The Chromatin Fingerprint of Gene Enhancer Elements*

Published, JBC Papers in Press, September 5, 2012, DOI 10.1074/jbc.R111.296491
Gabriel E. Zentner* and Peter C. Scacheri†

From the *Department of Genetics and Genome Sciences and Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio 44106 and the †Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109

Different cell types within a single organism are generally distinguished by strikingly different patterns of gene expression, which are dynamic throughout development and adult life. Distal enhancer elements are key drivers of spatiotemporal specificity in gene regulation. Often located tens of kilobases from their target promoters and functioning in an orientation-independent manner, the identification of bona fide enhancers has proved a formidable challenge. With the development of ChIP-seq, global cataloging of putative enhancers has become feasible. Here, we review the current understanding of the chromatin landscape at enhancers and how these chromatin features enable robust identification of tissue-specific enhancers.

All multicellular organisms are faced with a daunting challenge: the establishment of tissue- and temporal-specific transcriptional programs from a single genome sequence. Spatiotemporal regulation of gene expression is a complex process involving the coordinated actions of transcription factors and chromatin-remodeling and chromatin-modifying enzymes as well as distinct classes of functional elements, including promoters, insulators, and enhancers. Enhancers are speculated to be the most abundant class of regulatory element, perhaps making up as much as 10% of the human genome (1).

Enhancers were initially identified in cell culture using transient reporter assays as sequences that up-regulated transcription without regard to orientation or distance (2, 3). This flexibility in location and function has posed a significant obstacle to comprehensive cataloging of enhancers in comparison with other elements such as promoters, which can be characterized by 5'-sequencing of genes, or insulators, which are generally bound by the CCCTC-binding factor and tend to be invariant across cell types (4, 5). Various techniques have been used to locate enhancers, including mapping of open chromatin by DNase I hypersensitivity (6, 7) or formaldehyde-assisted isolation of regulatory elements (FAIRE) (8, 9) and analysis of evolutionary conservation of putative enhancer sequences, with the assumption that functional sequences are under a high degree of evolutionary constraint (10). Although these methods have identified a large number of functional regulatory elements, there are limitations associated with each. Sites of open chromatin overlap many types of regulatory elements, including promoters, enhancers, and insulators (6–8); thus, it cannot be determined without functional studies or additional genomic data if a site of open chromatin is in fact an enhancer. It has also become apparent that many enhancers are not as highly conserved as predicted (10–15), likely leading to a poor rate of enhancer discovery using sequence conservation alone.

The development of chromatin immunoprecipitation with massively parallel short read sequencing (ChIP-seq) has enabled genome-wide mapping of hundreds of histone modifications, transcription factors, and other chromatin-bound proteins (16–19). In particular, the study of enhancers, whose identification had previously proved quite challenging for the reasons described above, has benefitted from the rapid maturation of ChIP-seq. Here, we discuss the current understanding of chromatin features at enhancers as determined by ChIP-seq.

H3K4me1 and P300

Early studies profiling the genomic distributions of histone modifications focused on H3K4me1 in the absence of H3K4me3, which is highly enriched at promoters, as a chromatin signature of enhancers. Using chromatin immunoprecipitation with tiled microarray analysis (ChIP-chip), Heintzman et al. (22, 23) identified binding sites for the acetyltransferase P300, a known enhancer-binding protein (20, 21). The majority of promoter-distal P300-binding sites showed robust enrichment of H3K4me1, suggesting that this histone mark constituted a chromatin signature of enhancers (22, 23). An important insight from initial genomic mapping of H3K4me1 was that its distribution tended to be highly cell type-specific, in contrast to H3K4me3, which tended to be invariant across cell types (5, 22, 23), consistent with the fact that enhancers generally act in a cell type-specific manner. However, H3K4me1 alone was found not to be absolutely predictive of enhancer activity. When a subset of distal H3K4me1 sites were tested in reporter assays, seven of nine sites were active (22). Additionally, further studies of enhancer-associated histone modifications by ChIP-seq showed that H3K4me3 was enriched at a subset of putative enhancers (24, 25), suggesting that enrichment of H3K4me1 in the absence of H3K4me3, although a robust predictor of activity, might not identify all active enhancers in the genome.

Other studies focused on P300 binding without regard to H3K4me1 enrichment. Visel et al. (26) performed P300 ChIP-seq in mouse embryonic forebrain, midbrain, and hind limb and cloned P300-binding sites from each tissue for in vivo

*This work was supported, in whole or in part, by National Institutes of Health Grant R01 HD056369 from NICHD and Grant SR01 HG004722 from the National Human Genome Research Institute (to P. C. S.). This first article in the Thematic Minireview Series on Results from the ENCODE Project: Integrative Global Analyses of Regulatory Regions in the Human Genome.

1 To whom correspondence should be addressed. E-mail: px183@case.edu.

2 The abbreviations used are: FAIRE, formaldehyde-assisted isolation of regulatory elements; mESC, mouse embryonic stem cell; hESC, human embryonic stem cell; pol II, RNA polymerase II; ncRNA, noncoding RNA.

3 H3K4me1 indicates histone H3 lysine 4 monomethylation. Because of the large number of histone modifications discussed in this minireview, this nomenclature will be adhered to throughout: histone name, residue (K lysine) for all discussed modifications, and modification state (monomethylation (me1), dimethylation (me2), trimethylation (me3), and acetylation (ac)).
reporter assays. This method yielded an overall 87% success rate in predicting tissue-specific enhancer activity. Notably, this was a robust increase in accuracy (87% versus 46%) compared with previous studies predicting enhancers on the basis of sequence conservation (27, 28). Further studies also showed that P300 binding could identify poorly conserved enhancers in mouse embryonic hearts with a 75% success rate (29). Thus, although P300, like H3K4me1, was a strong predictor of enhancer activity, the failure of P300 binding to completely predict enhancer activity suggested that additional chromatin features at enhancers might provide more power in detecting active enhancers.

