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The Yin and Yang of Histone Marks in Transcription

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Abstract

Nucleosomes wrap DNA and impede access for the machinery of transcription. The core histones that constitute nucleosomes are subject to a diversity of posttranslational modifications, or marks, that impact the transcription of genes. Their functions have sometimes been difficult to infer because the enzymes that write and read them are complex, multifunctional proteins. Here, we examine the evidence for the functions of marks and argue that the major marks perform a fairly small number of roles in either promoting transcription or preventing it. Acetylations and phosphorylations on the histone core disrupt histone–DNA contacts and/or destabilize nucleosomes to promote transcription. Ubiquitylations stimulate methylations that provide a scaffold for either the formation of silencing complexes or resistance to those complexes, and carry a memory of the transcriptional state. Tail phosphorylations deconstruct silencing complexes in particular contexts. We speculate that these fairly simple roles form the basis of transcriptional regulation by histone marks.

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1. HISTONE MARKS AND METAPHORS

Mammalian genomes are packaged in nucleosomes that wrap DNA around an octamer of two copies each of the four core histones: H2A, H2B, H3, and H4. The core histones are each characterized by a histone fold domain that enables them to dimerize in specific antiparallel pairs—H3 with H4, and H2A with H2B—that can further assemble by forming four-helix bundles between dimers, leading to a central H3–H4 tetramer flanked by two H2A–H2B dimers (81). Nucleosomes constrain negative DNA supercoils and compact DNA, but it has been argued that their primary function is to suppress transcription (67), necessitating nucleosome mobilization to carry out gene regulation (128). In addition to histone fold domains, core histones have unstructured tails that are subject to the addition and removal of dozens of posttranslational modifications or covalent marks, some with important roles in gene regulation (134). Well-studied marks are found on the highly conserved tails of H3 and H4, which have low turnover compared with H2A–H2B dimers (131). The higher turnover of H2A–H2B dimers may be the reason these histones have a smaller number of marks and their tails are less strictly conserved (83). Other marks are found on the nucleosome core and may directly affect nucleosome stability (61). While the functions of these marks are still imperfectly understood, histone marks can be divided between those that directly affect nucleosome–DNA interactions (primarily acetylation marks) and those that serve as scaffolds to bind effector proteins that modulate the chromatin environment. Chromatin proteins with domains that recognize specific marks can interact with nucleosomes to effect mobilization or compaction.

It has become common to refer to the enzymes that add modifications to histones metaphorically as writers, those that remove them as erasers, and proteins or modules that bind to histone marks as readers. There are, for example, at least 9 writers that trimethylate H3K4 (H3K4me3) in the human genome and have diverse other functions (20), 42 bromodomain reader proteins (31) that represent only one of at least three acetyl-lysine binding modules, and a dozen or so other distinct families of mark-binding modules, each with many individual versions occurring in a variety of multimodule proteins (reviewed in 137). The language of covalent histone modifications therefore seems to resemble less a histone code (126) than a set of Chinese characters, in which many characters may write the same sound, and reading the characters involves recognizing common elements that are repeated in innumerable different combinations, each with its own meaning for chromatin regulation.

Despite this complexity, much progress has been made this century in understanding histone marks, though much remains to be clarified. Histone marks largely mediate two major outcomes—transcription or nontranscription, the yang and yin of chromatin states—as well as a handful of other functions in DNA repair, recombination, and chromosome condensation. Understanding the roles of marks is complicated by the fact that the writers and readers of marks also write and read other stories on other proteins, which often makes it difficult to determine the meaning of the mark, as opposed to the calligraphy brush or the poem it finds itself in.

Here, we focus on mutations of residues that bear marks and other functional assays to give an overview of how these marks regulate mammalian gene expression, with examples from other organisms where they are informative. For example, assessing the functions of histone modifications by mutating specific residues is extremely challenging in mammals because the many histone genes are separately encoded and interspersed with one another over large regions. By contrast, engineering histone mutations into yeast is routine and is feasible in *Drosophila* because the canonical histone genes are all present in a single multicopy repeat cluster. Given the exceptionally high conservation of histones and their modifications, conservation of function between organisms is a reasonable assumption. We confine ourselves to the acetylations, methylations, ubiquitylations,

and phosphorylations that have major roles in regulating transcription and silencing, which are among the best-studied marks, and omit marks with specialized, nontranscriptional roles in DNA repair, recombination, centromere function, and other processes.

2. THE ORIGIN OF MAMMALIAN HISTONE MARKS

Histone marks, including histone acetylation, methylation, ubiquitylation, and phosphorylation, were already present in the last eukaryotic common ancestor (53, 132). Putatively early-diverging eukaryotes, such as *Trichomonas vaginalis*, have a simple promoter architecture, lack heptad repeats in the RNA polymerase II (RNAPII) carboxy-terminal domain (CTD), and differ from other eukaryotes in their general transcription factors, but still mark active genes with H3K27ac and H3K4me3 (122) and have E(z)-like methyltransferases predicted to act on H3K27 (53), while in *Giardia intestinalis*, histone deacetylases (HDACs) and H3K9me3, a silencing mark, are important for encystation and antigenic variation (10). Trypanosomes, also thought to be early-diverging eukaryotes with divergent histone tails, can have as many as 250 different histone marks, including acetylation, methylation, phosphorylation, and others not observed in other organisms (114). Unicellular holozoan animal relatives, such as the filasterean *Capsaspora owczarzaki*, have typical histone marks and a conventional promoter architecture but differ from metazoans in lacking distal enhancers (119), which appear to be an innovation associated with multicellularity. By contrast, in the simple sponge *Amphimedon queenslandica*, enhancer long noncoding RNAs are present and associated with chromatin that is marked by an increased ratio of H3K4me1/H3K4me3 compared with promoter long noncoding RNAs, which are associated with chromatin marked by a decreased H3K4me1/H3K4me3 ratio (33). Similarly, in human cells, H3K4me1 is a predictor of enhancers, while H3K4me3 predicts promoters (107). Approximately 70% of mammalian genes have promoters made up of CpG islands, ~1-kb tracts of unmethylated CpG-rich sequence, to which some chromatin regulators, such as H3K4 methyltransferases, can bind. Unlike some invertebrates, such as *Drosophila melanogaster* and *Caenorhabditis elegans*, mammalian genomes are also subject to DNA methylation in gene bodies, which interacts with histone marks, especially with the elongation mark H3K36me3 and the silencing mark H3K9me3 (110, 111).

Although the origins of these marks are lost in proto-eukaryote history, we find a general evolutionary framework to be helpful in imagining how and why such complexity exists for the two outcomes of transcription or nontranscription. While nucleosomes might have evolved to suppress transcription from viruses or other parasites (67), acetylation marks probably evolved simultaneously to mobilize nucleosomes for transcription. In the ongoing arms race with parasites, methylation marks became scaffolding to silence first transposons and other repetitive elements and then, as eukaryotes became multicellular, developmental programs. As a method to control the inappropriate spread of silencing, cotranscriptional antisilencing methylations evolved, as well as phosphorylations that counteract silencing scaffolds. While this scenario is speculative, we believe it can provide a framework for thinking about how the various histone marks interact in the regulation of transcription.

