Differentiation-dependent Alterations in Histone Methylation and Chromatin Architecture at the Inducible Chicken Lysozyme Gene*§

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It is now well established that locus-wide chromatin remodeling and dynamic alterations of histone modifications are required for the developmentally regulated activation of tissue-specific genes. However, little is known about the dynamics of these events during cell differentiation and how chromatin of an entire gene locus responds to signal transduction processes. To address this issue we investigated chromatin accessibility, linker histone distribution, and the histone methylation status at the macrophage-specific chicken lysozyme locus and the ubiquitously expressed gas41 locus in multipotent precursor cell lines and BM2 monoblast cells. The latter can be induced to go through macrophage maturation by treatment with phorbol-12-myristate acetate and can be further stimulated with bacterial lipopolysaccharide. We show that expression of the lysozyme gene in undifferentiated monoblasts is low and that a high level of gene expression requires both cell differentiation and lipopolysaccharide stimulation. However, depletion of the linker histone H1 is observed already in lysozyme non-expressing multipotent precursor cells. In undifferentiated monoblasts, the lysozyme regulatory regions are marked by the presence of monomethylated histone H3 lysine 4, which becomes increasingly converted into trimethylated H3 lysine K4 during cell differentiation. We also present evidence for extensive, differentiation-dependent alterations in nucleosome accessibility at the lysozyme promoter without alterations of nucleosome and transcription factor occupancy.

Epigenetic processes involve the establishment of patterns of gene expression via the coordinated and heritable alterations in chromatin structure. These alterations involve a change in the covalent modification of histone N-terminal tails as well as global reorganization of chromatin architecture, all of which is driven by sequence-specific transcription factors recruiting the enzymatic machinery performing these reactions. Such histone tail modifications are not only able to alter the charge or biochemical surface of the chromatin fiber, but also serve as "docking" sites for transcription factor and chromatin remodeling complexes, thus stabilizing the interaction of such complexes with chromatin templates (1). High levels of gene expression go along with an increase in the accessibility of the chromatin of extended genomic regions to nuclease digestion and hyperacetylation of the histone tails of all core histones. Another modification that is associated with active genes is the methylation of lysine 4 of histone H3 (H3K4)3 (2–4). An interesting feature of histone methylation is the fact that, although the same enzyme modifies the histone tails, H3K4 residues can be mono-, di-, or trimethylated, and such differences are of functional consequence. It was recently shown that di- and trimethylated lysines, but not monomethylated lysines, bind the chromatin-remodeling complex ISWI with high affinity (5). In turn, it was shown that the presence of any H3K4 methyl mark inhibits the binding of heterochromatic silencing factors (6). In this context several observations are noteworthy. In yeast it was shown that the Set1 methyltransferase enzyme that modifies H3K4 is recruited by the RNA-polymerase II complex and thus leaves a trail of trimethylated histone behind even when transcription has ceased (7). This is also seen in higher eukaryotes (8). Interestingly, the K4-dimethyl mark seems to be regulated differently, because this appears also on non-transcribed genes and may indicate a poised chromatin state. Little is known about the role of H3K4 monomethylated histones at genes of higher eukaryotes and the functional significance of this modification.

Our laboratory has been investigating the interplay between developmental gene locus activation, active histone marks, and transcription factor occupancy. As a model system we have adopted the chicken lysozyme locus, which is expressed in myeloid cells. The chicken lysozyme gene is located within a generally DNase I-sensitive chromatin domain that spans 24 kb (9, 10) and contains two differentially regulated genes in close proximity: the highly tissue-specific clys gene and the ubiquitously expressed gas41 gene (11). However, a high level of general DNase I sensitivity at both genes is only observed in lysozyme-expressing cells, and the reason for this puzzling finding is not clear. Constitutive expression of the gas41 gene is driven by a CpG island with dual origin/promoter function. Lysozyme gene expression in macrophages is controlled by at least five tissue-specifically active cis-regulatory elements, three enhancer elements situated 6.1, 3.9, and 2.7 kb upstream of the transcription start site, a silencer element at −2.4 kb and a complex promoter (12). Priming of chromatin structure as indicated by selective demethylation of DNA, a partial accessibility to transcription factor binding, and changes in DNA topology occurs already in multipotent precursor cells, which