**Subclassification of Enhancers: H3K27 Modifications**

Recent studies have focused on covalent modification of H3K27 as a means to subclassify enhancers. Global analysis of H3K27ac in mouse embryonic stem cells (mESCs) and several differentiated mouse cell types revealed association of this mark with a fraction of putative enhancers (30), a finding confirmed in human ESCs (hESCs) (31). Genes associated with H3K4me1+, H3K27ac+ enhancers in human and mouse cells displayed higher expression than those lacking H3K27ac and were also associated with functional annotations relevant to the analyzed cell type (30, 31). Thus, H3K4me1+, H3K27ac+ enhancers represent cell type-specific active enhancers in both human and mouse cells.

In contrast to active enhancers, H3K4me1+, H3K27ac− enhancers in ESCs were associated with differentiation-specific functions and genes encoding developmentally important transcription factors, suggesting that these enhancers might be poised for activation upon differentiation (30, 31). Indeed, a number of mESC poised enhancers gained H3K27ac and were associated with increased gene expression upon differentiation, whereas mESC-specific enhancers lost H3K27ac or both H3K4me1 and H3K27ac and were associated with decreased gene expression (30). A similar phenomenon was observed for hESC poised enhancers (31). It is noteworthy that enhancers designated poised in hESCs were marked with H3K27me3, a hallmark of Polycomb repression (32), in contrast to mESC poised enhancers. However, further studies of enhancer-associated histone marks in mESCs and differentiated mouse cells revealed that a fraction of enhancers did indeed contain H3K27me3 (33). Furthermore, it was found that active, H3K27−poised, and H3K27me3+ poised enhancers could be stratified into three distinct groups by the expression and function of their associated genes. H3K4me1+, H3K27− enhancers were therefore designated intermediate, and H3K4me1+, H3K27me3− enhancers were designated poised. Active enhancers could be subclassified based on their levels of H3K4me1 and H3K27ac, and poised enhancers could be subclassified based on their levels of H3K27me3. H3K9me3 was also able to distinguish poised enhancers, independent of H3K27me3 (33). This is an interesting observation in light of a previous report implicating SETD81, which trimethylates H3K9, in maintenance of the mESC state (34). Paired enhancers were associated with developmental functions, whereas intermediate enhancers were linked to a variety of general non-specific cellular functions (33). Paired enhancers in mESCs and hESCs were bound by Polycomb proteins (31, 33), consistent with the H3K27me3 enrichment seen at these sites. H3K27me3-marked poised enhancers were also detected in terminally differentiated mouse cell types, including adipocytes and macrophages, suggesting that Polycomb-mediated enhancer poising is not restricted to developmental cell types (33).

Further classification of enhancers was also carried out by Ernst et al. (18), who mapped chromatin dynamics in nine human cell lines. Two classes of active enhancers were defined based on aggregate enhancer characteristics across these cell types. One was characterized by robust enrichment of H3K4me1, H3K4me2, H3K4me3, H3K27ac, and H3K9ac. The other contained robust H3K4me1 and H3K27ac and moderate H3K4me2 and H3K9ac. Two classes of weak or poised enhancers were also characterized. One was marked with moderate H3K4me1 and robust H3K4me2, whereas the other contained only moderate H3K4me1. No H3K27me3 enrichment was detected at weak/poised enhancers, contrary to a previous report on human poised enhancers (31). It has been suggested that marking of enhancers with H3K27me3 is a developmental phenomenon (35), and thus, the detection of enhancer chromatin features by aggregate characteristics across nine cell lines, only one of which was developmental (hESCs), may have obscured this characteristic. Consistent with previous findings, genes associated with active enhancers were associated with cell type-specific functions in each of the nine cell lines.

The characterization of active, intermediate, and poised enhancer classes sheds light on several reasons why previous approaches to enhancer identification were unable to absolutely predict activity. The association of H3K4me1 with both active and inactive enhancers is a simple explanation for the failure of some H3K4me1-marked regions to show activity in reporter assays (22). It was also found in these studies that P300 was associated with all classes of enhancers (30, 31, 33); thus, the inactivity of a fraction of P300-bound regions in transgenic reporter assays (26, 29) is likely due to the fact that these were intermediate or poised enhancers. Finally, it was found that H3K27me3+ poised enhancers were more highly conserved than other enhancer classes (31, 33), suggesting that the inactivity of a large proportion of highly conserved elements in reporter assays (27, 28) may be due to a bias toward testing poised enhancers.

**Transcriptional Chromatin Signatures at Enhancers**

An intriguing recent finding potentially related to enhancer function is that a subset of active enhancers appear to produce a variety of noncoding transcripts (18, 36–38). ChIP-seq analysis of RNA polymerase II (pol II) and its serine 2/5-phosphorylated forms revealed their association with a large number of enhancers (33, 36, 38). Additionally, a recent study has demonstrated the presence of substantial H3K36me3, indicative of transcriptional elongation (39), at active enhancers (33), suggesting that active enhancers adopt a transcriptionally active chromatin state.

Given that active enhancers are associated with noncoding RNA (ncRNA) and show similar chromatin features to promoters, it has been suggested that active enhancers may actually be
promoters regulating ncRNA expression rather than enhancers per se. However, active enhancers have little or no H3K4me3, which is generally high at promoters. Another potential scenario is that enhancer-associated ncRNAs are the product of leaky transcription from pol II promoters during transient looping of enhancers to promoters. Indeed, production of ncRNA from the Arc enhancer requires the presence of the Arc promoter (36). Although the relation of enhancer transcription to enhancer function is largely unclear, there is some evidence that enhancer-associated transcripts are functional. The β-globin locus control region is unidirectionally transcribed into a ncRNA that promotes open chromatin throughout the β-globin locus (40). The Evf-2 ncRNA, transcribed from the Dlx-5/6 ultraconserved region, acts as a transcriptional coactivator via association with Dlx-2 (41). Finally, several predicted enhancers bound by pol II in macrophages were able to activate transcription in luciferase assays (38). However, in most cases, the true identity and function of enhancer-associated ncRNAs remain to be determined.