3. HOW DO ACETYLATION MARKS PROMOTE TRANSCRIPTION?

Since acetylations, methylations, and ubiquitylations all occur on lysine residues, they are mutually exclusive on any particular residue at any one time. Though different methylations can be found on actively transcribed or silenced chromatin, acetylations are largely synonymous with active transcription because of their ability to neutralize the positive charges on lysines, reducing DNA–histone binding and limiting the ability to form hydrogen bonds (142), and thereby destabilizing

histone–DNA interactions and helping to mobilize nucleosomes to promote transcription or other processes that access DNA. In addition to neutralizing charges on histone tails, some acetylations also occur on the nucleosome core, where they may disrupt or evict nucleosomes, and acetylation can interfere with nucleosome-to-nucleosome interactions to open chromatin. These charge-based alterations of histone–DNA interactions are purely chemical and do not require a reader to have their effects. Instead, readers of acetyl marks are involved in recruiting other chromatin proteins to active chromatin.

3.1. Acetylation of Histone Tails

Acetylation of histone tails improves the efficiency with which RNAPII traverses chromatin in vitro, as does removing histone tails entirely (106). Modeling of histone tail conformations indicates that histone tails bind nucleosomal and linker DNA extensively, with longer residence times for H3 and H4 tails than for N- and C-terminal tails of H2A or H2B (99). Tails thus compete with nucleosome-binding proteins for contacts with DNA. Lysine acetylation occurs on all four core histones: H3 (K4, K9, K14, K18, K23, K27, K36, and K56; **Figure 1**), H4 (K5, K8, K12, K16, K20, and K91), H2A (K5 and K9) and H2B (K5, K12, K15, K16, K20, and K120) (91). Turnover of acetylation on histones by lysine acetyltransferases and lysine deacetylases indicates that histone acetylation is an active regulatory mechanism. Though lysine acetyltransferases and lysine deacetylases acetylate or deacetylate both histones and nonhistone proteins (92), they are often referred to as histone acetyltransferases (HATs) and HDACs, respectively, in the context of chromatin. In addition to acetyl groups, they can add and remove longer-chain acyl groups, such as propionyl and crotonyl groups, which have been proposed to have metabolic regulatory functions (79, 113). In HeLa cells, propionylation is nearly as abundant as acetylation, but longer-chain acylations are more than 200-fold less abundant than acetylations (66), and their metabolic functions remain speculative.

In human cells, both HATs and HDACs associate with active genes and correlate with H3 and H4 acetylation levels, RNAPII levels, and gene expression levels (146). Inhibition of either HDACs or the activity of the HAT paralogs p300 and CBP, which dynamically acetylate H3K4me3-containing nucleosomes, inhibits gene expression and reduces RNAPII occupancy, suggesting that acetylation turnover is required for gene expression (19). In a yeast RNAPII mutant, acetylation of promoters does not depend on transcription (26). In zebrafish, H3K27 acetylation also appears to precede transcription (118). Tethering of the catalytic core of p300 (amino acids 1048–1664) to promoters or enhancers of inactive genes in HEK293T cells is sufficient to acetylate H3K27 and to drive their robust expression, while a single amino acid substitution in the catalytic core (D1399Y) abolishes this effect (44). Activation by a tethered p300 core is more robust than activation by a tethered transcription factor. HATs such as p300 are frequently found in multisubunit complexes and can be recruited to promoters by the interaction of these subunits with the acidic

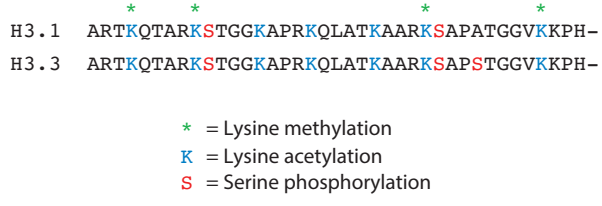

Figure 1

Marks on the H3 tail discussed in this review.

activation domains of transcription factors (6), suggesting that a major role of transcription factors may be to target acetylation to the genes and enhancers they activate. In MEF cells, p300 and CBP are necessary for more than 90% of the acetylation of H3K18 and H3K27, which is prior to and necessary for recruiting RNAPII. These marks are necessary for ligand-induced nuclear receptor-targeted expression of the *Angpt14* gene and appear within 10 min of induction (56). By contrast, the highly similar GCN5 family HATs GCN5 and PCAF (p300/CBP-associated factor) are more than 90% responsible for acetylating H3K9 and appear approximately 30 min after gene induction, coincident with RNAPII elongation, though a GCN5/PCAF double knockout has little effect on gene expression. H3K4me3, H3K36me3, and H3K79me3 appear within 4 h of induction, suggesting the priority of acetylation over these other active marks.

3.2. Destabilizing Nucleosomes

Acetylation of H3K56 occurs on the nucleosome core (**Figure 2**) rather than on the tail, destabilizing the ends of the nucleosomal DNA and promoting transient unwrapping, or DNA breathing (94). In budding yeast, approximately 30% of nucleosomes are acetylated on H3K56, which facilitates deposition of H3–H4 into new nucleosomes during replication (77). H3K56ac destabilizes promoter nucleosomes, and unacetylated H3K56R delays promoter nucleosome disassembly and gene activation and hastens reassembly (148).

By contrast, in human cell lines, H3K56ac is found on only 0.04–0.2% of H3 molecules (125). It colocalizes with p300 and is preferentially localized to transcriptionally active sites, with RNAPII–CTD phosphorylated on S5, characteristic of transcription initiation. Inhibition of RNAPII elongation with α-amanitin has no effect on H3K56ac localization, but inhibition of preinitiation-complex formation by juglone leads to rapid loss of nuclear H3K56ac signal. In mouse neural precursor cells, at the *Folr1* gene that promotes gliogenesis, knockdown of the histone variant isoform H2A.Z.1 strongly reduces H3K56ac but not H3K9ac, H3K27ac, H3K4me3, or H3K36me3.

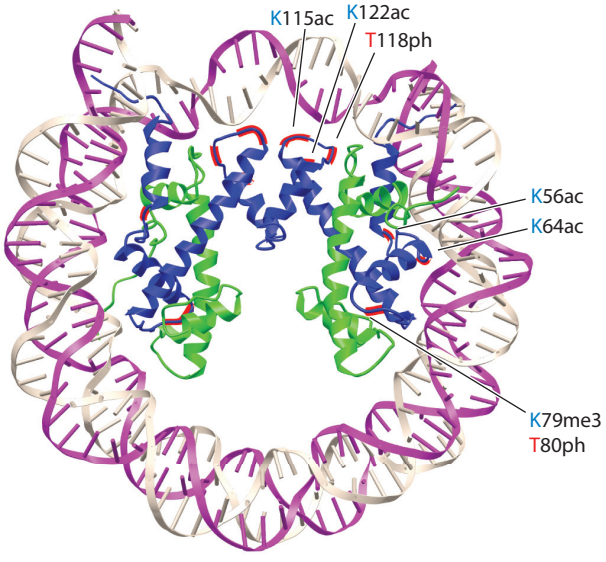

Figure 2

Marks on the nucleosome core. Modified residues are highlighted in red on both H3 molecules (*blue*) and labeled on one molecule. For clarity, H2A and H2B are not shown.

H2A.Z.1 is bound by the chaperone Asf1, suggesting they cooperate to promote incorporation of H3K56ac at the promoter (129).

