Active chromatin domains are defined by acetylation islands revealed by genome-wide mapping

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The identity and developmental potential of a human cell is specified by its epigenome that is largely defined by patterns of chromatin modifications including histone acetylation. Here we report high-resolution genome-wide mapping of diacetylation of histone H3 at Lys 9 and Lys 14 in resting and activated human T cells by genome-wide mapping technique (GMAT). Our data show that high levels of the H3 acetylation are detected in gene-rich regions. The chromatin accessibility and gene expression of a genetic domain is correlated with hyperacetylation of promoters and other regulatory elements but not with generally elevated acetylation of the entire domain. Islands of acetylation are identified in the intergenic and transcribed regions. The locations of the 46,813 acetylation islands identified in this study are significantly correlated with conserved noncoding sequences (CNSs) and many of them are colocalized with known regulatory elements in T cells. TCR signaling induces 4045 new acetylation loci that may mediate the global chromatin remodeling and gene activation. We propose that the acetylation islands are epigenetic marks that allow prediction of functional regulatory elements.

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Acetylation islands are regulatory elements

Results

Sequencing the GMAT library

To generate GMAT libraries for sequencing analysis, resting CD3+ T cells were isolated from human blood using negative selections. The cells were either used immediately or activated with anti-CD3 and anti-CD28 for 24 h before ChIP with anti-K9/K14 diacetylated histone H3 antibodies. The GMAT libraries were prepared from the ChIP DNA as described previously (Roh et al. 2003, 2004). As shown in Table 1A, the human genome contains a total of 24,577,210 tags, which represent 18,559,414 kinds of tags (or different 21-bp sequences). By sequencing 32,544 GMAT clones, we obtained a total of 803,439 tags, which represent 414,655 kinds of tags. Therefore, 2.2% of the tag kinds in the human genome were detected in the GMAT library. To determine whether we have covered the genome sufficiently, we quantified the level of H3-K9/K14 acetylation in resting T cells using ChIP as described (Litt et al. 2001). The results showed that ~1.2% of nucleosomes in total chromatin is associated with K9/K14 acetylated H3 (data not shown). Since the repetitive sequences, which make up ~40% of the human genome, were associated with hypoacetylated histone H3 (Table 1B), we estimate that ~2% of the unique sequences in the genome are associated with the acetylated histone. This is consistent with the data that 2.1% of unique tags were detected in the GMAT library (Table 1B). We conclude that our sequencing has covered most of the acetylated regions.

Table 1B shows that 2.1% of the unique sequences [1 repeat] in the genome were detected in the GMAT library, while the percentage of detection decreased as the repetitiveness [repeat number] increased, indicating that higher repetitive sequences are associated with lower levels of the histone H3 acetylation. Since the repetitive sequences were not associated with significant levels of the H3 acetylation and the repetitive tags that are associated with acetylated H3 could not be mapped precisely in the human genome, we considered only the unique tags for further analysis.

The GMAT tags are truly associated with H3 acetylation

We detected 670,073 tags (Table 1A) derived from unique sequences. The detection frequency of the tags in the GMAT library ranges from 1 to 65 times (Table 1C). Of these tags, 40.7% were detected only once [single-copy tag] and 59.3% were detected multiple times in the library (Table 1C). Since the specific antibody against the diacetylated [K9/K14] histone H3 pulled down 100-fold more DNA than nonspecific rabbit IgGs in the ChIP experiments, the majority of the ChIP DNA and therefore the tags detected in the GMAT library were associated with the acetylated histone. However, we could not completely avoid low levels of nonspecific pull-down of DNA in the procedure. One nonspecific fragment may generate a maximum of two tags irrespective of the fragment size. To confirm that the majority of the tags detected in the library truly represent the levels of histone modification and are not derived from contamination during ChIP experiments, we randomly picked ~100 tags and analyzed them by quantitative PCR using specific primers. Our analysis of 15 loci with a two-copy or higher-copy GMAT tag indicates that all of them were enriched in the ChIP DNA. The enrichment was >10-fold over the control β-globin sequence [data not shown]. The results were reproduced with four different ChIP samples obtained from chromatin prepared from four independent cultures. Therefore, we can confidently con-
To reconfirm that the GMAT tags were derived from the acetylated but not from nonacetylated regions by contamination, we examined the GMAT library. We cannot conclusively determine if these three tags represent true low levels of acetylation in resting T cells. The 120-kb region contained 1016 predicted single-copy tags and most of the single-copy tags detected in the GMAT library were associated with the acetylated histone H3.