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1 The abbreviations used are: H3K4, lysine 4 of histone H3; H3K9, lysine 9 of histone H3; PMA, phorbol-12-myristate acetate; LPS, bacterial lipopolysaccharide; DHS, DNase I-hypersensitive site; DMS, dimethyl sulfate; ChIP, chromatin immunoprecipitation; LM-PCR, ligation-mediated PCR; MNaes, micrococcal nuclease.
do not yet express the gene (13–15). The mechanistic basis for this observation is unknown. Lysozyme gene expression is first observed in granulocyte-macrophage precursors and in mature macrophages is rapidly induced by pro-inflammatory stimuli such as bacterial lipopolysaccharide (LPS). As judged by DNase I-hypersensitive site (DHS) mapping, enhancer and promoter elements are only fully active in LPS-treated mature macrophages where maximum level gene expression is observed. At each successive step of the developmental activation of the lysozyme locus, levels of histone H3 (K9 and K14) acetylation at the enhancers are increased and levels of histone H3 (K9) methylation are decreased (14). However, up to date, H3K4 methylation levels were not studied and the dynamics of histone methylation levels at the cis-elements regulating the two juxtaposed gene loci is not known. In the study presented here, we addressed this issue and we also examined how histone methylation levels and chromatin architecture of the entire locus respond to the induction of differentiation and high level expression by external signals. We studied dynamic alterations in chromatin accessibility, linker histone distribution, and the histone methylation status within the lysozyme chromatin domain in lysozyme non-expressing multipotent precursor cells as well as BM2 cells representing monoblast cells, which represent committed macrophage precursor cells. The latter express the lysozyme gene at a low level, can be differentiated into monocytes by treatment with PMA, and can be further differentiated into macrophages by LPS stimulation. Both stimuli are required for maximal lysozyme gene expression. Monomethylation of histone H3K4 in the lysozyme regulatory region is found in undifferentiated monoblasts. Cell differentiation and induced gene expression leads to high levels of H3K4 trimethylation over the coding region. Interestingly, already in lysozyme non-expressing precursor cells histone H1 is depleted at the promoter and is further depleted in a locus-wide fashion in response to PMA, but not LPS treatment.

EXPERIMENTAL PROCEDURES

Cell Culture—The chicken monocytic BM2 cell line (18), HD50 MEP, and HD37 cells (17) were grown in Dulbecco’s modified Eagle’s medium containing 8% fetal calf serum, 2% chicken serum, 75 μg/ml conalbumin (Sigma), 0.03 units/ml insulin, 10 μg/ml penicillin, and 100 μg/ml streptomycin. When indicated the BM2 cells were stimulated with 50 ng/ml PMA (Sigma) for 48 h. BM2 and HD37 cells were plated in a ratio of lysozyme or control genes (Ras or α-tubulin) at 100,000 cells/ml with DMEM containing 8% fetal calf serum, 2% chicken serum, 75 μg/ml conalbumin (Sigma), 0.03 units/ml insulin, 10 μg/ml penicillin, and 100 μg/ml streptomycin. When indicated the BM2 cells were stimulated with 50 ng/ml PMA (Sigma) for 48 h.

