Minireview

Hyperacetylated Chromatin Domains: Lessons from Heterochromatin*

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A small but growing number of loci that exhibit covalent histone modifications, such as hyperacetylation, over broad regions of 10 kb or more have been characterized. These hyperacetylated domains occur exclusively at loci containing highly expressed, tissue-specific genes, and the available evidence suggests that they are involved in the activation of these genes. Although to date little is known concerning the formation or function of these domains, rather more is known concerning repressive, heterochromatic domains, and the example provided by heterochromatin may be instructive in considering mechanisms of active domain formation.

In eukaryotes, genomic DNA is packaged with histones to form chromatin, which in turn condenses to form more compact structures (1, 2). The condensation of chromatin has an obvious impact on any process, such as transcription, replication, DNA repair, or recombination, requiring access to genomic DNA, and not surprisingly a wide array of studies has shown that chromatin structure plays a crucial role in the regulation of all of these processes. In particular, transcriptional regulation involves the modification of chromatin structure as a necessary step in the establishment and maintenance of active or repressed states. A common mechanism of modifying chromatin structure is the covalent addition (or removal) of acetyl, methyl, phosphoryl, or other moieties to the amino-terminal tail domains of the core histones by specific enzymes. For example, nucleosomes near active gene promoters and enhancers are nearly always acetylated, due to the recruitment of histone acetyltransferases (HATs)1 by transcription factors.

Function of Histone Acetylation

Two different but not mutually exclusive models exist to explain the role of histone acetylation in transcriptional activation. In the first, acetylation of core histone lysine residues directly inhibits chromatin condensation by disrupting normal core histone tail domain function. In vitro studies have shown that above a certain threshold of acetyl groups per nucleosome condensation of chromatin is blocked (3).

In the second model, covalently modified core histone amino termini serve as a signal for the binding of trans-acting factors. Evidence in support of this model is abundant. For example, some activating factors, including several HATs, possess a region termed the bromodomain that interacts specifically with acetylated core histones (4, 5). Methylation of lysine 9 of histone H3, a mark of repressive chromatin in organisms ranging from fission yeast to mammals, is recognized by the heterochromatin-associated protein HP1 (6). Moreover, a pattern of “cross-talk,” in which some histone modifications are required for or can inhibit others, has emerged from a number of studies (7).

Although current genome-wide studies of histone modification patterns as they relate to gene expression argue against such modifications comprising a histone “code” of high complexity (8), gene repression and activation are clearly associated with different modification patterns. At the very least, post-translational histone modifications serve the same purpose as on other cellular proteins and form binding surfaces of differing affinity for regulatory factors (9).

Histone Acetylation Patterns across the Genome

In the budding yeast Saccharomyces cerevisiae, HATs and their counterparts, histone deacetylases (HDACs), can modify the genome in a global untargeted fashion, as shown by genome-wide alterations in acetylation levels upon deletion of specific HATs or HDACs (9). Against this global background, histone hyperacetylation is targeted to the promoters of active genes via the recruitment of HATs by transcription factors. Similarly, HDACs can be targeted to specific sequences to achieve histone hypoacetylation. Finally, heterochromatic regions of the yeast genome exhibit histone hypoacetylation over much larger but still well defined regions (see below), such as at the silent mating-type loci and telomeric regions (6, 10).

These modes of histone modification also occur in vertebrates. At some vertebrate loci, however, histone hyperacetylation is observed over large regions of 10–100 kb. The first such region to be identified was the β-globin locus in chicken erythroid cells, which exhibits histone hyperacetylation over 30 kb of DNA (11), although the precise levels of acetylation are not uniform (12). In addition, methylation of lysine 4 of histone H3 has been shown to coincide with histone hyperacetylation at this locus (13), and so hyperacetylation of histones H3 and H4 represents only a subset of the modifications that mark the active locus in this generalized fashion.

Several other loci have since been shown to harbor broadly distributed patterns of histone hyperacetylation, including the β-globin locus in mouse (14–16), the α-globin locus in erythroid cells of chicken, mouse, and human (17), a transgenic human growth hormone locus in mouse pituitary and placenta (18, 19), the loci for the immunoglobulin heavy chain (IGH) gene, and the major histocompatibility complex class II gene HLA-DRA in B-lymphocytes (20, 21), the interferon-γ (Ifng) locus in T-lymphocytes (22), and HOX loci in lung fibroblasts (23), among others. I term these regions “hyperacetylated domains,” although other modifications are known to accompany acetylation. In some cases, e.g. the human growth hormone locus in placenta, different histone modifications can occur with different patterns, suggesting additional levels of complexity in domain formation.