Why is it important to incorporate destabilized H3K56ac nucleosomes into the promoter? H3K56ac appears to have a role in overcoming polymerase pausing. The SIRT6 HDAC forms a complex with RNAPII, removes acetyls from H3K9ac and H3K56ac, and stabilizes NELF-E to effect promoter-proximal pausing of RNAPII, which typically occurs 30–60 base pairs downstream of the transcription start site (TSS) in the +1 nucleosome (28). Knockout of SIRT6 in embryonic stem cells (ESCs) increases H3K9ac and H3K56ac in promoter-proximal regions and gene bodies; increases binding of the BET (bromodomain and extraterminal domain) protein BRD4, together with MYC; and increases the progression of elongating RNAPII. In HeLa cells, H3K9ac recruits the super elongation complex to overcome polymerase pausing. In *D. melanogaster*, the homolog of p300, dCBP, helps to recruit RNAPII through an interaction with TFIIB and is required to overcome the barrier of the +1 nucleosome, which it acetylates (5), suggesting conservation of the control of polymerase pausing by histone acetylation.

H3K64 is on the lateral surface of the nucleosome near the α1 helix (**Figure 2**) and can also be acetylated (24). H3K64 acetylation is thought to break water-mediated hydrogen bonds to the DNA, destabilizing and disassembling nucleosomes. H3K64ac is found at promoters and enhancers and is acetylated by p300 and CBP. It is enriched on the replacement H3 variant, H3.3, with H3.3K64ac promoting more nucleosome eviction than wild-type H3.3 or H3.3K64R. It is present in mouse elongating spermatids undergoing the transition to protamines. Like H3K56, H3K64 can also be methylated instead of acetylated, which is associated with heterochromatic regions. H3K122ac, acetylated near the dyad axis by p300, is found together with H3K64ac at promoters and enhancers, usually with H3K27ac, but also in a subset of enhancers without H3K27ac (105). In vitro, H3K122ac can stimulate transcription relative to H3K122+, H3K122R, and H3K18ac and increases histone eviction (138). In vivo, it decondenses chromosomes when overexpressed. Thus, these core marks appear to destabilize or evict nucleosomes for transcription or for the protamine transition.

In *Drosophila*, mutations converting H3K56, H3K115, or H3K122 to R, mimicking unacetylated lysine, or to Q, mimicking acetylation, are lethal, except H3K122R (40). Similarly, mutations converting the nearby phosphorylatable residues H3T80 and H3T118 to A, to prevent phosphorylation, or to Q or I, to mimic phosphorylation, are also lethal in embryogenesis (H3K115 and H3T118 mutants), third-instar larvae (H3K122Q), or pharate adults (H3K56 and H3T80 mutants). Thus, all of these core residues (**Figure 2**) or their modifications appear to be essential for fly development.

3.3. Disrupting Internucleosomal Contacts

Acetylation of H4K16 disrupts the interaction of the H4 tail with the acidic patch, a cluster of eight acidic residues on H2A and H2B, of an adjacent nucleosome (59). This interaction is important for chromatin fiber folding in vitro, and deacetylation of H4K16ac by the NAD^+-dependent HDAC SIR2 is necessary for the formation of silent chromatin in budding yeast (52). An H4K16A mutation, which may mimic a lysine neutralized by acetylation, abolishes silencing, while H4K16R, where R may mimic unacetylated lysine, has little effect (97). In *Drosophila*, the Mof acetyltransferase of the dosage compensation complex in males hyperacetylates H4K16, increasing expression from the single X chromosome. An H4K16R mutation is lethal in males at the end of larval development, but morphologically normal females survive to adulthood, though they die prematurely (17). This suggests that acetylation of H4K16 is not required for normal female development but is necessary for dosage compensation in males. An H4K16Q mutation survives to adulthood

in both females and males, while an H4K16A mutation is lethal in both sexes at the end of embryogenesis, suggesting a requirement for H4K16ac at that point in development. In human cells, H4K16 acetylation is carried out largely by the Mof homolog, hMOF or MYST1, which is not necessary for H4K5 or H4K8 acetylation (130).

Deacetylation of histones is necessary in mitosis (108) and meiosis, with hyperacetylated H4K16 leading to reduced CENP-A at the mouse meiotic kinetochore and fewer microtubule–kinetochore attachments (82). Hyperacetylation disrupts pericentric heterochromatin, which might lead indirectly to kinetochore dysfunction (108), but in budding yeast, which lacks pericentric heterochromatin and has only a single Cse4 (CENP-A) nucleosome (32), increased H4K16ac is synthetic lethal in *cse4* mutants. H4K16Q but not H4K16R mutants cause chromosome loss (13), suggesting a direct effect of H4K16ac on kinetochore function. In mouse oocytes, overexpressing H4K16R has little effect on kinetochore attachments, while overexpressing H4K16Q increases detachments (74). H4K16 hyperacetylation increases in aged mouse oocytes, which are subject to kinetochore attachment errors that can be ameliorated by melatonin, which promotes expression of the deacetylase SIRT2. Overexpressing H4K16R can partially rescue kinetochore detachments in aged oocytes, whereas detachments are not rescued by melatonin when H4K16Q is overexpressed, suggesting that attachment to kinetochores requires CENP-A nucleosomes that are deacetylated on H4K16.

3.4. Acetylation Versus Ubiquitylation of H2BK120

In mammals, enhancers and promoters can be predicted by their acetylation marks, independent of the H3K4me1/H3K4me3 ratio, with promoters enriched in H3K9ac, H3K27ac, H3K56ac, and H2AK5ac, while enhancers are enriched in H2BK20ac and H2BK120ac (107). The relative enrichment of H2BK120ac at enhancers may reflect loss of initial p300/CBP-mediated acetylation on H2BK120 at promoters and gene bodies and its subsequent monoubiquitylation following gene induction (36). Ubiquitin is a 76-amino-acid protein that can be attached to lysines by a flexible C terminus. Monoubiquitylation of H2B depends on transcription and is carried out by the ubiquitin-conjugating (E2) and ubiquitin ligase (E3) enzymes RAD6 (UB2A) and BRE1A/B (RNF20/RNF40), respectively, which are recruited to transcribing genes by the polymerase-associated factor complex and the histone chaperone FACT (98). H2BK120ub1, but not H2BK120A, increases the efficiency of FACT-dependent elongation in a reconstituted in vitro system, suggesting that it facilitates the ability of FACT to displace H2A–H2B dimers to overcome the nucleosome barriers to transcription.

3.5. Anchoring to Acetylated Promoters

In addition to a structural role in loosening DNA–histone tail contacts, acetyl-lysine provides binding sites for acetyl-binding protein domains. These reader or effector domains exist in multidomain proteins or protein complexes with other chromatin-binding and enzymatic domains (31) and play a role in localizing and/or regulating these proteins at promoters, likely through multivalent interactions with DNA, nucleosome cores, histone tail modifications, and transcription factors (112). Bromodomains, YEATS domains, and double plant homeodomain fingers are each known to bind acetyl-lysine.

The initial finding that the bromodomains of the HATs PCAF and GCN5 bind acetyl-lysine suggested that this binding could stabilize these proteins at promoters (23). More recently, bromodomains in the remodelers BRM and BRG1 of the BAF complex have been shown not only to bind H3K14ac with moderate specificity but also to bind DNA cooperatively with an AT hook

adjacent to the bromodomain. This DNA binding is largely responsible for the nucleosome affinity of BRG1 in vitro, raising the possibility that the bromodomain may help to displace the H3 tail from binding to the nucleosome to facilitate direct DNA binding of BRM/BRG1.

The bromodomain of the p300 paralog CBP is required for acetylation of H3K56 by CBP but not for acetylation of H3K27. Rather than (or perhaps in addition to) anchoring CBP at acetylated promoters, the bromodomain binds the chaperone Asf1 together with an H3–H4 dimer to acetylate H3K56, and the acetyl group then binds to the CBP bromodomain to release H3K56ac–H4/Asf1 from the catalytic site for subsequent incorporation into nucleosomes (151). Since the bromodomain of p300 is 96% identical to that of CBP, it is likely that it has a similar role in H3K56 acetylation.