Reverse Transcription—Total RNA was prepared using TRIzol (Invitrogen) according to the manufacturer’s instructions, and genomic DNA was removed by DNase I treatment. First-strand cDNA synthesis from RNA samples was carried out using oligo(dT)15 primer and MoltDNA was removed by DNase I treatment. First-strand cDNA synthesis (Invitrogen) according to the manufacturer’s instructions, and genomic DNA was removed by DNase I treatment. Primers or primers specific for the coding region of the lysozyme promoter, LM-PCR was performed using gene-specific nested primers P1–P3 and P351–P353. LM-PCR on mRNA-treated material was performed on a robotic workstation as described in detail before (19). Briefly, DNase I was labelled by primer extension using γ-32P-labeled nested primers and primers were selected on 6% denaturing polyacrylamide gels. The lysozyme promoter, LM-PCR was performed using gene-specific nested primers P1–P3 and P351–P353. LM-PCR on mRNA-treated material was performed on a robotic workstation as described in detail before (19). Briefly, DNA obtained as input samples for ChIP was phosphorylated with polynucleotide kinase and directly ligated to the linker, LP25–21. Primer extension was performed by using biotinylated gene-specific primers. Primer extension products were bound to magnetic beads and then amplified by LM-PCR as described above. Primers P1–P3 and P351–P353 and β-actin promoter-specific primers are shown in Supplemental Table 2A.

Chromatin Immunoprecipitation Assays and Real-time PCR Analysis—Chromatin immunoprecipitation using sonicated material was performed exactly as previously described (14). Chromatin immunoprecipitation assays using MNase-treated material are described in (20). After preclearing, 50 μl of the chromatin preparation was kept as control (Input), and 500 μl was incubated with 1 mg of rabbit IgG (Upstate Biotechnology, 12-370) to estimate the background level for each ampiclon (data not shown), anti-histone H3 C-terminal (Abcam ab1791), anti-histone H3 1me-, 2me-, or 3me-Lys-4 (Abcam ab9889, ab7766, and ab8590), histone H1 (Abcam ab7759–290) or anti-histone H3 1me- or 2me-Lys-9 (Abcam ab9045 and ab7312) antibodies. Primers were designed using Primer Express 1.5 software and tested against a standard curve of sonicated genomic DNA. Primer sequences are shown in Supplemental Table S1. PCR signals from immunoprecipitated material were first calculated in percentage of the Input for all primers. To correct for the variation of the quality of different batches of chromatin from different cells and for varying background signals generated by different antibodies, signals were normalized to those obtained with internal control primers such as the hepatocyte-specific gene VLDL-apo2 primers or primers specific for the coding region of the β-actin gene. Non-normalized data are shown in Supplemental Fig. S2 for five different primers, including primers specific for the control genes.

RESULTS

Maximal Expression of the Chicken Lysozyme Gene Requires Both PMA-mediated Monocytic Differentiation and LPS Signaling—In previous studies we measured transcription factor occupancy and chromatin in retrovirally transformed cell lines representing different stages of myeloid differentiation (16, 17, 21) and identified the transcription factors binding to the different cis-regulatory elements by chromatin immunoprecipitation (ChIP) assays and in vivo footprinting experiments. The results of these experiments are summarized in Fig. 1A. Fig. 1B depicts the cell models used in this study. These include multi-potent progenitor cells (HD50 MEP) as well as HD37 representing a non-macrophage cell type (erythroblasts). HD50 myl cells are bi-potent granulocyte-macrophage precursors. BM2 cells are v-myb-transformed committed macrophage precursors (monoblasts). BM2 cells grow in suspension and differentiate into adherent monocytes after treatment with PMA and can be further differentiated to activated macrophages after treatment
with LPS. We measured lysozyme and gas41 mRNA expression in the different cell types by real-time PCR (Fig. 1C). gas41 was expressed at low level in all cell types analyzed. In contrast, lysozyme mRNA was not detected in HD37 erythroblasts and HD50 MEP progenitor cells. Un-stimulated BM2 cells showed a low level of expression, which was not significantly increased by treatment with PMA alone, whereas stimulation with LPS alone up-regulated mRNA levels. Treatment with both agonists leads to a synergistic increase in mRNA levels, indicating that both signals are required for high levels of lysozyme gene expression.

