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Genomic views of chromatin

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With the availability of complete genome sequences for a number of organisms, a major challenge has become to understand how chromatin and its epigenetic modifications regulate genome function. High-throughput microarray and sequencing technologies are being combined with biochemical and immunological enrichment methods to obtain genome-scale views of chromatin in a variety of organisms. The data pinpoint novel, genomic elements and expansive chromatin domains, and offer insight into the functions of histone modifications. In parallel, state-of-the-art imaging techniques are being used to investigate higher-order chromatin organization, and are beginning to bridge our understanding of chromatin biology with that of chromosome structure.

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Introduction

A major challenge in genomics research is to understand the epigenetic factors that regulate how DNA sequence is manifested at the level of an individual cell or organism. Much excitement has centered on post-translational histone-modifications and their roles in regulating chromatin structure and function. Several recent studies have applied genomic technologies to obtain global views of histone modifications in addition to other aspects of chromatin. The findings offer unique insight into the complex mechanisms that have evolved to package the genome and to maintain the accessibility of appropriate sequences for transcription.

Here, we review several genomic studies of chromatin structure and function. These include investigations of nuclease accessibility, nucleosome distribution, histone modifications and higher-order structure. Though the

techniques employed by the studies vary widely, each has contributed to our understanding of chromatin as a dynamic regulatory structure that acts both locally at individual genes and globally across broad genomic loci.

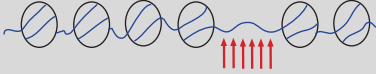
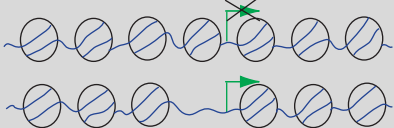


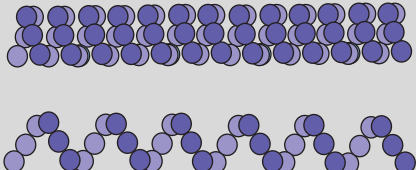
Genomic assays for chromatin accessibility

It has long been known that the accessibility of a given gene to digestion by nucleases varies according to its transcriptional status [1]. Highly accessible ‘hypersensitive’ genomic sites coincide with promoters, enhancers and other functional elements (reviewed in [2]). Two recent studies [3,4] used high-throughput sequencing methods to screen for hypersensitive sites in the human genome. Both confirmed the correlation between the hypersensitive sites and the expected genomic elements on a global scale, and also revealed thousands of potentially novel elements. Hypersensitive sites most frequently coincided with the 5' ends of genes, which tend to be highly enriched with transcription factors and other regulatory proteins. Other sites coincided with evolutionarily conserved sequences, including many non-coding sites clustered near expressed genes. Given that many hypersensitive sites are cell type-specific, this screening technology has the potential to rapidly generate ‘digital’ readouts, or profiles, of chromatin structure for any given cell line. Importantly, the experimental designs implemented by both studies facilitated the relatively precise localization of the most markedly hypersensitive sites. By contrast, Weil and colleagues [5] resolved, on a broad scale, condensed genomic loci from accessible loci by hybridizing nuclease resistant DNA to microarrays. Together, these studies reveal a multitude of novel hypersensitive sites, genomic elements containing multiple such sites, and broader loci that are relatively inaccessible to nucleases. This hierarchy probably reflects local and global alterations in nucleosome structure and histone modification patterns, in addition to higher-order structures (Table 1).

One structural change that can underlie hypersensitivity is the relative absence of nucleosomes [2]. In *Saccharomyces cerevisiae*, nucleosome depletion appears to be widespread, according to two recent studies that globally evaluated nucleosome occupancy by combining chromatin immunoprecipitation (ChIP) and DNA microarrays [6,7]. Marked depletion of nucleosomes was observed in the promoters of highly active genes, but not in coding regions, and was confirmed by micrococcal nuclease digestion. Depletion appeared to be highly dynamic, because it could be rapidly induced upon gene activation and relieved upon gene repression (e.g. during a stress response). Depletion was especially prevalent in

Table 1

Hierarchy of chromatin structure.

Chromatin structural alteration	Genomic technique	References
Nuclease hypersensitivity 	Nuclease hypersensitivity Nuclease digestion and high-throughput screening. Digital analysis of chromatin structure.	[3*,4*]
Nucleosome depletion 	Nucleosome depletion ChIP-on-chip.	[6,7]
Punctate modification 	Histone modifications ChIP-on-chip. ChIP and SAGE.	[9*,19,23*,25**]
Broad modification 		
Condensed versus accessible chromatin 	Higher-order structures Chromatin fractionation, microarrays, <i>in situ</i> methods. Chromosome conformation capture (3C).	[43,45**,46–48]

A range of genomic techniques have been applied to investigate local alterations in chromatin structure (e.g. nuclease hypersensitivity and nucleosome depletion), local and global histone modification patterns, in addition to various aspects of higher-order chromatin structure and genome organization.

promoters containing multiple transcription-factor binding motifs, an association that might reflect competition between transcription factors and nucleosomes for access to the DNA. Although there is some evidence that variations in nucleosome occupancy play an analogous role in mammalian cells [8], a global analysis of human chromosomes 21 and 22 failed to detect systematic depletion at the promoters of active genes [9*].

