Chromatin signatures of active enhancers

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Gene-distal cis-regulatory sequences, such as enhancers, are key contributors of tissue-specific gene expression. In particular, enhancers can be located up to hundreds of kilobases from the promoters that they control, making their identification challenging. Thanks to the recent technological advances to map histone modifications and chromatin-associated factors genome-wide, several studies have begun to characterize chromatin signatures of active enhancers. Here, we discuss some of these results and how they provide new insights into the tissue-specific organization of enhancer repertoires.

Introduction

Functional enhancers are cis-regulatory DNA elements that are independent of orientation and position and can act at variable distances from the transcription start site (TSS) of the genes they regulate.1,2 They are able to establish long-range interactions with the promoters of regulated genes and are key contributors of tissue-specific gene regulation.3 Recent technological advances, particularly the combination of chromatin immunoprecipitation (ChIP) with either microarrays (ChIP-on-chip) or high-throughput sequencing (ChIP-seq), and the development of refined computational tools for the analysis of large data sets, now allow us to precisely investigate the genome-wide distribution of chromatin-binding proteins and histone modifications in any sequenced genome.4 The application of this technique, and related approaches, to a variety of developmental and differentiation systems in mammals has provided global views of the cis-regulatory elements, transcription factor (TF) functions and epigenetic processes involved in the control of gene transcription.5 In particular, they offer an increasingly clear picture of the basic enhancer organizations and functions.1,2 This review focuses on the recent finding of chromatin signatures allowing the genome-wide identification of enhancer elements and the characterization of their cell-type specific activities.

Genome-Wide Characterization of Enhancers

The flexible localization of enhancers with respect to the regulated genes have for a longtime impaired the global identification and characterization of enhancers at the whole genome level. In the past, isolation of enhancers have been based on laborious molecular approaches based on either chromatin structure (e.g., DNase I hypersensitivity assay) and/or transactivation activity (e.g., gene reporter assays), sometimes coupled with the analysis of non-coding sequence conservation.1,2 With the recent development of microarray-based and high throughput sequencing technologies, it is now possible to identify enhancers at the genome-wide scale (Table 1).5 The most straightforward strategy has been to adapt DNase I-based approaches genome wide.6,7,23-25 A similar method, named NA-Seq, used specific sequencing of restriction enzymes accessible regions for identifying cis-regulatory elements.8 An alternative approach is provided by the formaldehyde assisted isolation of regulatory elements (FAIRE) assay, which allows the recovery of the soluble (i.e., nucleosome-free) fraction of the chromatin.9,10 In parallel, ChIP-on-chip and ChIP-seq studies have shed light...
on the distribution of epigenetic marks and the striking correlations between the local enrichment for histone modifications and chromatin-associated factors and the presence and, possibly, the functional state, of transcriptional cis-regulatory elements.40–41 In particular, these studies have led to define chromatin signatures characteristic of discrete types of cis-regulatory regions (Table 1). In a pioneering study, the monomethylation of histone H3 lysine 4 (H3K4me1) was found to be a hallmark of promoter-distal cis-regulatory elements (i.e., enhancers) in HeLa cells.16 This signature was based on the relative abundance of H3K4me1 as compared with trimethylation (H3K4me3) at promoters and enhancers, the latter being defined as the gene-distal genomic regions bound by the transcription factor p300, a general transcriptional co-activator. The same group used a similar approach to demonstrate that histone modifications and chromatin-associated factors and the striking correlations between the local enrichment for histone modifications and chromatin-associated factors and the presence and, possibly, the functional state, of transcriptional cis-regulatory elements, its systematic usage to identify enhancers genome-wide might present several caveats. On the one hand, H3K4me1 enriched regions are generally larger than the associated cis-regulatory elements (for examples, see refs. 18 and 27). Consequently, it is difficult to define the exact location of the active enhancer site(s) they contain. On the other hand, the presence of H3K4me1 per se does not strictly correlate with the functional activity of these elements.16–18,33,34 In this respect, the efficiency to accurately predict functional enhancements based solely on the H3K4me1 profiles is relatively low.16,33,35 Taking these observations into consideration, it is believed that H3K4me1 patterns rather mark a wide spectrum of enhancers associated with tissue-specific genes that become further activated or repressed in a lineage-restricted manner.