Specificity of Hyperacetylated Domains

Hyperacetylated domains are not a universal feature of active gene loci. Genome-wide surveys of histone acetylation have
failed to reveal a general pattern of histone hyperacetylation, or most other modifications, over active genes outside of gene promoters (24, 25). In fact, a survey of histone H3 acetylation and methylation across human chromosomes 21 and 22, using chromatin immunoprecipitated from lung fibroblasts, failed to reveal any such domains, although this same study found them at the HOX gene loci (23). A microarray-based analysis of 88 genes in green tobacco shoots, however, revealed histone H4 hyperacetylation 1 kb upstream or downstream (or both) of 25 promoters (28% of the sample), suggesting that histone hyperacetylation beyond gene promoters may still occur at a significant number of gene loci (26). Another genome-wide survey using human T-lymphocytes employed a novel combination of the chromatin immunoprecipitation and serial analysis of gene expression techniques (27). This study found most histone hyperacetylation to be associated with gene promoters but found broader patterns of hyperacetylation at some loci, including those of the CD4 and IL2Rα genes.

Thus, hyperacetylated domains appear to reflect specific modes of gene regulation and only at certain loci. The α-globin gene cluster, for example, is embedded within a region of the genome that contains several other genes that are widely expressed. A domain characterized by histone hyperacetylation extends over the α-globin genes and a neighboring constitutively expressed gene but only in erythroid cells (17). In fact, to date, the known examples of domain formation are limited to tissue-specific genes and function of domains, defined in part by histone modifications under the control of distal regulatory elements.

Genes within a transgenic human growth hormone cluster are expressed in both pituitary and placenta in mice. In pituitary, a 32-kb domain of histone hyperacetylation encompasses the single active member of the cluster along with several upstream regulatory elements (18). Mutation of tandem binding sites for the transcription factor Pit-1, found within an enhancer 15 kb upstream of the active gene, results in both the complete loss of gene expression and the elimination of the hyperacetylated domain (28). In placenta, where a different subset of genes is expressed from within the cluster, a hyperacetylated domain extends across all of the expressed genes but not the upstream regulatory elements (19). These studies suggest that different modes of gene regulation within the same locus are reflected in different patterns of histone modification and in distinct hyperacetylated domains.

In pre-B-lymphocytes, the locus harboring the λ5 and VpreB1 genes exhibits extensive histone hyperacetylation and H3K4 methylation, albeit with different patterns, throughout a domain of more than 20 kb. Examination of cell lines corresponding to different stages of B cell differentiation revealed the presence of a sequence located between the two genes that was associated with modified histones even in embryonic stem cells, and it was proposed that histone modifications spread from this element to encompass the entire locus (29). In addition, mature B-lymphocytes, which express neither gene, are also devoid of histone hyperacetylation and H3K4 methylation.

These studies demonstrate a strong correlation between domains of histone modification and high level, tissue-specific gene expression mediated by distal regulatory elements. Two questions arise at this point. First, what is the function of a hyperacetylated domain within the context of enhancer-mediated gene activation? Second, by what mechanism is the domain formed and maintained? Unfortunately, existing studies of hyperacetylated domains do not provide answers to either question. Rather more is known, however, about the formation and function of domains, defined in part by histone modifications, that are involved in gene repression.

**FIG. 1. A model for hyperacetylated domain formation, based on the mechanisms of heterochromatin assembly.** A complex is nucleated at a regulatory sequence (A), such as an enhancer, perhaps targeted by noncoding RNA (blue line). This complex includes a HAT and/or other histone-modifying activity, which modifies nearby nucleosomes (B). Modified nucleosomes in turn represent high affinity binding sites for a subset of the complex, including the histone-modifying activity, resulting in the progressive spread of the complex and of the characteristic modification pattern (C). In some cases, additional sequences may be bound by factors that block the further spread of the complex and thus serve as boundaries (D, yellow oval).

**Heterochromatic Domains: Formation and Function**

In eukaryotes, the genome is packaged into two general types of chromatin: heterochromatin, which appears compact or condensed throughout the cell cycle, and euchromatin, which appears condensed only prior to mitosis. Heterochromatin is gene-poor, relatively inaccessible to DNA-binding factors, and transcriptionally silent, whereas euchromatin is more accessible and comprises the bulk of transcriptionally active regions within the nucleus. Domains of heterochromatin occur both in large blocks, such as those at and near centromeric and telomeric sequences, and in smaller regions dispersed throughout the genome. Studies of heterochromatic domains provide four general principles that might apply to hyperacetylated domains (Fig. 1).