Developmental Regulation of Histone H3 Lysine 9 and Lysine 4 Methylation Patterns—We next examined the distribution of histone H3K9 methylation and H3K4 methylation across the lysozyme chromatin domain. Using sonicated chromatin, we had previously measured the distribution of Histone H3K9 methylation by ChIP using an antibody that did not distinguish between mono (1me)-, di (2me)-, or tri (3me)-methylated K9. These experiments showed that H3K9 methylation levels were high in HD37 erythroblasts and HD50 MEP progenitor cells, but dramatically decreased in mature macrophages (14). Here we asked the question at which stage of macrophage differentiation the H3K9 methyl mark was lost, and we also examined whether H3K9 methylation levels were altered during the differentiation of BM2 monoblasts to activated macrophages. This question is of importance, because the H3K9 methyl mark is very stable (22) and PMA-treated differentiating cells cease proliferating (data not shown). To answer these questions we used antibodies against 1me, 2me, and 3me Lys-9. To increase resolution without having to purify mononucleosomes, we prepared cross-linked chromatin from the indicated cell types (HD37, HD50 MEP, untreated BM2 cells, and BM2 cells treated with LPS, PMA, and PMA plus LPS) and then digested such chromatin preparations with micrococcal nuclease. It has been shown before that this avoids nucleosome movement during the preparation of native chromatin while generating chromatin fragments of mostly nucleosome length (23) (an example for such a digestion profile can be seen later in Fig. 5D). To be able to normalize signals and to precisely measure the relative representation of each amplicon, this material (input) was subjected to a real-time PCR analysis using 13 primer pairs across the lysozyme chromatin domain, plus different internal control primers. The results of a ChIP experiment with an antibody against H3 1meK9 are depicted in Fig. 2A and show that histones in the lysozyme cis-regulatory elements have already lost most of the H3 1meK9 mark in undifferentiated BM2 cells. The same results were obtained with an antibody against the H3 2meK9 (data not shown), and methylation levels remained low during further differentiation. Significant histone H3K9 methylation was still observed between cis-elements at all stages of differentiation. We could not detect any H3 3meK9 anywhere across the locus (data not shown). No histone H3K9 methylation was seen at the gas41 CpG island, whereas the histones at the promoter of the liver-specific VLDLapo2 gene, which is inactive in all examined cell types, were highly meth-
FIG. 2. ChIP assay using MNase-treated chromatin and examining histone H3 1meK9 (A), H3 1meK4 (B), and H3 3meK4 (C) status during myeloid differentiation. The top part of the figure presents the map of the lysozyme locus with DNase I-hypersensitive sites in white arrows for the cis-regulatory elements and in black for the constitutive DHS corresponding to the gas41 CpG island. A dotted arrow marks the ~7.9-kb constitutive DHS. Amplicons are represented by gray and white rectangles for flanking/coding region and cis-regulatory elements, respectively, with the exception of the silencer element which is shown in black. Each chart contains results obtained with one or two different amplicons and with the cells indicated on the x-axis as HD37 (1, HD50 MEP (2), and BM2 (3), and PMA-treated BM2 (4), LPS-treated BM2 (5) and PMA- and LPS-treated BM2 (6). Data are expressed as precipitate/input (IP/Input) of test amplicons versus precipitate/input for control amplicons (IPc/Inputc) as outlined under "Experimental Procedures." The antibody used is indicated on the left. Results represent the average of at least two independent chromatin preparations assayed in triplicate.
lated at H3K9 (Supplemental Fig. S2D).

We next analyzed H3K4 methylation and performed ChIP assays with antibodies against 1me, 2me, and 3me H3K4. No H3 1meK4 was observed anywhere across clys in lysozyme non-expressing cells (Fig. 2B), but it was present across the entire 5’ regulatory region in lysozyme-expressing cells (even at the far-upstream amplicon at −10 kb), and increased slightly upon cell differentiation. H3 1meK4 levels in the lysozyme coding region were low, and it was absent in the gas41 gene and absent in the 5’ coding region of the β-actin gene (data not shown). H3 2meK4 and 3meK4 patterns were similar to each other, but different to the H3 1meK4 patterns (Fig. 2C).