### Chromatin Signatures of Active Enhancers

It is clear from the studies described above that H3K4me1 might not be the only histone modification present at enhancers elements and that a more accurate definition of the chromatin signature of active enhancers is required. By analyzing a large set of histone modifications in human CD4+ T cells, the laboratory of Keji Zhao has actually shown that putative enhancer regions are enriched in several histone marks, including the three levels of H3K4 methylation, H3K27 acetylation (H3K27ac) and the histone variant H2A.Z.27,36 More recently, three independent studies compared the epigenetic profiles of ES cells to several differentiated tissues,28,37,38,39 all finding that H3K27ac was specifically associated with active enhancers.16,27,30 Interestingly, while the class of active enhancers was found to be enriched in both H3K4me1 and H3K27ac, another class, termed “poised” enhancers (see below), was enriched in H3K4me1 only and linked to genes inactive in ES cells, but involved in orchestrating early steps in embryogenesis. Mechanistically, the presence of H3K27ac at active enhancers might reflect the recruitment of specific histone acetyl-transferases (HAT), such as p300 and CREB, which are able to acetylate H3K27 in vivo.35

As mentioned above, it has been suggested that distal regulatory elements are characterized by the presence of H3K4me3 in the absence of significant levels of H3K4me1, which is in turn associated with active gene promoters.16 However, this sharp difference between the two marks is not obvious in other studies (for examples, see refs. 27, 30, 38 and 39). In order to define more precisely the chromatin signature of active enhancers, we recently investigated the combinatorial of H3K4 methylation status in enhancer elements during the process of T lymphoid cell differentiation in the adult mouse thymus.28 Upon analyzing the dynamic of histone modifications at well-defined T-cell loci, active enhancers were found to be generally associated with the presence of both H3K4me2 and H3K4me3, while H3K4me1 was present at enhancers regardless of their functional state (Fig. 1). Interestingly, enhancer activity, as assessed by gene reporter assay, specifically correlated with the presence of H3K4me2 and H3K4me3, but not with H3K4me1 alone.18 Furthermore, gain or loss of H3K4me2/3 at distal genomic regions correlated with, respectively, the induction or the repression of associated genes during T-cell development.18 All in all, these findings strongly support the notion that distal

Table 1. Current approaches used to map distal enhancers genome-wide

| Approach      | Activity† | Specificity‡ | Examples                        |
|---------------|-----------|--------------|---------------------------------|
| DNase I-seq   | Open      | Non          | refs. 6, 7                      |
| NA-seq       | Open      | Non          | ref. 8                          |
| FAIRE        | Open      | Non          | refs. 9, 10                     |
| P0000 (ChIP-seq) | Open     | Enh. > Prom. | refs. 11–13                     |
| H3K4me1 (ChIP-seq) | Open     | Enh. > Prom. | refs. 11, 14                    |
| H3K4me2 (ChIP-seq) | Open     | Enh. > Prom. | ref. 15                         |
| H3K27ac (ChIP-seq) | Active   | Enh. > Prom. | refs. 16, 17                    |
| H3K4me3 (ChIP-seq) | Active   | Prom. > Enh. | ref. 18                         |
| Pol II (ChIP-seq) | Active   | Prom. > Enh. | refs. 19–21                     |
| BRG1 (ChIP-seq) | Active   | Enh. = Prom. | ref. 22                         |

†Refers to whether the approach allows the discrimination between poised and active enhancers. "Open" means that the approach identifies both poised and active enhancers without discrimination. In some cases, a quantitative difference in either chromatin accessibility or the enrichment of the specified factor or histone modification might be observed between poised and active enhancers.

‡Refers to whether the approach preferentially marks enhancers (Prom. > Enh.) or both regions (Enh. = Prom.).
H3K4me1 domains are enriched for lineage specific enhancers, whereas the functional activity of these regulatory regions can be assessed from the presence of additional histone modifications (Table 1).

Is there a functional redundancy between the presence of H3K27ac and H3K4me3 at active enhancers? A comprehensive study mapped nine histone modifications across nine human cell types, including common lines designated by the ENCODE consortium and primary cell types. By the recurrent combination of histone marks they were able to define 15 chromatin states, from which four corresponded to distinct categories of weak (or poised) and strong (or active) enhancers. In agreement with the results described above, active enhancers could be defined by the co-occurrence of H3K4me1, H3K4me2, H3K27ac and, for a subset of active enhancers, the additional presence of H3K4me3 and H3K9ac. It will be of interest to determine whether the presence of H3K27ac and H3K4me3 marks distinct classes of active enhancers.

Enhancer Transcription and Pol II-Dependent Deposition of H3K4me3

What could be the functional significance of H3K4 trimethylation at enhancers? H3K4me3 is closely linked to RNA-polymerase II (Pol II) recruitment and the onset of transcription initiation at gene promoters. Pol II binding at enhancers has been described at several loci and seems to be a common trait of distal regulatory elements, most probably associated with the setting of enhancers’ activity. Interestingly, active enhancers are specifically associated to the initiating (Serine 5 phosphorylated) form of Pol II and undergo localized transcription. These findings suggest the possibility that H3K4me3 may be present in enhancer regions as a consequence of local Pol II occupancy. Strikingly, distal genomic regions selected by the co-occurrence of CBP (a p300 homolog), H3K4me1, H3K4me3 and Pol II are more frequently associated with tissue-specific genes than a selection based on CBP and H3K4me1 only.