**Nucleation**—First, the characteristic chromatin structure of the domain nucleates at specific sequences. At yeast silent mating-type loci, sequence-specific DNA-binding factors associate with silencer elements and recruit a complex of Sir proteins, Sir1p–Sir4p (10). Of these proteins, Sir2p is a NAD+-dependent histone deacetylase, which modifies core histone H3 and H4 amino termini. Telomeres in budding yeast contain repeated binding sites for Rap1p, which also recruits Sir proteins. At centromeres and other heterochromatic domains in organisms ranging from the fission yeast Schizosaccharomyces pombe to humans, repetitive DNA sequences are transiently transcribed, which produces double-stranded RNAs by inter- or intramolecular pairing of repeat-containing RNAs. These double-stranded RNAs enter the RNA interference pathway, involving cleavage and processing by the Dicer ribonuclease and the association of the products, termed siRNAs, with a complex termed RITS. The RITS complex then localizes to repetitive DNA in the nucleus, presumably by pairing interactions between siRNAs and DNA or nascent RNA, which in turn results
in targeting of histone methyltransferases to nucleosomes at the repeat sequences (30, 31).

**Spreading**—After the recruitment of factor complexes to specific sequences such as silencers or repetitive DNA elements, the heterochromatic structure spreads outward to at least partially "coat" the chromatin fiber over a large region. This process involves a complex that contains a subset of the factors present at the nucleation sites and includes histone-modifying enzymes that establish the modification pattern specific to the domain. Thus, at yeast silent mating-type loci, Sir2p deacetylates H3/H4 histones adjacent to the silencers, which facilitates the binding of Sir3p/Sir4p, which in turn positions more Sir2p to deacetylate the next nucleosomes, and so on (10, 30). At heterochromatic regions in other eukaryotes, H3K9Me is specifically bound by HP1, which in turn associates with the H3K9 histone methyltransferase, resulting in another progressive mechanism for the spread of the heterochromatic signature (30, 32). Such spreading can be continuous, but in some cases discontinuous spreading has been observed, dependent upon the activity of "protosilencers," which may interact with the original silencer element(s) directly by looping (33).

An extreme example of spreading of heterochromatic structure occurs in mammalian dosage compensation, which is accomplished by the transcriptional silencing of nearly all genes on one X chromosome in cells of XX females (34–36). This silencing requires expression of the X-linked noncoding RNA Xist, which spreads from its site of transcription to coat the entire chromosome. The mechanism by which this occurs is thought to resemble the formation of other, more limited heterochromatic domains, involving the processive spread of a ribonucleoprotein complex containing Xist RNA and histone-modifying activities.

**Boundaries**—In some cases specific sequences serve to block the further spread of the repressive chromatin structure, thus constraining its extent (37, 38). In yeast, such boundary sequences restrict Sir proteins to a well defined interval encompassing the silent mating-type loci. Deletion of the boundary elements results in spreading of heterochromatin proteins to the neighboring, euchromatic regions. The mechanism by which such spreading is blocked has been attributed to targeted recruitment of HAT activity to boundary elements, with histone acetylation acting as a chain terminator for the heterochromatin modification pattern. An alternative model proposes that a short (100–300 bp) nucleosome-free region is formed at the boundary, which the processive mechanism of heterochromatin spreading cannot span (39).

At some heterochromatic regions, clear boundary elements are not evident, and the extent of heterochromatic structure is subject to the cellular levels of opposing HAT and HDAC activities. For example, spreading of Sir proteins from telomeres in budding yeast can be modulated by altering the level of a specific HAT (40). Other histone modifications, such as methylation of lysine 79 of histone H3 (41), and even histone variants, such as histone H2AZ (42), have been implicated in regulating the extent of heterochromatic domains by inhibiting the association of silencing proteins.

**Stable Maintenance**—Although nucleation of heterochromatin at silencers is clearly sequence-dependent, the subsequent spreading step encompasses sequences that do not in themselves specify heterochromatin. Coupled with the self-reinforcing properties of heterochromatin, spreading therefore provides a mechanism for a transition to maintenance of the repressed state independent of the nucleation site. This is seen at silent mating-type loci in yeast, where the silenced state is stably maintained in the absence of cell cycle progression after excision of the actual silencer sequence (43). Similarly, in non-dividing thymocytes, irreversible silencing of the mouse terminal transferase (Dntt) gene involves nucleation of a repressive chromatin structure at the gene promoter, which then spreads outwards for at least 10 kb to either side (44). In a transformed thymocyte cell line, however, the repressive structure fails to spread, and gene repression is reversible. Such examples indicate that it is the structure of the domain itself, and not continued activity of the nucleation sequence, that is responsible for transcriptional silencing.