Including different ChIP DNA samples (Table 2). When the nearest detected tag was found at a distance of >5 kb, only 36% of the tags were enriched more than threefold [Table 1D]. However, only 7.9% of the tags fell into this category. Therefore, we conclude that all of the multiple-copy tags and most of the single-copy tags detected in the GMAT library were associated with the acetylated histone H3.
Promoter regions are highly acetylated in the human genome

To determine if the H3 acetylation is biased toward any functional regions in the human genome, we arbitrarily defined the genome as consisting of three parts: 2-kb promoter regions including 1 kb upstream and 1 kb downstream of the transcription initiation site, gene body regions including introns and exons, and intergenic regions [Fig. 1A]. The 2-kb promoter region makes up 0.8% of the genome [Fig. 1B]. The gene body and intergenic regions make up 27.3% and 71.9% of the genome, respectively. Interestingly, 23.5% of the tags detected in the GMAT library were derived from the promoter region, and 30.0% and 46.4% were from the gene body and intergenic regions, respectively. These results indicate that the distribution of the acetylated H3 is biased toward the promoter region in the genome, which is consistent with the results obtained from the analysis of 57 human genes [Liang et al. 2004]. We aligned 21,355 annotated genes relative to their transcription initiation sites and plotted the tag density, which is derived by normalizing the detected number of tags in the GMAT library to the number of expected NlaIII sites in a 50-bp window across a 10-kb region [Fig. 1C]. The green line represents the calculated value, which shows that the calculated tags are distributed evenly across the whole region. Interestingly, a significant peak of tags was detected in the GMAT library in the 2-kb promoter region, as revealed by the black line for all the genes. A chi-squared statistical test indicates...
the enrichment of the H3 acetylation in the promoter region is significant \( p < 0.0001 \). To rule out the possibility that the enrichment of the GMAT tags in the promoter region was caused by the exclusion of repetitive tags from the data, we analyzed the distribution of the repetitive tags detected in the GMAT library. As shown in Supplementary Figure 1B, the repetitive tags were distributed in the genome with a slight enrichment in the promoter region. Therefore, removal of these tags from the analysis should not generate a bias in favor of detecting the unique tags in the promoter region. The pink line represents the acetylation level of the 8000 highly expressed genes (van der Kuyl et al. 2002) and the blue line indicates the acetylation level of 14,000 genes whose expression was not detected. The data showed that more active promoters have higher levels of histone H3 acetylation than less active promoters. A similar observation indicates the acetylation level of 14,000 genes whose expression was not detected. The data showed that more active promoters have higher levels of histone H3 acetylation than less active promoters. A similar observation has been made from the genome-wide analysis of Drosophila cells (Schubeler et al. 2004). It is noteworthy that the 1-kb region 3’ of the transcription initiation site has the highest acetylation level. Since the genome-wide expression data in literature did not allow us to distinguish true silent genes from the genes expressed at lower levels, we examined the acetylation of several genes including β-globin, BAF53b (Olave et al. 2002), NEUROD1 [Naya et al. 1995], and PITX2 (Semina et al. 1996), which are not expressed in T cells and are true silent genes. The analysis indicates that no acetylation was detected in their promoter regions (data not shown).