The YEATS domain protein Yaf9 preferentially binds H3K27ac (65) and is a component of the yeast SWR1 complex, which deposits H2A.Z at the +1 nucleosome, and of the NuA4 complex, which acetylates H2A.Z (144). Similarly, in mouse ESCs, the YEATS domain of Gas41, a component of the TIP60 and SRCAP complexes, which deposit H2A.Z, binds H3K27ac and is necessary for H2A.Z deposition at bivalent promoters (promoters marked with both H3K4me3 and H3K27me3) (46). The YEATS domain protein AF9 is found in the super elongation complex (35), the DOT1L H3K79 methyltransferase complex, and the polymerase-associated factor complex, all of which are involved in transcriptional elongation, and has a greater preference for crotonylated over acetylated lysines (78), though it is unclear whether this substrate preference is relevant to the recruitment of these elongation complexes to transcribing genes.

In human cells, double plant homeodomain fingers in the HATs MOZ and MORF bind H3 tails unmodified by H3K4me3. MOZ can acetylate H3K9 and H3K14 to reinforce its own binding and facilitate its association with chromatin (1). In mice, *Moz* mutants have anterior homeotic transformations. At the Hox genes, H3 tails are hypoacetylated and hypermethylated on H3K9 and have reduced recruitment of the H3K4me3 methyltransferase MLL1 (143). MLL1 activity is enhanced by H3K9ac and H3K14ac (88), suggesting that Moz acts to acetylate Hox genes in order to recruit MLL1 and promote H3K4 methylation for gene activation.

These examples indicate that, in addition to their direct effects on histone–DNA contacts, acetylated histone tails can localize chromatin remodelers, histone chaperones, elongation factors, HATs, and methyltransferases at or around promoters to further mobilize nucleosomes.

4. SCAFFOLDS FOR SILENCING

Transposons and other repetitive elements make up a large fraction of eukaryotic genomes that must be kept silent. In mammals, this occurs primarily through trimethylation of H3K9 and H3K27 by the SET domain methyltransferases SUV39H1/2 and EZH1/2. These marks might have originally served merely to block H3K9 and H3K27 acetylation to prevent transcription of transposons and other repetitive sequences, prior to their elaboration into silencing complexes based on the trimethyl-binding chromodomain proteins of the HP1 (heterochromatin protein 1) and Polycomb families. These complexes serve to physically compact the chromatin to make it inaccessible to transcription factors, acetyltransferases, and other activating proteins, in contrast to the chromatin decompaction mediated by acetylation. It seems likely that both H3K9me3 and H3K27me3 originally functioned to silence repetitive elements, as in *Chlamydomonas* (120), fungi (25, 55), or ciliates (104). As eukaryotes grew more complex and multicellular, the H3K27me3/Polycomb system was adapted for developmental silencing. Methylation marks are metabolically more stable than acetylation marks, and a crucial function of these marks is to establish a memory of silent chromatin than can survive replication and be inherited by daughter cells.

4.1. H3K9me3 and H4K20me3: Constitutive Silencing

In mammals, DNA and H3K9 methylation coordinate to maintain constitutive heterochromatin through replication. The maintenance DNA methyltransferase DNMT1 interacts with PCNA at the replication fork (14) and is recruited to hemimethylated DNA by UHFR1, which also binds to H3K9me3 (111). DNMT1 methylates the newly synthesized strand, and MBD1 (methyl-CpG-binding domain protein 1) binds the methylated DNA and forms a complex with the histone methyltransferase SETDB1 and the large subunit of the replication-coupled histone chaperone CAF-1, so that SETDB1 monomethylates H3K9 before CAF-1 deposits it into chromatin (116). H3K9me1 is a substrate for the histone methyltransferases SUV39H1 and SUV39H2 to trimethylate H3K9. The chromodomain protein HP1 binds to the heterochromatic mark H3K9me3, forms bridges across nucleosomes, and recruits SUV39H1, SUV39H2, and the H4 methyltransferase SUV420H, which catalyzes H4K20 trimethylation (68, 123). These enzymes methylate adjacent nucleosomes, spreading a local heterochromatic environment. H3.3 is deposited into heterochromatin by the chaperones ATRX and DAXX (27), where SETDB1, SUV39H1, and SUV39H2 methylate H3.3K9 (139). Deletion of H3.3 in mouse ESCs results in decreased H3K9me3, H4K20me3, and ATRX at telomeres and derepression of transcription from telomeres (139) and from endogenous retroviruses (27), consistent with the role of H3K9me3 and H3.3K9me3 in silencing repeats. These effects can be rescued by wild-type H3.3 expression but not by H3.3K9A (139).

In *Drosophila*, H3K9R mutants have decreased H3K9me3, HP1 binding, and nucleosome occupancy at pericentric heterochromatin and increased transcription of transposons (101). A few H3K9R mutants survive to adulthood, but H3K9R H3.3K9R double mutants die soon after embryogenesis, having increased transcripts from heterochromatic regions and having many decreased transcripts from regions enriched for H3K9ac in control animals, indicating that acetylation of H3K9 is important to the transcription of many genes (102).

In contrast to H3K9R, H4K20A mutants are viable in flies, though with developmental delay (85). In mice, however, catalytically inactive mutants in the monomethyltransferase SETD8, which must first methylate H4K20 for all methylation states, are lethal, with defects in chromosome condensation (95). The ubiquitous H4K20me2 mark, unlike the heterochromatic mark H4K20me3, has roles in DNA repair and replication (reviewed in 57).

4.2. H3K27me3: Developmental Silencing

H3K27me3 and Polycomb repressive complexes 1 and 2 (PRC1 and PRC2) have central roles in cell type–specific developmental gene silencing in multicellular eukaryotes (**Figure 3**). The H3K27 methyltransferase component of PRC2, enhancer of zeste [E(z) in *Drosophila*, or EZH1 and EZH2 in humans], methylates H3K27, and H3K27me3-directed gene silencing is carried out by PRC1 (70, 90). Classical PRC2 in *Drosophila* contains four subunits—E(z), Su(z)12, p55, and Esc (120)—while vertebrates have two E(z) paralogs, which form two PRC2 complexes, PRC2.1 and PRC2.2, that share EZH1 or EZH2, SUZ12, EED (Esc), and RBBP4/7 (p55) core components but differ in additional Polycomb-like (PCL) substoichiometric components (141). PRC2.1 contains one of three PCL proteins (MTF2, PHF1, or PHF19) in complex with either EPOP or PALI1/2. The PCL subunits target PRC2.1 to CpG islands in mouse cells (75). PRC2.2 contains JARID2 and AEBP2, which bind to H2AK119ub1-containing nucleosomes and stimulate EZH2 activity (60) at the affected promoters. H3K27me3 is usually present in large contiguous domains over genes, which form when the human PRC2 member EED binds H3K27me3 and positions the E(z) homolog EZH2 to methylate an adjacent nucleosome, facilitating H3K27me3 spread (103).