Genomic views of site-specific histone methylation and acetylation

Post-translational histone modifications play central roles in chromatin regulation [10] and in the epigenetic maintenance of lineage-specific gene-expression patterns [11]. The global distributions of modified histones have been examined in several organisms. The studies reviewed here combined ChIP with microarrays or with a variation of the serial analysis of gene expression technology (SAGE). However, alternative approaches for profiling chromatin proteins on the basis of exogenous adenine methylation (for example, by DamID) [12] or biotin ligation [13] have also been described and should prove highly complementary to ChIP-based approaches.

ChIP-microarray studies in *S. cerevisiae* revealed that methylation of histone H3 at K4 (H3K4) occurs in coding

regions and correlates globally with transcriptional status [14]. More detailed analysis at specific genes has since shown that highly active genes predominantly exhibit trimethylation at H3K4 across the 5' portions of their coding regions [15,16]. This distribution might reflect an interaction between RNA polymerase and Set1, the yeast K4 methylase [16,17], and implies a role for histone methylation in transcriptional elongation [18]. Interestingly, unbiased analysis by ChIP-SAGE indicates that histone acetylation (of H3 and H4) in yeast also peaks over the 5' portions of active protein-coding regions [19]. However, global analyses of yeast histone acetylases and deacetylases suggest that they also function by modulating histone acetylation levels in gene promoters [14,19–22].

Global views of histone modifications in mammalian cells have also been obtained, using approaches originally developed for yeast experiments. A combination of ChIP and high-resolution tiling oligonucleotide arrays was used to map the patterns of histone methylation and acetylation across human chromosomes 21 and 22 and at a series of human and mouse loci [9*]. Methylation and acetylation both typically affect short stretches of genome sequence (<1 kilobase), and approximately two-thirds of such 'punctate' modified sites map to the 5' end of a known gene. A separate study that combined ChIP and

SAGE to map H3 acetylation genome-wide in T-cells also identified punctate sites enriched for modified histones that coincide with 5' ends of genes [23[•]]. These punctate sites of modified histones at gene starts are reminiscent of those described previously in yeast and, therefore, are likely to have highly conserved functions.

Interestingly, both genomic studies [9[•],23[•]] of mammalian histone modification patterns also identified multiple 'islands' of modified histones within gene-rich, active chromosomal regions that were more distant from gene starts. Some of these islands coincide with highly conserved sequences and/or with known regulatory elements. However, others overlay sequences that do not show high conservation. Nonetheless, both studies provided evidence that these sites might also be functional. The first study compared human with mouse chromatin and found that the patterns of histone methylation at orthologous loci are highly conserved, even when the underlying DNA sequence is not [9[•]]. The second demonstrated that the DNA sequence underlying an acetylated island dramatically enhanced transcription in a reporter assay, even though it did not show high sequence conservation [23[•]]. At a minimum, these intriguing findings demonstrate a role for 'epigenomic' mapping studies in the identification of functional genomic elements that are not discernable on the basis of sequence alone.

One caveat of the previous studies is that highly-repetitive genomic regions were not considered, a significant oversight given that nearly half of the human genome is composed of repetitive sequences. Though the functional relevance of repetitive elements remains obscure, there is mounting evidence that they can mediate epigenetic silencing (see review by Lippman and Martienssen [24]). Lippman *et al.* [25^{••}] included such sequences in their tiling array analysis of a heterochromatic region of *Arabidopsis* chromosome 4. They found that transposable elements and tandem repeats are associated with heterochromatic modifications, including DNA methylation and H3K9 methylation. They also identified small inhibitory RNAs corresponding to these elements. The authors argue that the elements play a role in epigenetic silencing of proximal genes, including *FWA*, the promoter of which contains tandem repeats (see review by D Zilberman and S Henikoff [26], this issue). Despite this progress in *Arabidopsis*, it remains technically challenging to determine the chromatin status of repetitive elements, especially in organisms that contain much higher proportions of repetitive DNA. Martens *et al.* [27] used ChIP–microarray- and ChIP–PCR-based approaches to examine the different classes of repeats in the mouse genome. Their approach quantified the degree to which specific modifications associate with a particular repeat class but did not distinguish between repeats at different genomic locations. Distinct patterns of repressive methylation marks — at H3K9, H3K27 and H4K20 — were associated

with long and short interspersed nucleotide elements (LINEs and SINEs, respectively), tandem repeats, DNA transposons and retrotransposons. Tandem repeats uniquely appeared to generate double-stranded RNAs, consistent with a role for RNA interference in silencing them. Intriguingly, the methylation status of certain repeats varied according to cell type, suggesting these elements might have more critical developmental functions than is presently appreciated.