**CpG islands are highly acetylated**

Many human promoters contain CpG islands, which are important transcription-controlling elements and are unmethylated under normal circumstances [Bird et al. 1985; Gardiner-Garden and Frommer 1987]. The mechanisms preventing CpG islands from being methylated remain elusive. We decided to examine the acetylation status of CpG islands. The human genome project has identified 27,058 CpG islands in the human genome [Krolchik et al. 2003]. As suggested by previous studies [Larsen et al. 1992], we found that the CpG islands were highly concentrated in a 1-kb region surrounding the transcription initiation site of human genes (Supplementary Fig. 2), which is consistent with the overrepresentation of the CpG dinucleotides in human promoter sequences [Marino-Ramirez et al. 2004]. Our data indicate that 64.2% of the CpG islands were acetylated [Fig. 2A], which is much higher than the 1.2% average acetylation level in the whole genome. In active genes and the genes whose expression was not detected [from \(-10\) kb to the gene end], 78.2% and 67.8% of CpG islands, respectively, were acetylated. Interestingly, \( \sim 33\% \) of CpG islands were located in the intergenic regions and were also acetylated at a level of 49.1%. A chi-squared test indicates that the association of H3 acetylation with CpG islands is significant \( p = 0.0001 \). To study the relationship between CpG islands and associated chromatin acetylation status, we analyzed acetylation boundaries of 4973 CpG islands that have a size ranging from 1 to 2 kb [Fig. 2B]. The analysis revealed that the acetylation was quite evenly distributed within the CpG islands. However, sequences 200 bp away from CpG islands showed a significant decrease in the acetylation. Furthermore, some of the GMAT tags neighboring CpG islands may have been brought down by the acetylation within the CpG islands because of the heterogeneous size of the chromatin fragments used for ChIP experiments. The acetylation level decreased rapidly as the distance to CpG islands increased. It reached background level when the distance was \( \sim 1\) kb. These data suggest that the H3 hyperacetylation may serve as a mechanism to prevent the CpG islands from being methylated.

**The highest H3 acetylation is detected in gene-rich regions**

Based on the sequence information, we mapped the GMAT tags onto the 24 chromosomes in the human
Active chromatin domains are not uniformly hyperacetylated

Supplementary Figure 4 indicates that active chromatin domains correlate with high levels of H3 acetylation. Is an open chromatin domain generally highly acetylated? To answer this question, we examined the high-resolution map of the H3 acetylation of the STAT2 locus on chromosome 12 (Fig. 3A). The 160-kb region (chromosome 12: 54,920,000–55,080,000) harbors five expressed genes as indicated by the arrows in Figure 3A. Interestingly, there were no sustained high levels of the H3 acetylation throughout the entire domain. Instead, high peaks of acetylation were detected within promoter regions. To determine whether this is a general phenomenon, we examined the high-resolution maps of more active loci. BAF53A (Zhao et al. 1998) and Sp1 are constitutively expressed in almost every cell type, and STAT5B (Liu et al. 1995) are constitutively expressed in T cells. As shown in Figure 3B, C, and D, all of these loci have high levels of acetylation in their promoter regions. However, no general hyperacetylation was detected in their transcribed regions, except for a few isolated clusters of tags in some genes. The same is also true for the CD4 locus (Fig. 4A, data not shown). These results indicate that active domains are not uniformly hyperacetylated but correlated with hyperacetylation of critical regulatory elements, suggesting that localized acetylation may contribute to maintain the openness of the entire domain.

Identification of acetylation islands

Examination of the high-resolution maps revealed that there were clusters of two or more acetylation tags along the chromatin fiber, which we have named “acetylation islands” [highlighted and numbered in Figs. 3, 4]. The acetylation islands were detected both in the intergenic and transcribed regions. We identified a total of 21,481 and 25,332 acetylation islands in the intergenic and transcribed regions, respectively. Our discovery of the acetylation islands suggests that besides promoters, other regulatory elements may also be marked by histone acetylation.

Most acetylation islands colocalize with CNSs

Comparative genomics studies have identified ~240,000 CNSs in the human and mouse genomes (for review, see Hardison 2000), which are believed to be regulatory elements in the mammalian genomes. Because of the general role that histone acetylation plays in the regulation of transcription and chromatin structure, we investigated whether there is a correlation between the acetylation islands and CNSs. As shown in Figure 4, most of the acetylation islands are associated with CNSs that are
revealed by the VISTA analysis [http://pipeline.lbl.gov/cgi-bin/gateway2] at the lower part of the figure.