The Depth of Histone Modification at Enhancers

Although the studies described above have focused on only a handful of marks, there are several dozen characterized histone modifications, with more being discovered at a rapid pace (42–44), suggesting that the spectrum of histone modifications at enhancers is likely much broader than is currently appreciated. Indeed, Wang et al. (24), mapping 39 histone modifications in CD4+ T cells by ChIP-seq, detected enrichment of many other modifications at putative enhancers, including multiple acetylations on H2A, H2B, and H4, H3K18ac, and H3K9me1. Mapping of histone marks in hESCs and IMR90 fibroblasts also revealed association of additional modifications at a subset of putative enhancers, including H2BK5ac, H3K18ac, and H4K5ac (45). H3K18 can be acetylated by P300 (46–48), suggesting a further link between this modification and enhancer chromatin structure.

Histone Variants

In addition to histone modifications, variant forms of the canonical H2A and H3 histones (49) have been suggested to play a role in modulating enhancer chromatin structure. Previous studies have found enrichment of the universally conserved H2A variant H2A.Z (50) at putative enhancers (24, 25). Enhancers have generally been considered to be nucleosome-depleted based on the results of DNase-seq and FAIRE-seq experiments (6, 31, 33). Previous studies of nucleosomes containing the H3 variant H3.3 and the H2A variant H2A.Z indicated that these nucleosomes were unstable and disrupted by moderate salt conditions (51). Subsequent genome-wide mapping of H3.3 and H2A.Z under conditions of low ionic strength demonstrated that H3.3/H2A.Z double variant nucleosomes were strongly enriched at putative enhancers (52). This finding suggests that the chromatin accessibility of enhancers is not mediated by nucleosome depletion per se, but by the fragility of H3.3/H2A.Z nucleosomes, and that standard protocols for assaying open chromatin involving moderate or high salt conditions lead to loss of double variant nucleosomes.

Table 1

| Feature                  | No. in human genome | No. in mouse genome |
|--------------------------|---------------------|---------------------|
| DNase-seq/FAIRE-seq      | 80,000–225,000 (9, 98, 99) | 121,378–720,631 (71, 100) |
| Distal H3K4me1 ± H3K27ac | 24,566–58,023 (22, 45) | 17,809–76,001 (30, 33, 55) |
| Distal P300              | 1664–8703 (31, 63, 67) | 540–10,741 (22, 26, 29, 33, 62, 64, 91) |

Cell Type-specific Transcription Factors

Transcription factor motif analysis has also been used to predict cell type-specific active enhancers. It is known that distal H3K4me1 sites are often bound by cell type-specific transcription factors, i.e. OCT4 in ESCs (30), FOXA1/2 in liver (53, 54), and PU.1 in macrophages and B cells (55). Ernst et al. (18) carried out an extensive analysis of transcription factor motifs within cell type-specific active enhancers across nine human cell lines and found strong enrichment of motifs for cell type-specific factors. For instance, robust enrichment of OCT4 motifs was found within hESC-specific active enhancers, whereas enrichment of motifs for liver transcription factors (HNF1, HNF4, and peroxisome proliferator-activated receptor-γ) was detected in active enhancers in the HepG2 hepatocellular carcinoma line. Further analysis revealed robust correlations between enrichment of transcription factor motifs within active enhancers and the expression of their cognate factors across all nine cell lines. Thus, it may be inferred that cell type-specific transcription factors are key drivers of enhancer activity. Indeed, analysis of HepG2 enhancers containing HNF4 motifs in reporter assays showed that enhancer activity was partially dependent on this motif in many cases.

Certain cell type-specific transcription factors may promote enhancer activity by establishing an active chromatin state. These proteins, referred to as pioneer factors, bind closed chromatin and enable the binding of additional regulatory factors (56). For instance, FOXA1, a transcription factor involved in endoderm development (57), binds regions throughout the genome in breast and prostate cancer cells to promote nucleosome remodeling upon hormone stimulation (58–60). It appears that nucleosome displacement is a key aspect of enhancer activation, as histone modifications such as H3K4me2 are already present at enhancers prior to activation (54, 58, 59, 61).

General Enhancer-binding Proteins

In addition to cell type-specific transcription factors, which function in a restricted range of tissues, it is also known that there are factors that associate with enhancers across a wide range of cell types. As indicated above, the acetyltransferase P300 binds enhancers across a wide range of cell types and tissues (22, 23, 26, 29–31, 33, 62–68). P300 generally binds to a small subset of H3K4me1-defined enhancers in the genome (Table 1). It has also been shown that the chromatin-remodeling protein CHD7, mutated in the developmental disorder CHARGE syndrome (69), binds putative enhancers in several cell types (70). CHD7 occupies many P300-bound enhancers in
mESCs but binds a larger fraction of H3K4me1-predicted enhancers than does P300 (33, 71). Another chromatin-remodeling enzyme, BRG1, also appears to function as an enhancer-binding factor in several cell types (31, 72–74). BRG1 expression is altered in cancers (75–79), and its loss may play a role in the pathogenesis of CHARGE syndrome (80). Some evidence also suggests that CHD7 may be involved in cancer (81, 82). These findings highlight the general importance of chromatin remodeling at enhancers and also suggest that dysregulation of enhancer chromatin structure is pathogenic.