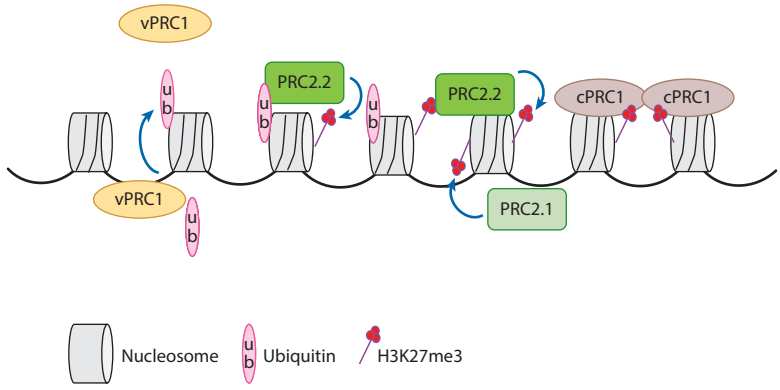

Figure 3

Polycomb repressive complexes. Variant PRC1 (vPRC1) complexes bind DNA and ubiquitylate nucleosomes on H2AK119. PRC2.2 complexes bind and are stimulated by ubiquitin to trimethylate H3K27 more efficiently. PRC2.2 complexes can also bind to H3K27me3 to trimethylate H3K27 on adjacent nucleosomes. PRC2.1 can bind DNA at CpG islands to trimethylate H3K27. Canonical PRC1 (cPRC1) binds H3K27me3 and is thought to compact chromatin to silence transcription.

By contrast, H3K27me2 is found widely over the genome, where it may result from a less stable and less localized PRC2 activity.

Classical PRC1 in *Drosophila* also contains four core subunits, including the chromodomain protein Polycomb, which binds to H3K27me3, and two RING-type zinc finger E3 ubiquitin ligases (Sce and Psc) that together ubiquitylate H2AK119 (**Figure 3**). There are also variable substoichiometric subunits, including HDACs and the DNA-binding protein Pleiohomeotic, which targets PRC1 to specific genes (41). H2AK119ub1 is essential for viability but not for PRC1 silencing in *Drosophila* (69, 100). However, in ESCs, H2AK119ub1 is essential to maintain PRC2.2 and transcriptional silencing (133). It stimulates PCR2.2, forming a positive feedback loop between PRC2.2 and PRC1 (60), and inhibits H3K36 methyltransferases (155), contributing to the mutual exclusivity of H3K27me3 and H3K36me3. In *Drosophila*, H3K27me3 silencing is thought to occur through inhibition of transcription initiation and elongation by PRC1-mediated chromatin compaction (4). Silencing changes during differentiation and depends on the PRC1 component Polyhomeotic (69), which can polymerize (109), potentially bringing disparate PRC1 complexes together. Mammals have three to five Polycomb homologs (CBXs), two paralogs of the PRC1 Sce (RING1A and RING1B), and six Psc (BMI or PCGF) homologs that define six groups of PRC1 complexes (34, 41). While all PRC1 complexes have RING1A/B, they can be divided between canonical PRC1 (cPRC1) complexes, which have CBX chromodomain proteins that bind to H3K27me3 and effect silencing, and variant PRC1 (vPRC1) complexes, which bind to DNA and appear to more efficiently ubiquitylate H2AK119 (34).

In *Drosophila*, H3K27A and H3K27R mutants fail to maintain silencing of PRC2 gene targets, with ectopic expression of homeobox genes and homeotic transformation, similar to the effects of mutations in Polycomb or other PRC components (85). In mouse ESCs, a mutation in the EZH2 catalytic domain substantially decreases H3K7me3, but cells can still differentiate, whereas EZH2-null cells cannot (87). However, mutant cells do not stay differentiated, needing H3K27me3 to maintain differentiation rather than to initiate it. H3K27me3 is thus an essential link in Polycomb silencing. In humans, heterozygous loss-of-function mutations in EZH2, SUZ12, or EED reduce levels of H3K27me3 and cause Weaver syndrome, an overgrowth syndrome characterized by tall stature, distinctive facial characteristics, and variable intellectual disability (51).

5. COTRANSCRIPTIONAL METHYLATIONS: RESISTING SILENCING

Similar to the stimulation of the EZH2 methyltransferase by H2AK119ub1, H2BK120ub1 stimulates trimethylation of H3K4, H3K36, and H3K79 by their respective methyltransferases (3, 63). H2BK120ub1 is bound by mammalian MLL H3K4 methyltransferase complexes through the RBB5 subunit (152); to SET2, the methyltransferase that methylates H3K36me3 (3); and to DOT1L, which methylates H3K79 (150). Cryogenic electron microscopy structures suggest that H2BK120ub1 flexibly helps to position, orient, and stabilize the methyltransferases to carry out their reactions (22). Thus, H2BK120ub1 appears to function not only to stimulate FACT-mediated transcription but also to serve as a catcher's mitt for methyltransferases. Like H2BK120 ubiquitylation, these methylations are largely or entirely dependent on transcription and appear up to 4 h after activation-linked acetylations (56). Their functions are not entirely clear, but they seem to be generally describable as antisilencing, preventing the encroachment of the silencing systems on transcribing genes. The same SET family of proteins that includes SUV39H, SUV420H, and EZH2 has evolved members that methylate H3K4 and H3K36, while a different family, DOT1L, distantly related to arginine methyltransferases, evolved to methylate H3K79, perhaps because of its location on the surface of the nucleosome core rather than on the tail (**Figure 2**). Similar to the memory function of silencing methyl marks, a possible function of all three of these methyl marks is to act as a memory of transcription.

5.1. H3K4me: What Is It For?

Budding yeast gets by with only a single SET1 methyltransferase that methylates all H3K4s, while *Drosophila* has three SET1 family proteins, dSET1, TRX, and TRR, and mammals have three pairs of SET1 proteins, duplicated relative to the *Drosophila* proteins: SETD1A and SETD1B, MLL1 and MLL2, and MLL3 and MLL4, respectively (reviewed in 20). Each SET1 family methyltransferase requires accessory proteins and has nonredundant functions. H3K4 methylation levels are strongly correlated with transcription, with H3K4me3 peaking at the +1 nucleosome of TSSs for both coding and noncoding transcripts, H3K4me2 peaking further downstream, and H3K4me1 peaking even further downstream or at enhancers. Cfp1 is an accessory protein with a PHD domain that may bind to H3K4me2 to direct SET1A/B to form H3K4me3, and a CxxC zinc finger that binds the CpG islands found at approximately 70% of promoters. Deletion of Cfp1 results in embryonic lethality in mice (9), whereas in ESCs the cells are viable but unable to differentiate (8). H3K4me3 is lost from approximately half of all wild-type H3K4me3-marked promoters in *Cfp1*$^{-/-}$ cells, nearly all with CpG islands, and with more highly expressed genes more severely affected (16). Loss of H3K4me3 has little effect on transcription of CpG island genes, suggesting that H3K4me3 is not necessary for transcription. Transcription in *Cfp1*$^{-/-}$ cells is increased at some enhancers that have ectopic peaks of H3K4me3. The DNA-binding capacity of Cfp1 is necessary to prevent ectopic H3K4me3 peaks at enhancers but is not needed to trimethylate H3K4 at promoters. Accumulation of H3K4me3 at p53-induced genes following doxorubicin treatment depends on Cfp1, but transcription does not (15). Together, these observations suggest that H3K4 methylation is downstream of transcription. H3K9 acetylation is linked to H3K4 trimethylation, perhaps through recruitment of the GCN5 HAT by H3K4me3.

CxxC fingers are also present in MLL1 and MLL2. H3K4 trimethylation at bivalent promoters, in which both H3K4me3 and H3K27me3 are present, is carried out largely by MLL2. Knockdown of MLL2 leads to loss of H3K4me3 at bivalent promoters but has little effect on gene expression (48). MLL2 also methylates H3K4 at some enhancers, including those required to specify primordial germ cell development (47). Expression of *Prdm1* and *Prdm14* genes proximal to these enhancers is dependent on both the CxxC finger and the methyltransferase activity, indicating a

requirement for H3K4me3 at some genes. Loss of H3K4me3 and reduced expression of *Prdm1* and *Prdm14* is accompanied by reduced p300 and H3K27ac but does not affect the formation of enhancer/promoter loops. The purpose of the H3K4me3 requirement at these genes may be to prevent DNA methylation, since DNA methyltransferases recognize unmodified H3K4 (96).

