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Dynamic Regulation of Histone H3 Methylated at Lysine 79 within a Tissue-specific Chromatin Domain*

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Post-translational modifications of individual lysine residues of core histones can exert unique functional consequences. For example, methylation of histone H3 at lysine 79 (H3-meK79) has been implicated recently in gene silencing in *Saccharomyces cerevisiae*. However, the distribution and function of H3-meK79 in mammalian chromatin are not known. We found that H3-meK79 has a variable distribution within the murine β-globin locus in adult erythroid cells, being preferentially enriched at the active *βmajor* gene. By contrast, acetylated H3 and H4 and H3 methylated at lysine 4 were enriched both at *βmajor* and at the upstream locus control region. H3-meK79 was also enriched at the active *cad* gene, whereas the transcriptionally inactive loci *necdin* and *MyoD1* contained very little H3-meK79. As the pattern of H3-meK79 at the β-globin locus differed between adult and embryonic erythroid cells, establishment and/or maintenance of H3-meK79 was developmentally dynamic. Genetic complementation analysis in null cells lacking the erythroid and megakaryocyte-specific transcription factor p45/NF-E2 showed that p45/NF-E2 preferentially establishes H3-meK79 at the *βmajor* promoter. These results support a model in which H3-meK79 is strongly enriched in mammalian chromatin at active genes but not uniformly throughout active chromatin domains. As H3-meK79 is highly regulated at the β-globin locus, we propose that the murine ortholog of Disruptor of Telomeric Silencing-1-like (mDOT1L) methyltransferase, which synthesizes H3-meK79, regulates β-globin transcription.

Post-translational modification of core histones in chromatin represents a common epigenetic mechanism that controls diverse nuclear processes. An almost endless number of histone modifications has been documented, and it has been hypothesized that a histone code exists in which unique combinations of histone modifications confer distinct functional consequences (1). Acetylation of core histones enhances factor access to the chromatin template by increasing accessibility of nucleosomal DNA (2, 3). Increased accessibility does not appear to be accompanied by a major change in nucleosome structure *in vitro* (4, 5). However, a 3-fold increase in acetylation strongly inhibits folding of chromatin fibers into higher order structures (6). Furthermore, the acetylated amino-terminal tails of core histones are recognized by bromodomains (7, 8), which are often present in coactivators that mediate transcriptional activation. Although the structure of acetylated chromatin and the functional implications of acetylation have been studied for many years (9), considerably less is known about other histone modifications such as methylation.

The concept of a histone code is illustrated by methylation of histone H3 at amino acids 4 and 9. Histone H3 methylated at lysine 9 (H3-meK9),[1] but not at lysine 4 (H3-meK4), is selectively recognized by heterochromatin protein 1 (HP1) (10–14). HP1 serves an important role in the assembly of repressive chromatin structures (15), thus linking a site-specific histone modification to heterochromatin formation. By contrast, H3-meK4 is preferentially enriched in transcriptionally active chromatin (16–18), including transcriptional regulatory elements such as the murine β-globin locus control region (LCR) (Fig. 1) (18, 19). However, H3-meK4 is not uniformly present throughout the active β-globin locus in adult murine erythroid cells (18). The central portion of the locus, which contains the *Ey* and *βH1* embryonic β-globin genes and is characterized by broad, low level acetylation (19–21), also has low level H3-meK4. H3 dimethylated at Lys-4 is enriched within coding regions of transcribed genes in yeast, and this modification is established by the methyltransferase Set1 (22). A more recent analysis in yeast revealed that H3 dimethylated at Lys-4 was enriched at active and inactive genes, whereas H3 trimethylated at Lys-4 marked only active genes (23). The functional consequences of H3-meK4 include inhibiting interaction of the

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[1] The abbreviations used are: H3-meK9, methylation of histone H3 at lysine 9; *cad*, carbamoyl-phosphate synthase/aspartate transcarbamoylase/dihydroorotase; ChIP, chromatin immunoprecipitation; Me_2SO, dimethyl sulfoxide; DOT1, disruptor of telomeric silencing-1; DOT1L, disruptor of telomeric silencing-1-like protein; EryP, primitive erythroid cell colony derived from murine ES cells; ES, embryonic stem cell; H3-meK4, methylation of histone H3 at lysine 4; H3-meK79, methylation of histone H3 at lysine 79; HP1, heterochromatin protein 1; HS, DNase I-hypersensitive site; IVR, intervening region; LCR, locus control region; MEL, mouse erythroleukemia cell; mDOT1L, murine DOT1L; RT, reverse transcriptase; SIR, silent information regulator.

nucleosome remodeling and deacetylase repressor complex with the amino terminus of H3; H3-meK9 does not prevent nucleosome remodeling and deacetylase repressor complex binding (24, 25).