### How Many Enhancers Are in the Genome?

The number of enhancers present in the human genome has been the subject of much speculation. Heintzman et al. (22) detected ~55,000 enhancers between the HeLa cervical carcinoma and K562 leukemia cell lines based on H3K4me1 enrichment, most of which were cell type-specific. By extrapolation to the 200 cell types of the human body, it was speculated that there could be on the order of ~10^5–10^6 enhancers in the genome. However, because it is not known if H3K4me1 is present at all potential enhancer sites, this is only a rough estimate at present.

Within a given cell type, the number of enhancers as defined by promoter-distal H3K4me1 and/or H3K27ac enrichment is quite variable, ranging from ~18,000 to 76,000 in human and mouse cell lines and tissues (Table 1). The number of distal H3K4me1 and/or H3K27ac sites in each cell type is generally far lower than the number of sites of open chromatin detected by DNase-seq and FAIRE-seq, highlighting the utility of histone modification ChIP-seq in locating enhancers (Table 1). However, the figures presented here should be taken only as approximations. Variations in experimental and analytical parameters likely contribute a degree of variability to these enhancer surveys, and other histone modifications may mark enhancers independent of H3K4me1.

### Summary and Future Challenges

Although the classification of enhancers by chromatin state is in its early stages, some general trends have emerged. Active enhancers, i.e. enhancers associated with high levels of gene expression and functions consistent with the current cell type, are marked with both H3K4me1 and H3K27ac (30, 31, 33). Active enhancers may demonstrate transcription of ncRNA and display a transcriptional chromatin signature (H3K36me3 and pol II enrichment) (33, 36, 38). Within a given cell type, active enhancers often harbor motifs for cell type-specific transcription factors (18). Poised enhancers tend to be associated with low levels of gene expression and functions required later in development or upon stimulation. They are marked with H3K4me1 and may also contain H3K27me3 (30, 31, 33). Histone modifications associated with enhancers are summarized in Table 2.

Although our understanding of enhancer chromatin states has been greatly enhanced by the use of ChIP-seq, many unresolved issues related to chromatin state and enhancer function remain. Several of these are summarized below.

1) A major issue facing the field of enhancer genomics is the gap between prediction and functional validation of putative enhancers. Enhancer trap (86) and transgenic reporter (26) assays, which involve integration of reporter vectors into the genome, may be advantageous in this regard. However, these assays are relatively low throughput, expensive, and impractical for most laboratories. Luciferase reporter assays are generally accepted as a validation method, but there are several limitations to this approach. First, reporter assays require the use of plasmids, which lack native chromatin conformation and are thus not representative of the chromatin context in which the putative enhancer sequence would normally function. Second, the plasmids used in enhancer assays usually contain a general promoter such as SV40 rather than the enhancer’s endogenous promoter, and it is therefore not clear how to interpret negative results. Finally, most genes are thought to be regulated by more than one enhancer, yet it is typically only a single enhancer that is tested in a reporter assay. Thus, in instances in which gene activation requires multiple enhancers, reporter activity may not be observed when a single enhancer is tested, although that enhancer might still be functional in vivo.

2) It is known that additional histone modifications localize to putative enhancers (24, 45), but it is unclear how they relate to the currently understood enhancer chromatin states. It is also possible that entirely novel enhancer chromatin signatures will be discovered, as there is no reason a priori why all enhancers would be marked with H3K4me1.

3) It is unclear how histone marks are maintained at enhancers, where there is nucleosome remodeling (59, 60), variant deposition (52), and rapid histone turnover (83). However, because robust ChIP-seq signals can be obtained for histone marks at enhancers, it stands to reason that there must be a mechanism in place to rapidly modify newly deposited histones. Histone-modifying enzymes such as P300 and EZH2 may remain bound to enhancers and modify histones as they are present. Additionally, histone-modifying enzymes such as CREB-binding protein and p300 may be recruited to enhancers to facilitate chromatin remodeling and transcriptional activation.

### Table 2

**Characteristics of enhancer-associated histone modifications**

| Modification            | Description                                                                 | Refs.     |
|-------------------------|-----------------------------------------------------------------------------|-----------|
| H3K4me1/2               | Associated with active, intermediate, and poised enhancers                  | 22–24, 30, 31, 33, 58 |
|                         | Widely considered to be the chromatin signature of enhancer elements        |           |
|                         | May be necessary for recruitment of pioneer factors (i.e. FOXA1)            |           |
| H3K9me3                 | Associated with poised enhancers                                           | 33, 34    |
|                         | Established by SETDB1                                                      |           |
| H3K27ac                 | Associated with active enhancers                                          | 30, 31, 33, 46–48 |
|                         | Established by P300                                                        |           |
| H3K27me3                | Associated with poised enhancers                                          | 31–33     |
|                         | Established by Polycomb-group protein EZH2                                |           |
| H3K36me3                | Associated with active enhancers                                          | 33, 36, 38 |
|                         | pol II is also present at H3K36me3-marked enhancers, consistent with ncRNA transcription from these enhancers |           |

For a complete list of references, please consult the original article.
deposited. However, this mechanism is speculative, and the kinetics of the erasure and re-establishment of histone marks at enhancers are not precisely known.

4) Although putative enhancers can efficiently be identified based on chromatin signatures, it is currently difficult to accurately determine the genes associated with enhancers on a large scale. Current computational strategies include associating an enhancer with its nearest gene and associating an enhancer with all genes located within a certain distance. There are several issues associated with these methods: enhancers can often be located quite far from their target genes and/or within other genes (for instance, a limb-specific Shh enhancer is located within an intron of the Lmbr1 gene, over 1 megabase away from its target promoter (84, 85)), enhancers may not regulate all genes within a given distance, and current computational methods do not take into account enhancer-blocking CCCTC-binding factor boundaries (4). Global approaches such as chromosomal conformation capture (3C)-based technologies (86–88), chromatin interaction analysis with paired-end tag sequencing (ChIA-PET) (89–91), and 3C with DNA selection and ligation (3D-DSL) (92) have been used to study long-range chromatin interactions but are difficult to execute and may not have the resolution needed to see interactions over a broad range of distances. Thus, to conclusively associate an enhancer with a target promoter, low throughput 3C must be used.