Comparative analysis of human chromosome 21 with syntenic regions of the mouse genome has revealed 2262 CNSs in the intergenic regions (Dermitzakis et al. 2002). Comparison between the CNSs and H3 acetylation indicates that there are 187 acetylated CNSs, which accounts for 8.3% of CNSs. Genome-wide analysis of the acetylation status of CNSs revealed that 15.7% of the 241,222 CNSs identified in the VISTA database [http://pipeline.lbl.gov/cgi-bin/gateway2] were associated with the acetylated H3. Chi-squared statistical tests suggest that the correlation between CNSs and the H3 acetylation is significant ($p = 0.001$).

**Colocalization of acetylation islands with known regulatory elements in T cells**

The significant colocalization of acetylation islands with CNSs suggests that the acetylation islands may represent functional regulatory elements in T cells. Therefore, we examined whether they are correlated with known regulatory elements in T cells.

CD4 is a critical T-cell coreceptor that assists antibody production. DNase hypersensitive sites [HS] mapping combined with transgenic studies has identified several regulatory elements that collectively mediate the specific expression of the CD4 gene (for reviews, see Ellmeier et al. 1999; Siu 2002). As expected, strong acetylation was detected in the promoter [Fig. 4A, highlight 7]. Interestingly, the proximal enhancer, which is conserved between human and mouse and is located 6.5 kb upstream of its transcription initiation site in human, is colocalized with an acetylation island [Fig. 4A, highlight 5]. The distal enhancer located upstream of the LAG3 gene was also acetylated [Fig. 4A, highlight 1]. The CD4 gene is also regulated by a locus control region [LCR] and a thymocyte enhancer [TE] that are located 30 kb downstream of the CD4 gene with several intervening genes. A significant acetylation island was detected in the LCR/TE region [Fig. 4A, highlight 12]. Besides the known regulatory elements, we detected several other significant acetylation islands within the locus [Fig. 4A, highlights 2–4, 6, and 9–11]. It will be interesting to determine if these also represent important regulatory elements for the CD4 gene.

The CD8α and CD8β coreceptors play critical roles in mediating cell killing. The minimal functional elements of the human CD8 genes, which render their specific expression, are contained in a 95-kb region (Kieffer et al. 1997). Six clusters of DNase HSs are present in the locus (Kieffer et al. 2002), as summarized in Figure 4B. Interestingly, most of these HS sites coincided well with significant acetylation islands. However, HS cluster III was not colocalized with any significant acetylation islands. Instead, a significant acetylation island (Fig. 4A, highlight 5) was detected ∼5 kb away from HS III. Since the expression level of the CD8 genes contained in the 95-kb region varies depending on integration site, it does not contain a LCR, suggesting a LCR may be located outside of the region. Examining the acetylation map revealed a highly acetylated region and a TCR signaling-induced island, 100 and 40 kb downstream of the CD8α gene, respectively [data not shown]. It will be interesting to test whether these regions function as LCRs for the CD8 locus.
Examination of the IL2Rα [Lin and Leonard 1997] and BCL3 (Ohno et al. 1990) loci (Supplementary Fig. 5) also revealed that known transcription and chromatin regulatory elements are well correlated with the acetylation islands, suggesting that the acetylation islands may represent a functional regulatory network of gene expression programs in T cells.

Genome-wide change of histone H3 acetylation induced by TCR signaling

TCR signaling induces thousands of genes required for cellular differentiation and immunologic functions, accompanied by massive chromatin decondensation [Crabtree 1989]. To identify cis regulatory elements that initiate the global chromatin remodeling upon T-cell activation, we analyzed the histone H3 acetylation of T cells after 24 h of TCR signaling [Table 1A]. We determined the changes in acetylation levels between the resting and activated T cells by comparing the average tag density, which is obtained by dividing the total number of detected tags by the number of NlaIII sites in the region, within a 3-kb window. Changes of three times or more were plotted along chromosome coordinates [Fig. 5; Supplementary Fig. 6]. The analysis revealed that increased acetylation was detected at 4045 loci and decreased acetylation was detected at 4178 loci (Supplementary Table 1), indicating that the TCR signaling induced a genome-wide acetylation change.