H3K4me3 is bound by the PHD finger of TAF3, a component of the general transcription factor TFIID, and TAF3 occupancy at promoters is decreased in HCT116 cells when the PHD finger is mutated so that it does not recognize H3K4me3 (73). Chromatin assembled with the H3K4me3 analog trimethylaminoethylcysteine (H3Kc4me3) shows increased p53-induced transcription compared with unmodified H3 and increased preinitiation-complex formation. Depletion of TAF3 has a minor effect on constitutive transcription, but H3K4me3 and TAF3 are required for the regulation of certain p53 target genes. H3K4me3 also appears to have a role in directing acetylation to promoters. A minor fraction of HATs and HDACs are found at inactive human genes, and those inactive genes marked with H3K4me1, H3K4me2, or H3K4me3 at their promoters are more likely to become acetylated and occupied by RNAPII upon inhibition of HDACs (146), similar to the way that, in animals, p300/CBP is necessary for dynamic acetylation on H3 tails marked with K4me3 (19).

Despite these gene-specific effects, it remains perplexing that H3K4me3 is conserved at promoters throughout eukaryotes but seems largely dispensable for transcription. In nuclear transplantation experiments in enucleated *Xenopus* eggs or in human cells, failure of donor nuclei to reprogram is associated with the transcriptional memory of genes expressed in the donor nucleus and with increased H3K4me3 relative to donor nuclei that successfully reprogrammed, suggesting that H3K4me3 acts as a memory of prior transcription. Injection of one-celled *Xenopus* embryos with mRNA for the H3K4 demethylase KDM5B improved reprogramming.

Taken together, the data suggest a model in which most, but perhaps not all, trimethylation of H3K4 is a consequence of transcription, with methyltransferases facilitated by transcription-coupled H2BK120 ubiquitylation and with relatively little direct effect on gene expression, but with the ability to serve as a scaffold to localize other proteins to promoters and to aid in continued expression or reactivation of genes.

Monomethylation of H3K4 at enhancers is largely carried out by MLL3 and MLL4, which are recruited to enhancers by lineage-specific transcription factors (71). H3K4 mono- and dimethylation by MLL4 precedes and is necessary for acetylation of H3K27 by p300 and enhancer activation, and for RNAPII recruitment and lineage-specific gene expression. Mammalian BRWD2/PHIP, which is necessary for neural development, colocalizes extensively with H3K4me1, H3K4me2, and H3K4me3 through its cryptotudor domain, and depletion of the *D. melanogaster* homolog disrupts the pattern of H3K27ac (89). MLL3/4 increases enhancer–promoter interactions in mouse ESCs; such interactions are reduced in MLL3/4 double-knockout cells and in catalytically inactive mutants that lack methyltransferase activity (153). Monomethylation of H3K4 leads to accumulation of cohesin, suggesting a role for H3K4me1 and MLL3/4 in recruiting cohesin to mediate enhancer–promoter interactions.

In somatic clones of *D. melanogaster* cells expressing H3.3K4A or H3.3K4R in the absence of other H3 proteins, the only obvious phenotype is a reduced proliferative rate (45). In mouse ESCs, however, expression of H3.3K4A in place of H3.3K4 leads to widespread changes in gene expression, defects in the ability to differentiate into neural precursor cells or cardiac cells, and depletion of H3.3K4A and nucleosome remodelers NuRD and SWI/SNF from TSSs along with increased nucleosome turnover and increased transcription from these depleted TSSs (38). A similar depletion from TSSs is found with H3.3K4R, H3.3K4Q, and H3.1K4A, suggesting that the K4 residue itself is interdependent with remodelers to maintain occupancy at TSSs.

5.2. H3K79me: Maintaining Accessible Chromatin?

Methylation of H3K79 is carried out by DOT1L, the mammalian homolog of yeast Dot1, though only the N terminus of DOT1L is homologous to Dot1 (64). DOT1L binds to RNAPII–CTD phosphorylated on S2, S5, or both but not to the nonphosphorylated CTD or to a scrambled phosphorylated CTD heptad YSPTSP repeat, indicating that both phosphorylation and the heptad amino acid sequence are important for binding. H3K79me1, H3K79me2, and H3K79me3 are enriched at actively transcribed genes. As with the unrelated H3K4 methyltransferases, DOT1L activity is stimulated by H2BK120ub1, which reduces DOT1L mobility on the nucleosome and promotes higher methylation states of H3K79 (140). DOT1L is essential for mammalian development but not for ESC cycling, and it has a role in promoting transcriptional elongation that is independent of its catalytic activity (7). Like H3K4me3, loss of H3K79me seems to have relatively little direct effect on transcription. DOT1L has a role in leukemias with an MLL–AF9 translocation and is recruited by interactions with AF9 and other translocation protein partners, resulting in high levels of H3K79me2, H3K9ac, and H4K16ac at MLL–AF9 target genes (12). Inhibition of DOT1L results in silencing of these target genes, loss of H3K9ac, and increase of H3K9me2 by a complex that includes the HDAC SIRT1 and the H3K9 methyltransferase SUV39H1, suggesting that H3K79me2 inhibits SIRT1. Whether H3K79me has a similar, but subtle or redundant, role in maintaining accessible chromatin in all transcribing genes is an open question.

A subset of enhancers, called H3K79me2/3 enhancer elements, are marked with H3K79me2/3 and are present in MLL–AF4 leukemia cells, human ESCs, and other cells. One study found that inhibition of DOT1L results in a loss of H3K79me2/3 and reductions in local H3K27ac, transcription factor binding, interactions between H3K79me2/3 enhancer elements and promoters, and target gene expression (39). The authors proposed a model in which H3K79me2/3 maintains H3K27ac and promotes a more accessible chromatin structure at H3K79me2/3 enhancer elements.

5.3. H3K36me: RNA Maturation and Antisilencing

While budding yeast has only a single H3K36 methyltransferase (SET2), mammals have three families of H3K36 methyltransferases (49), of which SETD2 is the only methyltransferase that trimethylates H3K36 (156). SETD2 is recruited by RNAPII–CTD–S2ph (154) and is found distributed through the gene body (**Figure 4**). In the yeast counterpart SET2, H3K36me3 is a docking site for the HDAC Rpd3s, which prevents genes from becoming hyperacetylated and therefore subject to cryptic transcription initiation (62). In *Drosophila*, H3.2K36R mutants (which can still produce H3.3K36me3) do not eclose as adults (85). In third-instar larvae, transcription is dysregulated and acetylation is increased, but with little change in chromatin accessibility, cryptic transcription initiation, or alternative splicing. Instead, defects in mRNA maturation suggest that H3K36me may have a role in recruiting the transcription termination and polyadenylation machinery to the 3′ ends of genes (86). A role for H3K36me3 in mRNA processing has also been reported in mouse ESCs, where knockout of SETD2 or overexpression of the H3K36me3 demethylase KDM4A greatly reduces m^6A in total and in polyA RNAs (50). H3K36me3, but not H3K36me1/2, directly binds METTL14, a component of the m^6A methyltransferase complex, and facilitates binding of the complex to RNAPII, where it can deposit m^6A in nascent transcripts cotranscriptionally. H3K36me3 promotes differentiation, at least in part through the destabilizing effect of m^6A on pluripotency transcripts.