Poised Enhancers and Priming

As mentioned above, not all H3K4me1-marked enhancers are actively engaged in regulating transcription in a given cell type. Some H3K4me1-marked enhancers modulate transcription in response to differentiation cues or other cellular stimuli and are thus considered poised. It is possible that H3K4me1 marking of developmentally regulated enhancers before transcriptional activation may constitute (or reflect) an epigenetic priming mechanism.
scenario, poised enhancers might be pre-assembled in the form of an inactive nucleoprotein complex prior to activation. A striking example is provided by the inflammatory response during which the intersection of lineage determining TFs, such as PU.1 at macrophage enhancers, set the stage for the activity of stimulus-activated TFs like NF-κB, AP-1 and interferon regulatory factors (IRFs), therefore explaining cell-type specificity in inflammatory responses.43,46 Interestingly, PU.1 binds to most macrophage enhancers and is both necessary and sufficient to establish cell-type specific enhancers.43,44,46 Similarly, the C/EBPβ TF binds a large number of enhancers before induction of adipocyte differentiation and is required for their establishment.25 Subsequently, a subset of these regulatory elements remains accessible and is eventually occupied by more restricted TFs.25 Thus, simple combinations of lineage-determining and stage-specific TFs can specify the genomic sites ultimately responsible for both cell identity and cell-type-specific responses to diverse developmental and stimulatory signals (Fig. 2).

Whether poised enhancers are marked by repressive histone marks is not yet clear. On the one hand, some studies have found that CpGIs within developmentally-regulated38 or glucocorticoid-induced47 enhancers are methylated prior to activation and undergo cell-type specific DNA demethylation. On the other hand, a subset of poised enhancers in ES cells contains H3K4me1 along with the repressive histone modification H3K27me3 and, to a lesser extent, with H3K9me3.17,36 However, this does not seem to be a general rule. In our study, we found very little correlation between the presence of H3K27me3 or H3K9me2 at H3K4me1-poised enhancers.18 Similarly, in the study by Ernst et al. no correlation was found between poised enhancers and the presence of H3K27me3.22 Thus, repressive histone or DNA methylation marks are more likely to be specific to a subset of poised enhancers required during development and/or cell differentiation.

Apart from repressive histone modifications, nucleosomes present itself a major barrier for the access of TFs to their target sites in vivo. Thus, positioning of enhancer nucleosomes might be regulated to allow for binding of lineage-specific TFs during development and/or cell activation. Recent studies have suggested that the activation of transcription is indeed correlated with the reorganization of nucleosomes at enhancer elements.11,48-51 In this respect, master regulators are thought to initially bind the enhancer and remodel nucleosome positioning in order to allow other TFs to gain access to their target sequences (Fig. 2). This process is, at least, partially mediated by the recruitment of ATP-dependent remodeling factors such as BRG1, a subunit of the SWI/SNF remodeling complex.48 Interestingly, distal BRG1 binding is well correlated with the regulation of gene expression and represent a useful marker for identifying distal cis-regulatory regions.11,48 For instance, during erythroid differentiation, binding of TAL1 to enhancers is facilitated by GATA1 recruitment of BRG1 and
subsequent remodeling of enhancer associated nucleosomes.48

Conclusion

The recent technological advances to map histone modifications and chromatin-associated factors genome-wide have provided new and exciting insights into the tissue-specific organization of enhancer repertoires. The general view that comes out from these studies is summarized in Figure 2. The analysis of chromatin signatures of enhancers in different cell types has provided direct evidence that the activity of this elements show very little overlap across different tissues, suggesting that a sizable fraction of the enhancer repertoire may be cell-type specific. In other words, all of the possible regulatory regions in the genome, only a small subset is selected for activation in a given cell type, which is probably essential for cell differentiation. Discriminating between poised and active enhancers will be crucial to unveil transcriptional regulatory networks during normal development and disease. Furthermore, given the potential impact of genomic alterations at distal regulatory regions in cancer and disease, it will be critical to dispose in the near future of a comprehensive catalog of all enhancer elements potentially active in every cell type and their pathological counterparts. The current efforts by the International Human Epigenome Consortium, HepG2 (human-hepatoma cell line) project, and the ENCODE project (www.genome.gov/encode) to coordinate large-scale mapping and characterization of the epigenome will be essential to achieve this goal.

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