Interestingly, some H3 3meK4 could be found in HD50 MEP cells, but not in HD37 cells, and levels increased during cell differentiation at and in between the cis-elements. H3 3meK4 levels in the coding regions increased parallel to the increase in mRNA levels, which were consistently higher in cells treated with LPS (Fig. 1C). Irrespective of the cell type, H3 3meK4 levels at the gas41 CpG island were significantly higher than in the lysozyme gene and within the lysozyme gene were highest in the coding region.

Macrophage Maturation Leads to Site-specific Alterations in Chromatin Architecture and a Gene-wide Depletion of Histone H1—In macrophages, the chicken lysozyme locus is organized in a DNase I-sensitive domain that also harbors the gas41 gene (10). We have previously mapped nucleosome positions at low resolution using micrococcal nuclease digestion and have observed a significant reorganization of nucleosomal architecture at specific cis-regulatory elements after the activation of gene expression (24). In the experiments described here we wanted to further investigate this finding and also test whether the increase in general DNase I sensitivity goes along with a loss of the linker histone H1. The results of these experiments are summarized in Fig. 3.

In the ChIP experiments described above we noticed that not all amplicons were equally represented in the input fraction, indicating significant differences in MNase accessibility and chromatin architecture across the lysozyme locus. Fig. 3A depicts an analysis of the relative representation of input material of cross-linked and MNase-digested chromatin as measured by real-time PCR. From the results depicted in Fig. 3A it is obvious that sequences within the DNase I-hypersensitive cores of the enhancers and the promoter, but not the flanking or coding regions, were becoming progressively under-represented in the Input material the higher the gene was expressed (compare amplicons depicted in white or black to amplicons depicted in gray). The promoter was highly MNase-sensitive over more than 100 bp as measured by looking at two juxtaposed amplicons (−75 to −156 bp (black) and −190 to −255 bp (white)). Interestingly, this under-representation was not seen at the gas41 CpG island, even though this element harbors a 2.7-kb enhancer. This was less prominent at the other cis-elements or was not observed at all (including the VLDLap2 promoter (Supplemental Fig. S2B)). Interestingly, although we observed a strong reduction of input signal at the promoter after MNase digestion (Fig. 3A), with the same amplicon no difference in H3 content was observed between cell types. This indicated that, although histone H3 was partly removed from a subset of enhancers, the promoter and other elements retained their nucleosomal structure. The reduction in input signal at these elements therefore had to originate from an enhanced accessibility of these nucleosomes to nuclease digestion.

Our analysis of linker histone distribution in the different cell types is described in Fig. 3C. These experiments showed that H1 levels at the gas41 CpG island were consistently low. Except for the far-upstream amplicon, all regions of the lysozyme locus showed a progressive reduction of H1 signals in lysozyme-expressing cells with cell differentiation, which was most prominent over the cis-regulatory elements, and greatest at the promoter. However, except for the lysozyme promoter, which lost H1 occupancy very early in differentiation, it was clear that efficient linker histone removal required PMA treatment. The signals in cells treated with LPS alone were consistently higher than those from cells treated with PMA only. Another interesting observation was that, although HD50 MEP progenitor cells do not express the lysozyme gene, they have already clearly reduced levels of H1 across the locus. This was particularly prominent over the promoter, but the same trend was visible at other cis-regulatory elements as well.