Unlike *Drosophila*, mammals have gene body methylation, and H3K36me3 is bound by the de novo DNA methyltransferases DNMT3A and DNMT3B (110). In mouse ESCs, H3K36me3

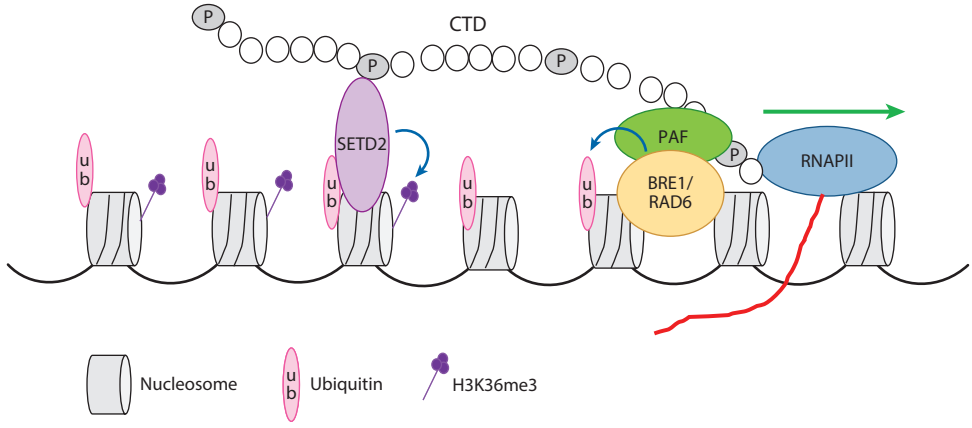

Figure 4

Cotranscriptional trimethylation of H3K36. BRE1 and RAD6 are associated with PAF and ligate ubiquitin to H2BK120. The SETD2 methyltransferase binds S2 of the RNAPII carboxy-terminal domain (CTD) and also binds to ubiquitin in order to trimethylate H3K36 in transcribed regions.

recruits DNMT3B to methylate gene bodies, which suppresses spurious transcription initiation within genes (93). DNMT3A and DNMT3B are inhibited at TSSs by H3K4me3 (96), which serves to keep promoters unmethylated and not silenced. In humans, heterozygous loss-of-function mutations in DNMT3A have an overgrowth and intellectual disability phenotype similar to that of Weaver syndrome, designated Tatton-Brown–Rahman syndrome (136). By contrast, a mutation in the PWWP domain of DNMT3A that prevents binding to H3K36me2/3 causes microcephalic dwarfism and gain-of-function hypermethylation of normally methylation-depleted Polycomb-regulated developmental genes and their H3K4me3/H3K27me3 bivalent promoters, silencing them and reducing H3K4me3 and H3K27me3 at these regions (43).

Besides SETD2, two other families of H3K36 methyltransferases, the NSD family and the ASH1L methyltransferase family, monomethylate or dimethylate H3K36 in mammals (49). In contrast to H3K36me3 found over gene bodies, H3K36me2 is abundant around TSSs and in intergenic regions, with lower levels throughout transcribed regions. Both H3K36me2 and H3K36me3 inhibit EZH2, the H3K27me3 methyltransferase (29, 157). Indeed, in HeLa cells, H3K36me2/3 and the developmental silencing mark H3K27me3 exist in largely mutually exclusive domains, establishing clear boundaries between transcribed and silenced regions. However, in ESCs, H3K36me2 and H3K27me2 commonly occur on the same peptides (58). Nsd1-mediated H3K36me2 and H3K27me2 colocalize in ESCs, where loss of Nsd1 leads to decreased H3K36me2 and increased H3K27me3, suggesting an antisilencing role for H3K36me2 and Nsd1 (127).

5.4. Balancing H3K27 and H3K36 Methylation in Human Health and Disease

Heterozygous loss of function of *NSD1* in humans leads to Sotos syndrome, which resembles Weaver syndrome and Tatton-Brown–Rahman syndrome in its characteristic overgrowth, intellectual disability, and facial dysmorphism (135). Given the antagonistic role of H3K36me2 to H3K27me3, it is counterintuitive that loss-of-function mutations in *NSD1* and *EZH2* lead to similar phenotypes, but a possible explanation lies in the fact that upon depletion of H3K36 methyltransferases or inhibition of their function by H3K36M or H3K36I mutations, which are found in chondroblastomas, there is an increase of H3K27me3 in intergenic regions, redistribution of

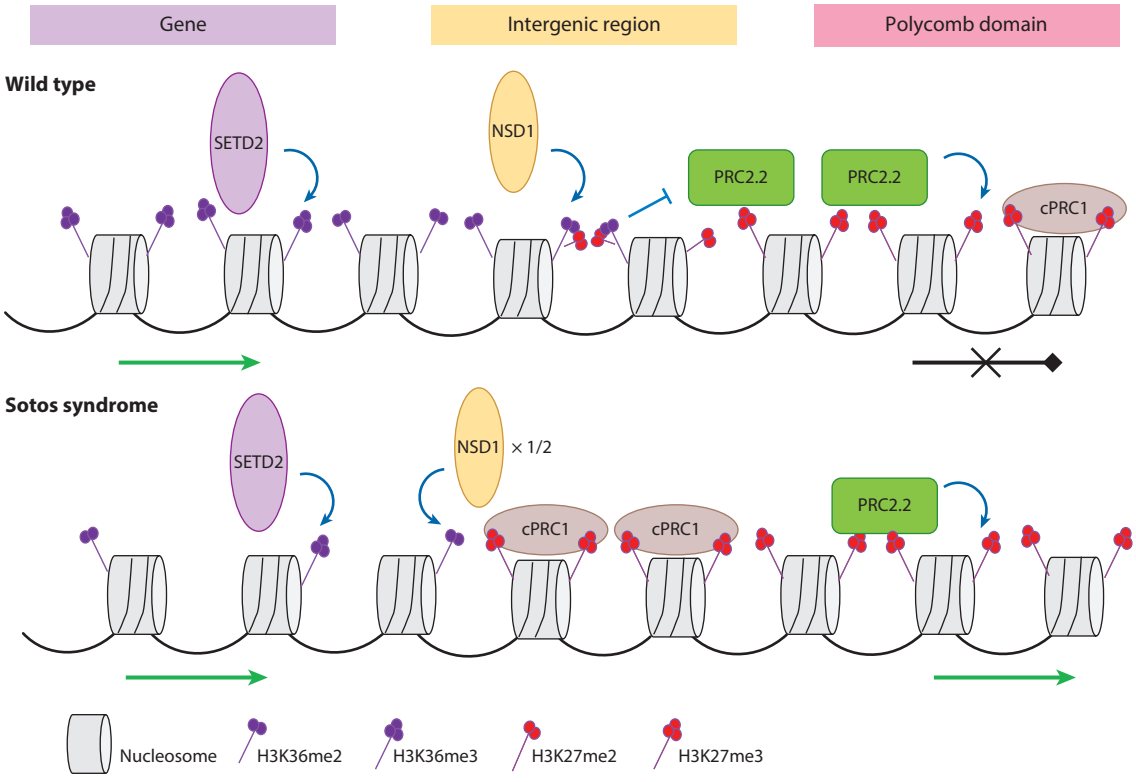

Figure 5

Model for Sotos syndrome. H3K36me2/3 inhibits the formation of H3K27me3. Loss of half of *NSD1* reduces H3K36me2/3, so that H3K27me3 spreads into intergenic regions and the PRC1 silencing complex redistributes, allowing misexpression from Polycomb domains.