To determine whether the acetylation islands induced by TCR signaling are involved in T-cell activation, we specifically examined the Th2 cytokine locus that harbors three TCR signaling-induced cytokine genes, IL5, IL13, and IL4 [Abbas et al. 1996]. Our analysis of resting T cells indicates that the IL13 and IL4 promoters were acetylated even in silent state [Fig. 6A, upper panel]. Interestingly, two acetylation islands [Fig. 6A, highlights 1 and 2] were induced downstream of the IL13 gene by TCR signaling [Fig. 6A, middle panel], even though there were no significant changes of histone H3 acetylation in the promoter regions. Acetylation Island 2 colocalized with a conserved sequence that is known to be required for the coordinated expression of the cytokine genes [Loots et al. 2000]. These data indicate that the TCR signaling-induced Acetylation Islands 1 and 2 may have an important function in initiating the chromatin opening and regulating the expression of the cytokine genes.

Figure 5. Genome-wide changes of acetylation induced by TCR signaling. The average tag density was derived by normalizing the total number of detected tags to the number of NlaIII site in a 3-kb window. The average tag densities from resting and activated T cells were compared directly to obtain the fold change. Changes of threefold or more between activated and resting T cells were plotted along the chromosome coordinates. The data for chromosome 12 are shown. The other chromosomes are shown in Supplementary Figure 6.
The TCR-induced acetylation islands act as transcription enhancers

Accompanying the induction of Acetylation Islands 1 and 2, the IL13 and IL4 genes were induced by the TCR signaling (Fig. 6B, cf. lanes 1 and 2). To demonstrate whether the TCR-signaling-induced acetylation islands are functional regulatory elements, we tested the activities of Acetylation Islands 1 and 2 in a luciferase reporter assay. As shown in Figure 6C, both Acetylation Islands 1 and 2 activated the GM-CSF1 promoter in pGL3 vector in Jurkat cells even without stimulation, while the control insert from the unacetylated neighboring region (chromosome 5: 132,080,058–132,081,677) did not have any detectable activity. Upon stimulation with ionomycin and PMA, which mimicked TCR signaling, Acetylation Island 2 further activated the promoter twofold. Next, we cloned these sequences into the episomal pREP4 vector, which replicates and forms a regular chromatin structure in cells. Both Acetylation Islands 1 and 2 only modestly increased the promoter activity without stimulation (Fig. 6D). Interestingly, they dramatically activated the promoter upon ionomycin and PMA stimulation (Fig. 6D). These results suggest that TCR signaling induced an activity that can overcome the inhibitory effect of chromatin, possibly by modification of the chromatin structure, which is consistent with the observation that the acetylation of Acetylation Islands 1 and 2 was induced by TCR signaling in T cells. It is noteworthy that even though Acetylation Island 1 was not conserved between human and mouse, it had strong activity in activating transcription, indicating that comparative genomics studies may miss important regulatory elements. These results indicate that Acetylation Islands 1 and 2 activate transcription in a chromatin-dependent manner and the TCR-signaling-induced acetylation islands are indeed epigenetic marks for functional regulatory elements.

Discussion

The human genome contains ~35,000 genes (Baltimore 2001; Lander et al. 2001). At least 10,000–15,000 genes are expressed in any tissues, which occupy ~15% of the human genome. We found that only 1.2% of the chromatin is associated with the K9/K14 diacetylated histone H3 in human T cells. Therefore, this level of acetylation does not allow all of the active chromatin domains to be uniformly highly acetylated, as suggested by the data from studies in yeast. Indeed, we found that most of intergenic and transcribed regions of active chromatin do not have generally elevated levels of histone acetylation. Our analysis of the 21,355 annotated human genes indicates that the promoter region is highly acetylated, as suggested by a previous smaller scale study (Liang et al. 2004). A higher acetylation level in the promoter region is correlated with more active genes, consistent with a previous study in Drosophila (Schubeler et al. 2004). Furthermore, we find that other functional regulatory elements such as enhancers, LCRs, and insulators are also marked by the histone acetylation. Our data argue for a model in which the chromatin accessibility and expression of a gene are controlled by histone modifications of a number of regulatory elements, which act to actively limit the spreading of neighboring heterochromatin and allow binding of transcription factors and assembly of the transcription machinery.