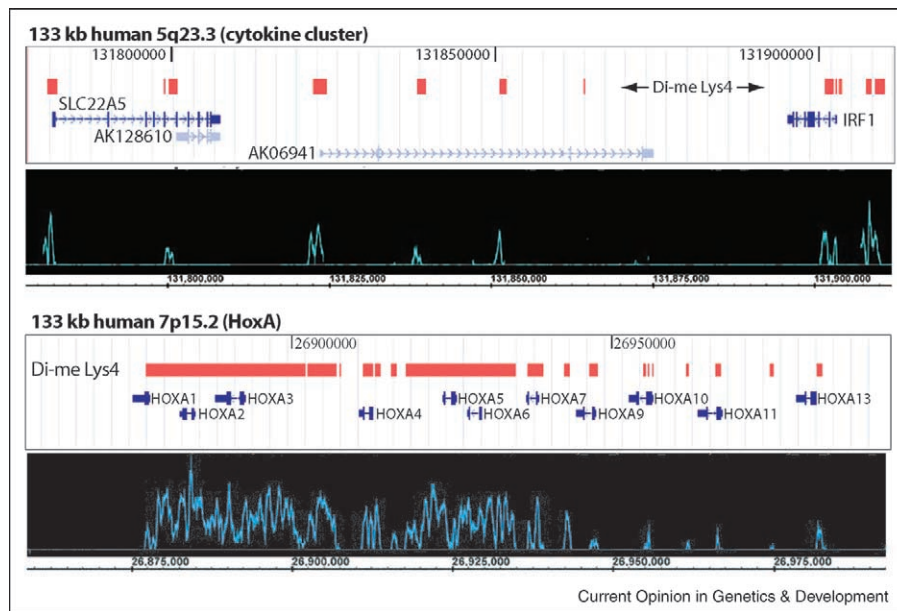
Combinatorial, specific and redundant roles for histone modifications

The large number and complexity of histone modifications has led to speculation that multiple modifications act in combinatorial fashion to mediate many distinct outcomes ('histone code') [10,28]. It has also been suggested that modifications act in a redundant fashion to ensure robust signaling [29] or function with specificity by creating protein-binding surfaces [30]. Genomic views of histone modifications from several groups offer insights into these potential models. A global study of histone modification patterns in *Drosophila* revealed a binary pattern of modifications [31]. Chromatin associated with genes generally assumed one of two states: active genes were hyperacetylated at H3 and H4 and hypermethylated at H3K4 and H3K79, whereas inactive genes lacked these modifications. Similarly, an analysis of human chromosomes 21 and 22 revealed an exquisite correlation between the distribution of histone H3K4 methylation and histone H3 acetylation [9[•]]. Whereas these studies are more consistent with redundant functions, an analysis of site-specific histone acetylation patterns in yeast identified distinct patterns of lysine acetylation for groups of biologically related genes, suggestive of specific and possibly combinatorial functions [32]. Further insight has come from two functional analyses [33,34] of the modifiable lysines in the tails of histones H3 and H4. The transcriptional consequences of mutating these residues individually, or in combination, were examined by genome-wide mRNA expression-profiling in yeast. Replacement of lysine 16 of H4 with arginine significantly reduced telomeric silencing. Previously, K16 acetylation was shown to modulate association of the SIR (silent information regulator) proteins, which mediate silencing [35]. By contrast, the other lysine residues on histones H3 and H4 altered gene expression only when mutated in combinations. Thus, whereas H4K16 appears to have specific functions that are largely related to transcriptional silencing, each of the other lysines appears to function in a 'cumulative', semi-redundant fashion [33]. Although far from decisive, these studies offer insights into the paradigms of chromatin function complementary to those gained from biochemical and genetic approaches [36].