5) Because it has been hypothesized that enhancers contact their target promoter(s) (1), it is possible that enhancer chromatin state is influenced by target promoter state or vice versa. Indeed, Rada-Iglesias et al. (31) showed that promoters associated with H3K27me3+ poised enhancers in hESCs also displayed appreciable levels of H3K27me3. Whether this is a general or Polycomb-specific phenomenon is not clear and bears further study.

6) It is not known if poised enhancers contact their target promoters. It may be argued that this is the case based on the finding that poised enhancers and their nearest promoters are both marked by H3K27me3 and/or H3K27me1 (31), but it may simply be that Polycomb proteins bind both enhancers and promoters independently and establish H3K27me3 to prevent looping. Analysis of chromatin conformation is necessary to address this issue.

7) As with most biological processes involving histone modification (93), a question of causality remains. Do histone marks define enhancers, or are they a consequence of the establishment of the enhancer state? The majority of distal H3K4me1 sites are cell type-specific (5, 22, 23, 30), indicating that this mark is established de novo at enhancers during differentiation and arguing that cells possess an intrinsic “knowledge” of all genomic regions that are enhancers independent of histone modification. This knowledge might be mediated by CpG methylation levels, as there appears to be an inverse correlation between DNA methylation and enhancer activity (94–97), although the mechanistic basis of this relationship is unclear. Because of the large number of potential enhancers in the genome and the fact that cells generally display far fewer distal H3K4me1 sites than potential enhancers, it stands to reason
that histone modifications, particularly H3K4me1, H3K27ac, and H3K27me3, may provide a means to “bookmark” these enhancers for immediate or later use. Thus, histone marks do not define enhancers per se but are used by the cell as a means to “arm” a subset of enhancers for activity by modulating chromatin structure and/or mediating interactions with non-histone proteins.

Despite these issues, the ChIP-seq studies summarized in this minireview provide a hypothetical model by which enhancer chromatin state might be established (Fig. 1). Prior to differentiation or stimulation, enhancer DNA is occupied by nucleosomes not modified at H3K4. Upon stimulation, the cell’s intrinsic memory of enhancers facilitates H3K4 methylation. H3K4me1/2, H3K27ac, H3K27me3, and H3K9me3. These models are speculative at present, and much work is needed to conclusively determine how enhancer chromatin state is established. However, with the increasing power of ChIP-seq, these mechanisms will surely not remain a mystery for long.

Acknowledgment—We thank Peggy Farnham for critical reading of the manuscript.

