. In all instances shown here the RbAp48–HDAC complex interacts with subnucleosomal particles. The transition between (b) and (c) represents chromatin maturation on nascent DNA following replication.

the coils of nucleosomal DNA. RbAp48 interacts with histone deacetylase directly and enhances enzymatic activity presumably by tethering the deacetylase next to the target site for deacetylation at the N-terminal tail of histone H4. The Mi-2/NuRD deacetylase complex resolves a paradox: the histone deacetylase catalytic subunit will modify free histone with low efficiency, but is without effect on nucleosomal histone. In the presence of ATP, the Mi-2 nucleosomal ATPase facilitates deacetylation of nucleosomal histone (102). This is presumably because the SWI/SNF nucleosomal ATPase will destabilize histone–DNA interactions to allow RbAp48 to gain access to histone H4 (Fig. 4).

The biochemical characterization a chromatin remodeling and histone deacetylase complex that contains methyl-CpG binding proteins provides mechanisms for DNA methylation to contribute to both the global repression of transcriptional noise and the targeted repression of genes. Like the histones, DNA methylation is not causal for specific gene control but greatly amplifies the range of transcriptional control that might be achieved. This amplification may follow from two phenomena: first, DNA methylation may encourage promoters that are destined for repression to become even more stably silenced than they would be by the association of chromatin with unmethylated DNA. Second, the more efficient exclusion of regulatory transcriptional activators and the basal transcriptional machinery from the vast bulk of nonproductive sites in chromatin as a consequence of DNA methylation will allow these factors to better focus their attention on the small fraction of potentially active promoters within the unmethylated CpG islands.

The connections between DNA methylation and histone deacetylation have been strengthened by the observation that the DNA methyltransferase Dnmt1 is associated with a histone deacetylase activity [(31), K. Robertson and A. P. Wolffe, unpublished].

DNA METHYLATION AND CHROMATIN MODIFICATION AS A MOLECULAR MECHANISM TO MAINTAIN A STATE OF EPIGENETICALLY DETERMINED GENE ACTIVITY THROUGH REPLICATION

The DNA methyltransferase Dnmt1 maintains the methyl-CpG content of both daughter DNA duplexes following replication (45). Methyltransferase localizes to the chromosomal replication complex and maintenance methylation takes place less than 1 min following replication (34,60). By contrast, chromatin assembly takes 10–20 min in a mammalian tissue culture cell (25). Histone deposition occurs in stages, and it is not until a complete histone octamer is assembled with DNA that histone H1 is stably sequestered (122). Comparable limitations might restrict the stable association of methylation-specific repressors. This would account for the lag time before methylated DNA is repressed following injection as a naked template into the nuclei of mammalian tissue culture cells or *Xenopus* oocytes (20,55).

A significant feature of transcriptional repression on methylated DNA is that it is not only time dependent but also potentially dominant (55). Thus, at early times when chromatin assembly is incomplete, the transcriptional machinery has the potential to associate with methylated regulatory DNA. As chromatin structure matures the basal transcriptional machinery is potentially erased from the template. This provides a general mechanism for the global silencing of transcription dependent only on DNA methylation state. The presence of acetylated histones would facilitate transcription factor access to nascent chromatin (92,

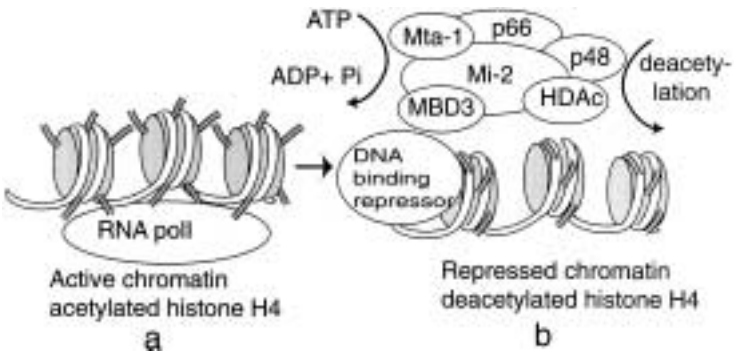

Figure 4. RbAp48 and HDAC are also components of the nucleosomal ATPase Mi-2 complex. (a) Active chromatin has transcriptionally engaged RNA polymerase II and the basal transcriptional machinery. Under these conditions the core histones in nucleosomes are hyperacetylated. (b) A DNA binding transcriptional repression recruits the Mi-2 complex. The nucleosomal ATPase disrupts the acetylated nucleosomal infrastructure to facilitate RbAp48 access to H4 and histone deacetylation. As a consequence transcription is repressed.