**Dosage Compensation and the Role of RNA**

In *Drosophila*, dosage compensation is achieved by doubling the transcriptional output of the X chromosome in males. Two noncoding RNAs transcribed from the X chromosome, *roX1* and *roX2*, are required for this process and are incorporated into a ribonucleoprotein complex termed the male-specific lethal (MSL) complex, which spreads bidirectionally from 30–40 entry sites on the X chromosome (including the *roX* genes) to coat much of the chromosome (35, 45). More recently, a study of rearrangements involving the *Drosophila* X chromosome has suggested that the number of entry sites may be much larger, which implies a correspondingly smaller extent of spreading from each site (46). The MSL complex includes a HAT as well as an ATP-dependent RNA helicase and a protein kinase that can phosphorylate histone H3 in *vitro*. *roX* RNAs are required for targeting of the MSL complex to the appropriate entry sites, whereas spreading requires the enzymatic activities of the HAT and the RNA helicase. The example of dosage compensation in *Drosophila* provides the clearest indication that the formation of hyperacetylated domains, which in this case encompass most of a chromosome, follows the same model as that governing the formation of heterochromatin.

Noncoding RNAs play an important role in dosage compensation both in mammals and in *Drosophila* and are required for heterochromatin formation in most eukaryotes. It is therefore notable that non-genic transcription occurs within several loci that exhibit hyperacetylated domains, such as the β-globin locus (47, 48), the λ5-VpreB1 locus (29), HOX gene loci (23), and the IgH (49) and HLA-DRA locus (21). As yet, no direct evidence links such transcription with domain formation, and in fact the patterns of transcription and histone modification do not coincide at the β-globin locus or HOX gene loci (23). This argues against models in which histone modification is a consequence of transcription elongation but leaves open the possibility that noncoding RNAs contribute to domain formation by another mechanism, perhaps analogous to the targeting function of RNA in heterochromatin formation.

**Extent of Hyperacetylated Domains**

Known hyperacetylated domains typically extend over spans of tens of kilobases of DNA; by comparison to heterochromatic domains, particularly at centromeres, this seems rather small. Microarray-based surveys of histone modifications across metazoan genomes have failed to reveal very large hyperacetylated domains (~200 kb or larger) (8, 23), and this has been contrasted with the known existence of large heterochromatic domains. This may not be a fair comparison, however, because the largest heterochromatic domains nucleate at repetitive sequences that are present in hundreds to thousands of copies, and so spreading within these regions does not necessarily occur over hundreds of kilobases either. Heterochromatic domains controlled by a single silencer, such as the mating-type loci in budding or fission yeast, are actually quite limited, 3–4 kb in the former case, 30–50 kb in the latter. Even when the boundary elements are deleted, the resulting extension of het-

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* M. Bulger, unpublished data.
eruchromatic structure is only 7–9 kb (50).

More extensive spreading has been observed when heterochromatic regions of the Drosophila genome are rearranged and juxtaposed with euchromatic regions (51). Even here, however, it is not clear that heterochromatin spreading occurs in a continuous fashion over large spans of chromatin because repetitive sequences that can serve as nucleation sites for heterochromatin are usually also present in euchromatin. Such sites could serve a role analogous to that of protosilencers identified in yeast, which are usually also present in euchromatin. Such sites could serve a role analogous to that of protosilencers identified in yeast, which

anticipate more practical benefits from a better understanding of this idea, genetic studies in Drosophila have demonstrated that on a large scale (hundreds of kilobases or greater) spreading is a discontinuous phenomenon (51). In contrast, similar spreading of MSL complexes is not observed in rearrangements involving the male X chromosome (46). This suggests that as opposed to heterochromatin, which can nucleate at repetitive sequences anywhere in the genome, hyperacetylated domains require more specific nucleation sequences.

Function of Domains

Studies of heterochromatic domain formation provide substantial clues as to how hyperacetylated domains might form. A separate but perhaps more important question concerns function: what purpose is served by an extended pattern of histone modification? Again, a consideration of heterochromatin presents several possibilities.

Regulation of Accessibility—The dominant model for the function of heterochromatin stems from its more condensed appearance and from molecular evidence for decreased accessibility of genomic DNA packaged in heterochromatin. Such inaccessibility is not absolute; in budding yeast, for example, silent chromatin is still accessible to binding by transcription factors but not to the general transcription machinery (52).

Regardless, a hyperacetylated domain may act in an analogous fashion to render large genomic regions more accessible, perhaps by interfering with higher order chromatin folding. Nuclear Localization—Domains of centromeric heterochromatin colocalize in metazoan nuclei; similarly, telomeres in budding yeast, for example, silent chromatin is still accessible to binding by transcription factors but not to the general transcription machinery (52).

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Conclusion

Studies of heterochromatin have greatly advanced our understanding of how large scale modulations of chromatin structure contribute to the establishment and maintenance of gene silencing. The small but growing number of loci that exhibit large scale patterns of histone modifications associated with gene activation present an emerging model for the maintenance of expression of some tissue-specific genes, and one which can benefit from the established example of heterochromat-