Comparison of the human and mouse genomic sequences reveals the existence of 240,000 conserved noncoding sequences that may function to direct the expression programs of the genomes [Hardison 2000]. However, even though all of the nucleated human cells have the same genome, each cell type has a different “epigenome.” Each cell type expresses a different set of genes, which requires a different set of regulatory elements. We show that the acetylation islands identified in this study are well correlated with known DNase HSs and functional regulatory elements, suggesting that the 46,813 acetylation islands may represent the functional regulatory network required for the expression programs in T cells. Even though many of the acetylation islands are not colocalized with any significant CN5s, they may have important regulatory functions, as revealed by the transcription enhancer activity of Acetylation Island 1 of the IL13 locus. It would be interesting to test whether the nonconserved syntenic locus in mouse is also acetylated and required for the coordinated expression of the cytokine locus. In summary, our data in this study provide valuable information for further identification of cis regulatory elements and for elucidation of regulatory mechanisms of transcription of almost every gene expressed in T cells.

Materials and methods

Isolation and stimulation of human T cell

Human resting T cells were purified sequentially using the lymphocyte separation medium (Mediatech) and Pan T-cell isolation kit II (Miltenyi Biotech). The final purity of T cells was >98%. T cells were activated with 1 µg/mL of anti-CD3 and anti-CD28 monoclonal antibodies (BD Pharmingen) for 24 h.

ChIP and preparation of the GMAT libraries

ChIP using anti-diacetylated K9/K14 histone H3 antibody [Upstate] and generation of GMAT library were performed as described (Roh et al. 2004). The method was refined based on our initial report (Roh et al. 2003).

Verification of H3 acetylation at randomly selected tag sites

An approximately 250-bp region encompassing a single or multicopy acetylation tag site was amplified. One region of β-globin domain (chromosome 11: 5,250,645–5,250,913) and one region on chromosome 4 [57,858,032–57,858,304] were used as controls. The PCR products from five DNA samples (input and four ChIP samples) were labeled by incorporating α-32P(dCTP) in the reaction and were separated on a denaturing polyacrylamide gel for quantification using the PhosphorImager (Molecular Dynamics). To calculate the fold of enrichment for each tag site, the signals from each sample were first normalized to the con-
Acetylation islands are regulatory elements

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control signals. Then, the normalized signals of each tag site from the ChiP samples were normalized to the input signals to obtain the fold of enrichment.

Data analysis

A theoretical reference library of 21-bp sequence tags was derived from the UCSC July 2003 human sequence [hg16] using SAGE2000 version 4.5 software [Johns Hopkins Oncology Center]. The GMAT library was generated by extracting 21-bp tags from raw sequencing data files using the SAGE2000 software. All other calculations and analyses were performed using in-house PERL programs. Detection frequency was determined by normalizing tag count to the genomic copy number. Tag density was calculated by dividing the detection frequency by the number of expected NlaIII sites in a 50-bp window.

An acetylation island was defined by the following criteria: (1) It is composed of tags from more than two adjacent NlaIII sites; (2) the detection frequency of all the tags is more than or equal to one; and (3) neighboring acetylation islands are separated by >500 bp.

The information on CpG islands and nonredundant RefSeq genes was obtained from the UCSC Genome Browser [Karolchik et al. 2003]. The list of highly transcribed active genes in CD4+ T cell was downloaded from the NCBI Genome Expression Omnibus (GEO) database. Gene names were synchronized using UniGene ClusterID and LocusLink RefSeq ID.

For comparative analysis of human and mouse genomes, graphic alignments were adopted from the VISTA browser [Courome et al. 2003] that shows gene information and sequences of >50% homology with October 2003 mouse genome assembly.

To compare the acetylation level between resting and activated T cells, the average detection frequency was calculated by normalizing the total number of tags to the number of NlaIII sites in the 3-kb window. The fold of change was calculated by dividing the average detection frequency of activated T cell by that of resting T cell.

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