Long-range domains in chromatin

High-resolution analysis of histone modification patterns in primary human and mouse fibroblasts revealed that

Figure 1



Punctate genomic sites and broad domains associated with modified histones. Histone modification patterns across human chromosomes 21 and 22, and at a series of orthologous human and mouse loci were recently evaluated in a ChIP-tiling array study. In most regions examined, short stretches of genome were found to associate with H3K4 methylated histones (see top panel depicting a representative portion of the human cytokine cluster; K4 methylation signal intensity is shown in blue, and regions meeting a significance threshold are shown in red; also shown are gene annotations from the UCSC Genome Browser [49]). By contrast, the *HOX* gene clusters contain broad K4 methylated regions (up to 60 kilobases in size) that overlay multiple active genes (see bottom panel depicting the human *HOXA* cluster).

remarkably large portions of the *HOX* clusters up to 60 kb in length are associated with H3K4 methylated histones [9^o]. These methylated 'domains' overlay multiple, active *HOX* genes in addition to intergenic regions that show abundant transcriptional activity (Figure 1). Such domains were not observed in the other regions examined in the mammalian tiling array study (e.g. chromosomes 21 and 22, and cytokine cluster interleukin-4 receptor loci), nor were they identified in the genome-wide ChIP-SAGE study of T-cell chromatin [23^o]. However, they are reminiscent of long-range acetylated domains described for the globin loci and for a few other genomic regions [37–40]. Though poorly understood, such active chromatin domains could be maintained through positive-feedback mechanisms analogous to those described for silenced chromatin domains [41]. The K4 methylated domains in *HOX* are highly cell type-specific and correlate with the expression of underlying genes, implying a role in the epigenetic maintenance of gene expression patterns. Given the importance of *HOX* genes in development and human disease, it will be important to determine how these domains are established and maintained, and how they affect *HOX* gene activity. It was recently shown that continuous intergenic transcription through a Polycomb response element in *Drosophila* is required to prevent epigenetic silencing and to maintain an activated state [42]. Thus, the pervasive intergenic

transcription associated with the activated mammalian *HOX* domains might itself contribute to their epigenetic maintenance.

Higher-order chromatin structures and long-range gene regulation

Insight into the temporal nature of chromatin domains and their relationship to higher-order chromatin organization can be found in a recent study by Chambeyron and Bickmore [43]. Using imaging techniques, these investigators found that *HOX* activation during cell differentiation is accompanied by chromatin decondensation and nuclear re-organization, including the looping out of *Hoxb1* from its chromosomal territory. This work, in addition to several other studies applying sophisticated imaging tools, underscores the importance of nuclear organization in defining the transcriptional status of a given gene or loci [44]. For example, whereas localization to the nuclear periphery is generally associated with gene repression, localization to the nuclear center is linked to gene-rich, active, chromosomal regions. Furthermore, looping out from core chromosomal structures or territories might be a general feature of active loci, as seen for *Hoxb1*. These large-scale structural reorganizations appear to be dynamic and developmentally determined and are likely to be regulated at the level of chromatin.

A small number of global studies are beginning to fill the gap between our understanding of the chromatin status of individual genes and loci — an understanding obtained through genomic analysis — and our understanding of global nuclear structure — obtained through imaging. Using microarrays and fluorescence *in situ* hybridization, Gilbert *et al.* [45**] investigated the distribution of compact and open chromatin fibers in human cell lines. In addition to finding a correlation between open chromatin fibers and gene-rich chromosomal regions, these investigators found that open fibers are cytologically decondensed and tend to reside outside chromosomal territories. Further insight into higher-order chromatin organization has emerged from the identifications of long-range regulatory interactions, such as those between the β -globin locus control region and specific globin genes. Carter *et al.* [46] used a novel *in situ* approach to tag and recover chromatin in the immediate vicinity of an actively transcribed β -globin gene. They found the gene to be in close physical proximity to an enhancer element within the locus control region located more than 50 kilobases away. Vakoc *et al.* [47] confirmed this higher-order looping using an orthogonal experimental approach, ‘chromosome conformation capture’ [48], and further defined a role for specific transcription factors in regulating the interaction between these distal loci. Though tools for higher-order structural analysis remain relatively low-throughput, this represents a key area of discovery with the potential to illuminate the mechanisms by which chromatin regulates genome organization and function.

Conclusions

With the availability of the complete sequence of the human genome, a key challenge is to understand its higher-level function and regulation. Valuable insight into the role of chromatin has emerged from genomic studies in human cells and in model organisms. Punctate structural elements, including nuclease hypersensitive sites and short stretches of modified histones, coincide with transcription start sites and regulatory elements. Broader regions with modified histones package the *HOX* clusters and might define epigenetically activated chromatin domains with a role in maintaining lineage fidelity. It remains to be determined how such structures are established and how their mis-regulation might contribute to the pathogenesis of disease. A smaller number of studies have begun to examine higher-order chromatin structure, its relationship to nuclear organization and its role in long-range gene regulation. In conclusion, emerging technologies in genomics and imaging are beginning to elucidate the structure of eukaryotic genomes at the level of individual genes and loci, and in terms of higher-order chromosomal organization. When interpreted in their proper biological context, these global views of chromatin should greatly enhance our understanding of the factors that determine how genome sequence is manifested at the level of an individual cell or an organism.

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