105). The subsequent recruitment of histone deacetylase by MeCP2 and other MBD proteins would create a dominant repressive chromatin environment (55). Although a strong activator such as Gal4-VP16 cannot function when chromatin is assembled on methylated DNA before exposure to the activator, the methylation-dependent transcriptional silencing does not occur in the presence of GAL-14-VP16 during the chromatin assembly. Therefore, under certain circumstances, regulatory nucleoprotein complexes might be assembled that resist this powerful silencing mechanism. Such a mechanism has been suggested to be dependent on SP1 sites in the promoter of a housekeeping gene in the mouse (adenine phosphoribosyltransferase) that is maintained in a methylation-free state (66). For example, if the components of regulatory complexes could bind to DNA immediately after replication with reasonable efficiency and before DNA methyltransferase can begin to modify the template, they might prevent DNA methylation around their binding sites. These sequences might then become progressively demethylated and eventually resist transcriptional repression. This would provide a mechanism for the demethylation of regulatory DNA in particular differentiated cell lines. Evidence in support of this hypothesis has recently been obtained for the demethylation of transcriptionally active promoters during the rapid replication cycles of *Xenopus* embryogenesis (67).

Other mechanisms might contribute to the maintenance of transcriptional repression through DNA synthesis. The assembly of a specialized chromatin structure on methylated DNA will result in the presence of additional proteins (e.g., MeCP2) and histone modifications (e.g., histone deacetylation) that could be maintained in daughter chromatids. Nucleosomes segregate dispersively in small groups to daughter DNA molecules at the replication fork (94). Particular modified histones and repressors such as MeCP2 would be anticipated to segregate within the nucleosomal context (82). These proteins could therefore provide at least 50% of the chromatin proteins necessary to restrict transcription. Their continued presence on DNA could help to reestablish transcriptional repression on both daughter chromatids. Therefore, demethylation alone might be insufficient to relieve transcriptional repression until successive cell divisions eventually unravel the repressive chromatin structure.

Although we focus on molecular mechanisms that might influence DNA methylation and gene expression in dividing cells, DNA demethylation is also important in nondividing terminally differentiated cells. Under these circumstances demethylation at particular promoters must occur in the absence of replication (90,98). Presumably mechanisms must also exist to destabilize any repressive chromatin structure associated with methylated DNA in order to allow the demethylation machinery access to the template.

DNA METHYLATION, HISTONE DEACETYLATION, AND HUMAN DEVELOPMENT AND DISEASE

Alterations in the controls of DNA methylation and histone deacetylation have other profound roles in human disease. A common form of inherited mental retardation in male children is connected with repression of the *Fragile X Mental Retardation gene 1 (FMR1)* gene. The expansion and methylation of a CGG trinucleotide repeat in the 5′ regulatory region leads to transcriptional inactivation of the *FMR1* gene. The *FMR1* gene can be reactivated using inhibitors of DNA methyltransferase, which leads to the reaccumulation of acetylated histones on the promoter (24). The CGG repeat is also the site of preferential chromosomal breakage. This may reflect alterations in chromosomal organization that are a consequence of repeat-induced silencing (47). Alterations in the expression of individual imprinted loci lead to developmental abnormalities. In humans, such abnormalities include Beckwith-Wiedemann, Angelman, and Prader-Willi syndromes (16,01).

While cancer cells often have reduced levels of 5-methylcytosine in the genome relative to normal tissues, many tumor suppressor genes are silenced in tumor cells by de novo methylation of their promoter regions (10,51). This aberrant methylation is suggested to have a causal role at the preneoplastic stage of cancer progression. Like the silenced *FMR1* gene, inhibitors of DNA methyltransferase and histone deacetylase will reactivate previously silent genes in cancer cells (21). Deregulation of genomic imprinting can also play a role in cancer development, as exemplified by loss of imprinting of the *IFG2* gene in Wilms' tumor (121). The epigenetic silencing of the *FMR1* and tumor-suppressor genes by DNA methylation offers the exciting clinical prospect of interfering with both the molecular pathways that target methylation per se, and those that mediate transcriptional silencing dependent on the recognition of DNA methylation (21,24).

The DNA methyltransferases themselves are now connected to a developmental syndrome in humans. Immunodeficiency in association with centromere instability of chromosomes 1, 9, and 16, and facial anomalies (ICF syndrome) is a rare autosomal recessive disorder. Afflicted individuals show a marked hypomethylation of their DNA with undermethyla-

tion of satellite II DNA being prevalent (91). Mutations in one of the three characterized DNA methyltransferases in humans, Dnmt3B, appears to be responsible for this disorder (38,80). In addition, recent observations indicate that the major form of genetically based mental retardation in female children, Rett syndrome, is associated with point mutations in the gene encoding MeCP2 (2,36,115,117). This observation firmly connects the function of MeCP2 to neural development and provides further emphasis and impetus for resolving the molecular details of MeCP2 function.

PERSPECTIVE

DNA methylation and histone deacetylation have major significance as epigenetic modifications in gene control. We have yet to resolve how both are targeted in normal development and in pathological situations. Understanding the molecular mechanisms involved is a challenge for the future, but due to the enzymology already associated with these processes, DNA methylation and histone deacetylation also present a wonderful opportunity for therapeutic intervention.

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