REFERENCES

1. Bulger, M., and Groudine, M. (2011) Functional and mechanistic diversity of distal transcriptional enhancers. Cell 144, 327–339
2. Gillies, S. D., Morrison, S. L., Oi, V. T., and Tonegawa, S. (1983) A tissue-specific transcriptional enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell 33, 717–728
3. Banerji, J., Olson, L., and Schaffner, W. (1983) A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell 33, 729–740
4. Phillips, J. E., and Corces, V. G. (2009) CTCF: master weaver of the genome. Cell 137, 1194–1211
5. Xi, H., Shulha, H. P., Lin, J. M., Vales, T. R., Fu, Y., Bodine, D. M., McKay, R. D., Chenoweth, J. G., Tesar, P. J., Furey, T. S., Ren, B., Weng, Z., and Crawford, G. E. (2007) Identification and characterization of cell type-specific and ubiquitous chromatin regulatory structures in the human genome. PLoS Genet. 3, e136
6. Crawford, G. E., Holt, I. E., Whittle, J., Webb, B. D., Tai, D., Davis, S., Margulies, E. H., Chen, Y., Bernat, J. A., Ginsburg, D., Zhou, D., Luo, S., Vasicek, T. J., Daly, M. J., Wolfsberg, T. G., and Collins, F. S. (2006) Genome-wide mapping of DNase-hypersensitive sites using massively parallel signature sequencing (MPPS). Genome Res. 16, 123–131
7. Hesselberth, J. R., Chen, X., Zhang, Z., Sabo, P. J., Sandstrom, R., Reynolds, A. P., Thurman, R. E., Nep, S., Kuehn, M. S., Noble, W. S., Fields, S., and Stamatoyannopoulos, J. A. (2009) Global mapping of protein-DNA interactions in vivo by digital genomic footprinting. Nat Methods 6, 283–289
8. Giresi, P. G., Kim, J., McDaniel, R. M., Iyer, V. R., and Lieb, J. D. (2007) FAIRE (formaldehyde-assisted isolation of regulatory elements) isolates active regulatory elements from human chromatin. Genome Res. 17, 877–885
9. Gaulton, K. J., Nammo, T., Pasquali, L., Simon, J. M., Giresi, P. G., Fogarty, M. P., Panhuis, T. M., Mieczkowski, P., Secchi, A., Bosco, D., Berner, T., Montanya, E., Mohlke, K. L., Lieb, J. D., and Ferrer, I. (2010) A map of open chromatin in human pancreatic islets. Nat. Genet. 42, 255–259
10. Noonan, J. P., and McCallion, A. S. (2010) Genomics of long-range regulatory elements. Annu. Rev. Genomics Hum. Genet. 11, 1–23
11. McGaughy, D. M., Vinton, R. M., Huynh, J., Al-Sail, A., Beer, M. A., and McCallion, A. S. (2008) Metrics of sequence constraint overlook regulatory sequences in an exhaustive analysis at phox2b. Genome Res. 18, 252–260
12. Taher, L., McGaughy, D. M., Maragh, S., Aneas, I., Bessling, S. L., Miller, W., Nobrega, M. A., McCallion, A. S., and Ovcharenko, I. (2011) Genome-wide identification of conserved regulatory function in diverged sequences. Genome Res. 21, 1139–1149
13. Fisher, S., Grice, E. A., Vinton, R. M., Bessling, S. L., and McCallion, A. S. (2006) Conservation of RET regulatory function from human to zebrafish without sequence similarity. Science 312, 275–279
14. Odom, D. T., Dowell, R. D., Jacobsen, E. S., Gordon, S. E., Danford, T. W., Danford, W. D., Danford, T. W., Maclsaac, K. D., Rolfe, P. A., Conboy, C. M., Gifford, D. K., and Fraenkel, E. (2007) Tissue-specific transcriptional regulatory function has diverged significantly between human and mouse. Nat. Genet. 39, 730–732
15. ENCODE Project Consortium (2007) Identification and analysis of functional elements in 1% of the human genome by the ENCODE Pilot Project. Nature 447, 799–816
16. Gerstein, M. B., Lu, Z. J., Van Nostrand, E. L., Cheng, C., Arshinoff, B. I., Liu, T., Yip, K. Y., Robinollo, R., Rechtsteiner, A., Ikegami, K., Alves, P., Chateignier, A., Perry, M., Morris, M., Auerbach, R. K., Feng, X., Leng, J., Vielle, A., Niu, W., Rhrissorrakrai, K., Agarwal, A., Alexander, R. P., Barber, G., Brdlik, C. M., Brennan, J., Brouillet, J. J., Carr, A., Cheung, M. S., Clawson, H., Contrino, S., Dannenberg, L. O., Dernburg, A. F., Desai, A., Dick, L., Dosé, A. C., Du, J., Egelhofer, T., Ercan, S., Euskirchen, G., Ewing, B., Feingold, E. A., Gassmann, B., Good, P. J., Green, P., Gu, T., Gutwein, M., Guyer, M. S., Habetler, J. C., Han, T., Henikoff, J. G., Henz, S. R., Hinrichs, A., Hölste, H., Hymann, T., Iniguez, A. L., Janet, J., Jensen, M., Kato, M., Kent, W. J., Kephart, E., Khivansara, V., Khurana, E., Kim, J. K., Kolasinska-Zwierz, P., Lai, E. C., Latorre, I., Leahy, A., Lewis, S., Lloyd, P., Lochovsky, L., Lowdon, R. F., Luebing, Y., Lyne, R., MacCoss, M., Mackowiak, S. D., Mangone, M., McKay, S., Mecenas, D., Merrell, G., Miller, D. M., Muroyama, A., Murray, J. I., Ooi, S. L., Pham, H., Phippen, T., Preston, E. A., Rajewsky, N., Rätsch, G., Rosenbaum, H., Rozovsky, I., Rutherford, K., Ruzanov, P., Sarov, M., Sasidharan, R., Shoner, A., Scheid, P., Segal, E., Shin, H., Shou, C., Slack, F. J., Slightam, C., Smith, R., Spencer, W. C., Stinson, E. O., Taing, S., Takasaki, T., Vafeados, D., Voronina, K., Wang, G., Washington, N. L., Whittle, C. M., Wu, B., Yan, K., Zeller, G., Zha, Z., Zhong, M., Zhou, X., modENCODE Consortium, Ahringer, J., Strome, S., Gunsalus, K. C., Micklem, G., Liu, X. S., Reinke, V., Kim, S. K., Hillier, L. W., Henikoff, S., Piano, F., Snyder, M., Stein, L., Lieb, J. D., and Waterston, R. H. (2010) Integrative analysis of the Caenorhabditis elegans genome by the modENCODE Project. Science 330, 1775–1787
17. modENCODE Consortium, Roy, S., Ernst, J., Kharchenko, P. V., Kheradpour, P., Negre, N., Eaton, M. L., Landolm, J. M., Bristow, C. A., Ma, L., Lin, M. F., Washietl, S., Arshinoff, B. I., Ay, F., Meyer, P. E., Robine, N., Washington, N. L., Di Stefano, L., Berezikov, E., Brown, C. D., Candeias, R., Carlson, J. W., Carr, A., Jungreis, I., Marbach, D., Seallon, R., Tolsto- rukov, M. Y., Will, S., Alekseyenko, A. A., Artieri, C., Booth, B. W., Brooks, A. N., Dai, Q., Davis, C. A., Duff, M. O., Feng, X., Gororchak, A. A., Gu, T., Henikoff, J. G., Kapranov, P., Li, R., MacAlpine, H. K., Malone, J., Minoda, A., Nordman, J., Okamura, K. P., Perry, M., Powell, S. K., Riddle, N. C., Sakai, A., and Samsonova, A. (2010) Identification of functional elements and regulatory circuits by Drosophila modENCODE. Science 330, 1787–1797
18. Ernst, J., Kheradpour, P., Mikkelsen, T. S., Shores, N., Ward, L. D., Epstine, C. B., Zhang, X., Wang, L., Issner, R., Coyne, M., Ku, M., Durham, T., Kellis, M., and Bernstein, B. E. (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 473, 43–49
19. Raney, B. J., Cline, M. S., Rosenbloom, K. R., Dreszer, T. R., Learned, K.
MINIREVIEW: Enhancer Chromatin States

Barber, G. P., Meyer, I. R., Sloan, C. A., Malladi, V. S., Roskin, K. M., Suh, B. B., Hinrichs, A. S., Clawson, H., Zweig, A. S., Kirkup, V., Fujita, P. A., Rheie, B., Smith, K. E., Pohl, A., Kuhn, R. M., Karolchik, D., Haussler, D., and Kent, W. J. (2011) ENCODE whole-genome data in the UCSC Genome Browser (2011 update). *Nucleic Acids Res.* 39, D871–D875

Merika, M., Williams, A. J., Chen, G., Collins, T., and Thanos, D. (1998) Recruitment of CBP/p300 by the IFNβ enhancerosome is required for synergistic activation of transcription. *Mol. Cell* 1, 277–287