The Stimulation of Lysozyme Gene Expression Does Not Alter the Degree of Transcription Factor Binding at the Lysozyme Promoter but Leads to Significant Changes in Chromatin Architecture—We next wanted to uncover the reason for the increased MNase accessibility at the lysozyme promoter and assayed transcription factor occupancy and nuclease accessibility by in vivo footprinting. DNS in vivo footprinting showed no difference in transcription factor occupancy between unstimulated and stimulated BM2 cells (Fig. 4A). As shown previously (13), HD37 cells and HD50MEP cells gave a pattern similar to naked DNA, indicating that no transcription factors were bound to the promoter or anywhere else (data not shown). The DNase I in vivo footprints show little difference between HD37 and MD50 MEP cells, although the pattern differs from naked DNA (Fig. 4B). In contrast, unstimulated and fully differentiated PMA- and LPS-treated BM2 cells showed a number of dramatic alterations in the chromatin structure at the promoter. In concordance with our DHS mapping studies the high resolution experiments demonstrated that promoter chromatin in lysozyme-expressing cells became highly nuclease-accessible. Strong DNS I-hypersensitive sites and protections from DNA I cleavage were observed at the known transcription factor binding sites. Again, pattern and intensity of footprints did not differ between unstimulated and stimulated BM2 cells. Our data therefore show that there was no alteration in the degree of transcription factor occupancy at the promoter as a result of differentiation of BM2 cells.

Our low resolution MNase mapping experiments had previously shown the presence of an array of regular MNase cuts over the promoter and downstream (one such MNase cleavage site is indicated in Fig. 5B). This pattern did not significantly change during cell differentiation, but we had observed an increased cleavage frequency (24). This agrees with the H3 C terminus ChiP assays described here (Fig. 3B). To examine nucleosome boundaries at nucleotide resolution, we used the same MNase-treated material as for ChiP assays (Fig. 5D) to
FIG. 3. A, representation of specific amplicons in input DNA after MNase treatment to prepare the chromatin assayed in Fig. 2. Relative representation of amplicons were calculated relative to the control amplicons depicted in Supplemental Fig. S2A. ChIP assay with sonicated chromatin measuring histone H3 using the H3 C terminus antibody (B) and histone H1 (C) during myeloid differentiation, and results were expressed as IP/Input versus IPc/Inputc. MNase-treated DNA used as a control or used antibody are indicated on the left. The relative representation of amplicons in the Input material from sonicated chromatin is shown in Supplemental Fig. S1. Results represent the average of at least two independent chromatin preparations assayed in triplicate. For all other details see legend of Fig. 2.
and DNase I reactivity are indicated as alterations in the DNase I digestion patterns were observed in lysozyme-expressing cells that were seen in more than one experiment are highlighted. In B multiple alterations in the DNase I digestion patterns were observed in lysozyme-expressing cells, which are not further highlighted.

**DISCUSSION**

Our studies demonstrate extensive alterations in chromatin modification, chromatin composition, and chromatin accessibility within the lysozyme gene, but not the gas41 gene, during macrophase differentiation, some of which are induced by external signaling. This indicates that, although in very close vicinity, both regulatory domains are quite separate and do not interact with each other. The mechanism mediating this independence in regulation is currently under investigation. However, in one respect the two genes appear to influence each other: There is no change in the intra-nuclear localization of the lysozyme locus with respect to the localization of centromeric heterochromatin after gene activation, which is most likely caused by the close vicinity of the active gas41 locus (26).

**Depletion of Histone H1 Is an Early Mark for clys Activation, and Macrophase Differentiation Leads to Extended Alterations in Chromatin Architecture and Nuclease Accessibility**—We have previously shown that the chromatin in multipotent progenitor cell lines (HD50 MEP cells) is partially reorganized and transiently accessible to the binding of transcription factors, although in these cells no stable transcription factor complexes and DHS are formed, and they do not contain hyper-acetylated histones (13, 14). Here we show that such cells contain a significantly reduced amount of histone H1 at the lysozyme promoter as compared with cells from an alternative lineage (HD37), and the same trend, although less dramatic, is observed at the other cis-regulatory elements as well (Fig. 3C). This result indicates a reduction in chromatin compaction and could account for some of the changes in DNA topology we have seen before (13). It is known that certain transcription factors, such as the retinoic acid receptor or the winged-helix factor HNF3 (a FOX family member) can lead to a displacement of histone H1 at specific sequences (27, 28). It is therefore tempting to speculate that the transient interaction of transcription factors in myeloid precursor cells leads to a reduced chromatin compaction via a partial displacement of H1.