PRC1 to these regions, and consequent derepression of normal PRC1 targets (80) (**Figure 5**). Similarly, in *Drosophila*, H3K36R mutants have reduced levels of H3K36me2 and H3K36me3, and reduced levels of H3K27me3 over PRC target genes, resulting in homeotic transformations reminiscent of Polycomb group mutations (29).

H3.1K27M and H3.3K27M mutations predispose toward pediatric diffuse midline gliomas and act as dominant negative inhibitors of PRC2 function, reducing H3K27me3 while increasing H3K36me2 on H3K27M tails (124). H3K27M is globally distributed in H3.1K27M glioma cell lines, consistent with the incorporation of H3.1 behind the replication fork, and H3K27me3 levels are globally reduced to 2–10% of other gliomas (117). By contrast, H3K27M in H3.3K27M glioma cell lines is present at low levels throughout the genome but is enriched at promoters of active genes, consistent with replication-independent nucleosome replacement by H3.3 nucleosomes. The number of H3K27me3-silenced domains is drastically reduced in H3.3K27M lines compared with gliomas with wild-type H3K27, but H3K27me3 and PRC2 levels are high in the remaining domains, which include many important developmental genes, indicating that EZH2 remains active in these cells. EZH2 at these silent domains may escape inhibition by H3.3K27M, which is largely incorporated at active sites.

H3.3-specific mutations of H3.3G34 (to R, W, or V), which can lead to giant cell tumors of bone and hemispheric high-grade gliomas, inhibit SETD2 but not NSD1/2, leading to loss of

H3.3K36me3 at a subset of enhancers, an increase of PRC2-mediated H3.3K27me3, and aberrant silencing of differentiation-promoting genes (54). H3.1G34W expressed from a transgene was depleted from active enhancers and failed to produce similar effects, probably because of high histone turnover at enhancers and replacement with H3.3.

6. PHOSPHORYLATIONS: DECONSTRUCTING SILENCING

Phosphorylation of serines and threonines resembles acetylation in changing the charge on histones, and H3T80ph and H3T118ph on the nucleosome core may have a similar role in disrupting nucleosomes (see Section 3.2). Some phosphorylations have specialized nontranscriptional roles, such as H3T3ph, which functions in centromeres, or H2A.XS139ph, which functions in DNA repair, and are outside the scope of this review. The common histone tail phosphorylations H3S10ph and H3S28ph, however, seem primarily to disrupt silencing scaffolds rather than histone–DNA interactions. The H3.3 replacement variant can additionally be phosphorylated on H3.3S31 and may have a similar role.

6.1. H3S10ph and H3S28ph

Temporary phosphorylation of H3S10 and H3S28 is prominent during mitosis, when they are phosphorylated by Aurora kinases (18). H3S10 and H3S28 are adjacent to H3K9 and H3K27, respectively. Both of these lysines can be acetylated, but they can also be methylated to give the silencing marks H3K9me3 and H3K27me3. H3K9me3 assembles heterochromatin HP1 proteins, but HP1 is evicted from chromatin during mitosis, which is thought to contribute to chromosome condensation, as a result of the phosphorylation of H3S10 to form H3K9me3S10ph nucleosomes (30). During interphase in *Drosophila* polytene cells, the JIL-1 kinase phosphorylates H3S10, which can stop the spreading of heterochromatin in position-effect variegation (145).

Similarly, H3S28 phosphorylation of genes regulated by mitogen- and stress-activated kinases (MSKs) to form H3K27me3S28ph can displace PRC1 and PRC2 proteins bound to H3K27me3 in stress-induced cells (37). Targeting MSK1 to the Polycomb-silenced α-globin gene activates gene expression, leading to loss of H3K27me3 and gain of H3K27acS28ph nucleosomes (72). Despite abundant H3S28ph on mitotic chromosomes, PRC2 proteins are not displaced in mitosis (37), perhaps suggesting that the Aurora kinase, unlike MSK1, does not significantly phosphorylate H3K27me3 nucleosomes.

6.2. H3.3S31ph

H3.3 differs from H3.1 and H3.2 in having serine 31 in the N-terminal tail (147), which is phosphorylated in the pericentromere (42) and in telomeres (149) during metaphase by CHK1 and Aurora B kinases (11, 76). In euchromatin of mouse ESCs, phosphorylation of H3.3S31 by CHK1 promotes p300-dependent acetylation at enhancers, facilitating differentiation of these cells (84). Similarly, in *Xenopus* embryos, H3.3S31 phosphorylation is necessary for blastopore closure, and nucleosomes bearing the phosphomimic H3.3S31D protein are enriched for K27ac (121). This suggests that H3.3S31ph may facilitate nucleosome acetylation and nucleosome turnover at promoters and enhancers. In addition, in macrophages, H3.3S31ph over gene bodies interacts with SETD2 to promote H3K36me3 in genes stimulated by bacterial lipopolysaccharides, suggesting a role in regulating rapid response genes (2). However, in *Caenorhabditis*, H3.3 is not required for viability (21), and in *Drosophila* H3.3 knockout mutants, an overexpressed H3.2 (H3A31) construct was able to rescue viability and nearly all transcription defects (115), implying minimally that the requirement for H3.3S31 differs in different organisms. In both flies and mice, H3.3 is required

for male fertility (115, 158). In H3.3-reduced male mice, apoptosis of spermatogonia and spermatocytes occurs and the transition to protamines is incomplete (158), implying a defect at or prior to meiosis.

The mechanisms by which H3.3S31ph promotes H3K27ac or H3K36me3, or promotes meiosis, are unknown. In view of the roles of phosphorylation of H3S10 and H2S28 in evicting HP1 and PRC2 complexes, it is tempting to speculate that H3.3S31ph might also evict or prevent binding of PRC2 complexes and thereby permit acetylation of H3K27 and trimethylation of H3K36.

7. CONCLUSIONS

While the writers and readers of histone marks are surprisingly complex, the marks themselves seem to have a relatively small number of roles in transcription, either promoting transcription or promoting chromatin compaction and silencing. Acetylations neutralize the charge of lysines, disrupt DNA–histone tail contacts, and destabilize nucleosomes cores, facilitating the mobilization of nucleosomes and paused polymerases to promote transcription. H3T80ph and H3T118ph may have similar roles in destabilizing nucleosome cores. Methylations form a scaffold on chromatin to enable the formation of silencing complexes or to resist silencing complexes. As more stable marks than acetylations or phosphorylations, they can serve a memory function of transcription or nontranscription. The balance between silencing and antisilencing methylations and their genomic locations are important for controlling the proliferation or differentiation of cells. Cotranscriptional methylations appear to have additional roles in directing DNA methylation away from promoters and into gene bodies to prevent cryptic transcription initiation, in inhibiting deacetylases, and in promoting mRNA processing. In most cases, methylation is stimulated by ubiquitin, which may help capture and orient methyltransferases to efficiently methylate histone tails for either silencing (H2AK119ub1) or antisilencing (H2BK120ub1). Tail phosphorylations of H3S10 and H3S28 can evict silencing complexes, probably because bulky charged phosphate groups destabilize adjacent non–covalently attached chromodomains anchoring to methyl groups. Many or most of these functions rely on fairly simple chemistry or steric constraints, with writers and erasers left to apply these tools to particular contexts or not, and readers left to respond to them. As less common and abundant marks are investigated, it will be interesting to see how they conform to or expand the roles of marks in promoting transcription or silencing.

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