Wang, Q., Carroll, J. S., and Brown, M. (2005) Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. *Mol. Cell* 19, 631–642

Heintzman, N. D., Stuart, R. K., Hon, G. C., Hawkins, R. D., Kheradpour, P., Stark, A., Harp, L. F., Ye, Z., Lee, L. K., Stuart, R. K., Ching, C. W., Ching, K. A., Antosiewicz-Bourget, J. E., Liu, H., Zhang, X., Green, R. D., Lobanenkov, V. V., Stewart, R., Thomson, J. A., Crawford, G. E., Kellis, M., and Ren, B. (2009) Histone modifications at human enhancers reflect global cell type-specific gene expression. *Nature* 459, 108–112

Heintzman, N. D., Stuart, R. K., Hon, G. C., Hawkins, R. D., Barrera, L. O., Van Calcar, S., Qu, C., Ching, K. A., Wang, W., Weng, Z., Green, R. D., Crawford, G. E., and Ren, B. (2007) Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* 39, 311–318

Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Peng, W., Zhang, M. Q., and Zhao, K. (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Genet.* 40, 897–903

Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. (2007) High resolution profiling of histone methylations in the human genome. *Cell* 129, 823–837

Visel, A., Blow, M. J., Li, Z., Zhang, T., Akiyama, J. A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., Afzal, V., Ren, B., Rubin, E. M., and Pennacchio, L. A. (2009) ChIP-seq accurately predicts tissue-specific enhancer activity of enhancers. *Nat. Biotechnol.* 27, 249–262

Visel, A., Blow, M. J., Li, Z., Zhang, T., Akiyama, J. A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., Afzal, V., Bristow, J., Ren, B., Black, B. L., Rubin, E. M., and Pennacchio, L. A. (2009) ChIP-seq identification of weakly conserved heart enhancers. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21931–21936

Antosiewicz-Bourget, J. E., Liu, H., Zhang, X., Greenberg, M. E. (2010) Widespread transcription at neuronal activity-regulated genes. *Nat. Neurosci.* 13, 1906–1917

Feng, J., Bi, C., Clark, B. S., Mady, R., Shah, P., and Kohtz, J. D. (2006) The Esv-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dsx-2 transcriptional coactivator. *Genes Dev.* 20, 1470–1484

Zhang, Y., Li, J., Liu, H., Zhu, J., Su, J., Wu, Q., Qi, Y., Wang, F., and Li, X. (2010) HHMD: the human histone modification database. *Nucleic Acids Res.* 38, D149–D154

Bannister, A. J., and Kouzarides, T. (2011) Regulation of chromatin by histone modifications. *Cell* 21, 381–395

Pasini, D., Malatesta, M., Jung, H. R., Walfridsson, J., Willer, A., Olsson, W., Antosiewicz-Bourget, J., Ye, Z., Khochbin, S., Ren, B., and Zhao, Y. (2011) Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* 146, 1016–1028

Hawkins, R. D., Hon, G. C., Lee, L. K., Ngo, Q., Lister, R., Pelizzola, M., Edsall, L. E., Kuan, S., Luu, Y., Klugman, S., Antosiewicz-Bourget, J., Ye, Z., Espinoza, C., Agarwah, S., Shen, L., Ruotti, V., Wang, W., Stewart, R., Thomson, J. A., Ecker, J. R., and Ren, B. (2010) Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. *Cell Stem Cell* 6, 479–491

Jin, Q., Yu, L. R., Wang, L., Zhang, Z., Kasper, L. H., Lee, J. E., Wang, C., Brindle, P. K., Dent, S. Y., and Ge, K. (2011) Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. *EMBO J.* 30, 249–262

Tie, F., Barnerje, R., Stratton, C. A., Prasad-Sinha, J., Stepanik, V., Zlobin, A., Diaz, M. O., Scacheri, P. C., and Harte, P. J. (2009) CBP-mediated acetylation of histone H3 lysine 27 antagonizes *Drosophila* Polycistron silencing. *Development* 136, 3131–3141

Pasini, D., Malatesta, M., Jung, H. R., Walfridsson, J., Willer, A., Olsson, L., Skotte, J., Wutz, A., Porse, B., Jensen, O. N., and Helin, K. (2010) Characterization of an antagonistic switch between histone H3 lysine 27 methylation and acetylation in the transcriptional regulation of Polycistron–target genes. *Nucleic Acids Res.* 38, 4958–4969

Talbert, P. B., and Henikoff, S. (2010) Histone variants–ancient wrap artists of the epigenome. *Nat. Rev. Mol. Cell Biol.* 11, 264–275

Zlatanova, J., and Thakar, A. (2008) H2A.Z: view from the top. *Structure* 16, 166–179

Jin, C., and Felsenfeld, G. (2007) Nucleosome stability mediated by histone variants H3.3 and H2A.Z. *Genes Dev.* 21, 1519–1529

Jin, C., Zang, C., Wei, G., Cui, K., Peng, W., Zhao, K., and Felsenfeld, G. (2009) H3.3/H2A.Z double variant-containing nucleosomes mark “nucleosome-free regions” of active promoters and other regulatory regions. *Nat. Genet.* 41, 941–945

Robertson, A. G., Bilenky, M., Tam, A., Zhao, Y., Zeng, T., Thiessen, N., Cezard, T., Fejes, A. P., Werederl, E. D., Cullum, R., Euskirchen, G., Krzywinski, M., Biro, L., Snyder, M., Hoodless, P. A., Hirst, M., Marra, M. A., and Jones, S. J. (2008) Genome-wide relationship between histone H3 lysine 4 mono- and trimethylation and transcription factor binding. * Genome Res.* 18, 1906–1917