We also found that PMA-induced macrophase differentiation, but not LPS induction alone leads to a significant further depletion of H1 from lysozyme cis-regulatory elements, although for maximum depletion both signals are needed. This and the fact that some reduction of H1 is already seen in multipotent precursor cells, indicates that it is the differentiation process itself and not high level gene expression that is responsible for H1 depletion. More elaborate experiments outside of the scope of this study using specific signal transduction inhibitors to block specific signal transduction pathways combined with ChIP assays will hopefully indicate which transcription factors drive this process.

All lysozyme cis-regulatory elements (but not the flanking regions) became progressively more nuclease accessible with increasing gene expression, as indicated by an under-representation of specific amplicons in the input fractions. For some elements, but not all, this correlated with the destabilization of nucleosome binding and nucleosome loss as measured by ChIP analysis with a H3 C-terminal antibody using sonicated chromatin. Such nucleosome loss is a genome-wide phenomenon (29), which has been carefully studied (30, 31) and indicates that care has to be taken when interpreting negative ChIP data. It also indicates that what is precipitated may be a transiently modified nucleosome population that is destined for eviction, as indicated previously (32). The lysozyme promoter clearly behaves differently. We show that although there are no detectable differences in nucleosome content (Fig. 3B), promoter structure becomes more and more nuclease-accessible with increasing transcription as measured by double strand-specific LM-PCR and real-time PCR, which indicates also the presence of single strand cuts (Fig. 3A). Curiously, our previous low resolution mapping experiments did not indicate major differences in nucleosome positions at the promoter between lysozyme expressing and non-expressing cells (24). However, although we can make statements of the precise position of nucleosomal linker regions in lysozyme non-expressing cells, this enhanced accessibility makes it very difficult to map precise nucleosomal boundaries in lysozyme-expressing cells by high resolution methods. The lysozyme promoter is highly inducible, and it is possible that weakened nucleosome-DNA contacts are a prerequisite for the rapid induction of gene expression, very similar to what has been observed with other inducible systems (33–35).

**Macrophase Differentiation Is Accompanied by Differential Alterations in Histone Tail Methylation at clys—**PMA-induced macrophase differentiation and LPS-induction leads to significant alterations in histone modification. Once transcription has started in BM2 cells and transcription factors have bound, H3 1meK9 and 2meK9 levels are drastically reduced at the
cis-elements, but remain relatively high between the elements. The inverse is observed with H3 1meK4, H3 2meK4, and H3 3meK4 levels. Two observations are noteworthy: the active lysozyme locus contains all three types of H3K4 methylation. In contrast, the gas41 CpG island only contains di- and trimethylated H3K4, which are also at much higher level compared with the lysozyme locus. Second, there are differences in the distribution of mono- and di/trimethylated K4 across the lysozyme gene. Monomethylated H3K4 is mainly seen in the regulatory region, whereas di/trimethylated H3K4 increases with transcription and is found in both the coding and the regulatory region. We speculate that this distribution also reflects the inducible nature of the lysozyme gene. We have previously shown that all enhancer complexes interact with the histone acetyltransferase CBP in lysozyme-expressing cells and that LPS induction leads to an increase in histone H3 acetylation without an increase in histone acetylase recruitment (14), indicating that outside signals are required for maximum histone modification. This strict correlation of histone acetylation and lysozyme mRNA levels is also true for differentiating BM2 cells (data not shown). Because the binding of active chromatin remodeling complexes is only promoted by H3 2me and 3meK4 and not by H3 1meK4 (5), our data support a model in which the transcription factors already bound to the cis-regulatory elements recruit a H3K4 methyltransferase activity that marks the lysozyme locus in undifferentiated monoblasts. This would block gene silencing (6), but would not promote chromatin remodeling. High nuclease accessibility and significant nucleosome eviction are only found in fully stimulated cells.
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