Hoffman, B. G., Robertson, G., Zavadila, B., Beach, M., Cullum, R., Lee, S.,
ing principles of the human genome. Science 326, 289–293
87. van Berkum, N. L., and Dekker, J. (2009) Determining spatial chromatin organization of large genomic regions using 5C technology. Methods Mol. Biol. 567, 189–213
88. Tiwari, V. K., Cope, L., McGarvey, K. M., Ohm, J. E., and Baylin, S. B. (2008) A novel 6C assay uncovers Polycomb-mediated higher order chromatin conformations. Genome Res. 18, 1171–1179
89. Fullwood, M. J., Liu, M. H., Pan, Y. F., Liu, J., Xu, H., Mohamed, Y. B., Orlov, Y. L., Velkov, S., Ho, A., Mei, P. H., Chew, E. G., Huang, P. Y., Welboren, W. J., Han, Y., Ooi, H. S., Ariyaratne, P. N., Vega, V. B., Luo, Y., Tan, P. Y., Choy, P. Y., Wansa, K. D., Zhao, B., Lim, K. S., Leow, S. C., Yow, J. S., Joseph, R., Li, H., Desai, K. V., Thomsen, J. S., Lee, Y. K., Karuturi, R. K., Bourque, G., Stunnenberg, H. G., Ruan, X., Cacheux-Rataboul, V., Sung, W. K., Liu, E. T., Cheung, E., and Ruan, Y. (2009) An estrogen receptor-α-bound human chromatin interactome. Nature 462, 58–64
90. Li, G., Fullwood, M. J., Xu, H., Mulawadi, F. H., Velkov, S., Vega, V., Ariyaratne, P. N., Mohamed, Y. B., Ooi, H. S., Tennakoon, C., Wei, C. L., Ruan, Y., and Sung, W. K. (2010) ChIA-PET tool for comprehensive chromatin interaction analysis with paired-end tag sequencing. Genome Biol. 11, R22
91. Handoko, L., Xu, H., Li, G., Ngan, C. Y., Chew, E., Schnapp, M., Lee, C. W., Ye, C., Ping, J. L., Mulawadi, F., Wong, E., Sheng, J., Zhang, Y., Poh, T., Chan, C. S., Kunarso, G., Shahab, A., Bourque, G., Cacheux-Rataboul, V., Sung, W. K., Ruan, Y., and Wei, C. L. (2011) CTCF-mediated functional chromatin interactome in pluripotent cells. Nat. Genet. 43, 630–638
92. Harismendy, O., Notani, D., Song, X., Rahim, N. G., Tanasa, B., Heintzman, N., Ren, B., Fu, X. D., Topol, E. J., Rosenfeld, M. G., and Frazer, K. A. (2011) 9p21 DNA variants associated with coronary artery disease impair interferon-γ signaling response. Nature 470, 264–268
93. Henikoff, S., and Shilatifard, A. (2011) Histone modification: cause or cog? Trends Genet. 27, 389–396
94. Schmidt, C., Klug, M., Boeld, T. J., Andreasen, R., Hoffmann, P., Edinger, M., and Rehli, M. (2009) Lineage-specific DNA methylation in T cells correlates with histone methylation and enhancer activity. Genome Res. 19, 1165–1174
95. Wiench, M., John, S., Baek, S., Johnson, T. A., Sung, M. H., Escobar, T., Simmons, C. A., Pearce, K. H., Biddie, S. C., Sabo, P. J., Thurman, R. E., Stamatoyannopoulos, J. A., and Hager, G. L. (2011) DNA methylation status predicts cell type-specific enhancer activity. EMBO J. 30, 3028–3039
96. Lister, R., Pelizzola, M., Downen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., Nery, J. R., Lee, L., Ye, Z., Ngo, Q. M., Edsall, L., Antosiewicz-Bourget, J., Stewart, R., Ruotti, V., Millar, A. H., Thomson, J. A., Ren, B., and Ecker, J. R. (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462, 315–322
97. Hoivik, E. A., Bjesanosy, T. E., Mai, O., Okamoto, S., Minokoshi, Y., Shima, Y., Morohashi, K., Boehm, U., and Bakke, M. (2011) DNA methylation of intronic enhancers directs tissue-specific expression of steroidogenic factor 1/adrenal 4 binding protein (SF-1/Ad4BP). Endocrinology 152, 2100–2112
98. Song, L., Zhang, Z., Grasfeder, I. L., Boyle, A. P., Giresi, P. G., Lee, B. K., Sheffield, N. C., Gräf, S., Huss, M., Keefe, D., Liu, Z., London, D., McDaniel, R. M., Shibata, Y., Showers, K. A., Simon, J. M., Vales, T., Wang, T., Winter, D., Zhang, Z., Clarke, N. D., Birney, E., Iyer, V. R., Crawford, G. E., Lieb, J. D., and Furey, T. S. (2011) Open chromatin defined by DNase I and FAIRE identifies regulatory elements that shape cell-type identity. Genome Res. 21, 1757–1767
99. Boyle, A. P., Davis, S., Shulha, H. P., Meltzer, P., Margulies, E. H., Weng, Z., Furey, T. S., and Crawford, G. E. (2008) High resolution mapping and characterization of open chromatin across the genome. Cell 132, 311–322
100. Wu, W., Cheng, Y., Keller, C. A., Ernst, J., Kumar, S. A., Mishra, T., Morrissey, C., Dorman, C. M., Chen, K. B., Drautz, D., Giardine, B., Shibata, Y., Song, L., Pimkin, M., Crawford, G. E., Furey, T. S., Kellis, M., Miller, W., Taylor, J., Schuster, S. C., Zhang, Y., Chiaromonte, F., Blobel, G. A., Weiss, M. J., and Hardison, R. C. (2011) Dynamics of the epigenetic landscape during erythroid differentiation after GATA1 restoration. Genome Res. 21, 1659–1671

MINIREVIEW: Enhancer Chromatin States