In addition to acetylation and methylation of Lys-4 and Lys-9 within the amino terminus of histone H3, H3 is modified in the central globular domain by methylation at Lys-79 (26–28). Lys-79 methylation is catalyzed by the histone methyltransferase disruptor of telomeric silencing 1 (DOT1) in yeast (26–28) and DOT1-like protein (DOT1L) in humans (29). Methylation of H3 at Lys-79 requires Rad6-mediated ubiquitination of Lys-123 of H2B (H2B-K123) (30, 31), analogous to the coupling of H2B ubiquitination and methylation of H3 at Lys-4 (32). DOT1 was identified by a genetic screen in yeast to isolate genes that disrupt telomeric silencing (33). DOT1 was also identified as a regulator of the pachytene checkpoint, which ensures proper chromosome segregation during the meiotic cell cycle (34). Mutation of Lys-79 and mutations that abrogate DOT1 catalytic activity impair telomeric silencing (26, 27), indicating that DOT1 mediates telomeric silencing via methylation of Lys-79. Taken together with results that H3-meK79 is abundant in bulk yeast histones and is deficient from telomeres, it has been proposed that H3-meK79 excludes silent information regulator (SIR) complexes (27). SIR complexes assemble at telomeres and mediate heterochromatin formation (35). The model assumes that H3-meK79 excludes SIR proteins from the bulk of genomic DNA, thus yielding sufficient amounts of SIR proteins to assemble repressive complexes at heterochromatic sites.

The studies described above have led to a compelling model to explain the functional consequences of H3-meK79 in yeast. However, given the considerably larger size of mammalian genomes *versus* the yeast genome, it would be striking if all active chromatin regions require H3-meK79 to prevent SIR-mediated heterochromatization. The distribution of H3-meK79 in mammalian chromatin has not been determined. Thus, we developed a quantitative chromatin immunoprecipitation (ChIP) assay to measure H3-meK79 levels at a mammalian chromatin domain, the erythroid-specific murine β-globin locus, and at other functionally distinct sites of mammalian chromatin. Although the distribution of H3-meK79 at these sites is consistent with the model in which H3-meK79 is enriched at active promoters, surprisingly, H3-meK79 levels were highly variable throughout the active β-globin domain in adult erythroid cells. As H3-meK79 levels were dynamically regulated by the hematopoietic activator p45/NF-E2 and differed between adult and embryonic erythroid cells, H3-meK79 is likely to be an important determinant of β-globin gene expression.

EXPERIMENTAL PROCEDURES

Cell Culture—MEL (36) and p45/NF-E2-null CB3 (37) cells were maintained in Dulbecco's modified Eagle's medium (Biofluids) containing 1% antibiotic/antimycotic (Invitrogen) and 5% calf serum, 5% fetal calf serum (MEL), or 10% fetal calf serum (CB3). Stably transfected clones of CB3 cells expressing p45/NF-E2 (WT) (38) were selected and maintained in 1 mg/ml G418 sulfate (Calbiochem). Cells were grown in a humidified incubator at 37 °C in the presence of 5% carbon dioxide.

Chromatin Immunoprecipitation Assay—ChIP was performed as described (18, 19, 39). Erythroid maturation of MEL cells was induced by treatment of 0.5×10^5 cells/ml with 1.5% Me_2SO (Sigma) for 4 days. Aliquots of immunoprecipitated chromatin (1.5 μl) were analyzed by real time PCR as described (18, 19, 39). Primers were designed by Primer Express R 1.0 software (PE Applied Biosystems) to amplify 50–150-bp products. Samples from at least two independent immunoprecipitations were analyzed.

ChIP primer pairs to amplify β-globin locus sequences were based on Hbb^d haplotype sequences (GenBank™ accession numbers Z13985, X14061, AF128269, and AF133300). Real time PCR primers (5′-3′) are as follows: HS2, AGTCAATTCTCTACTCCCCACCCT and ACTGCTGTGCTCAAGCCTGAT; IVR3, TGTGCTAGCCTCAAGCTCACA and TCCCAGCACTCAGAAGAAGGA; *βmajor*, CAGGGAGAAATATGCTTGTCATCA and GTGAGCAGATTGGCCCTTACC; *βmajor* I2, CTTCTCTCTCTCCTCTCTCTTTCTCTAATC and AATGAACTGAGGGAAAGGAAAGG; *necdin 5′*, TTCAGTAGCTGATGCCCAGGT and GGGAGGATACCAGAGATGGGA; *necdin* promoter, GGTCCTGCTCTGATCCGAAG and GGGTCGCTCAGGTCCTTACTT; *necdin 3′*, TTCAACTGGCACAGGAAGCA, AACAGTCCAGTTCAAATCAGTCCAT; *MyoD1 5′*, CCAGATCTCAGTGCTGCAGG and CCGCTTGCATAGCATAACCAG; *MyoD1* promoter, GGGTAGAGGACAGCCGGTGT and GTACAATGACAAAGGTTCTGTGGGT; *MyoD1 3′*, TACCCAAGGTGGAGATCCTGC and GCAGACCTTCGATGTAGCGGAT; *cad*, TTCTAACTTGACCGGCTGGTTT and GGACCATAGGATGGTTCCACAG; and *cad* exon2, GCCCTCACTGACCCTTCCTAC and TTGCCGATGAGAGGATACGTT.

FIG. 1. **Organization of the murine Hbb^d β-globin locus.** The murine β-globin locus contains embryonic (*Ey* and *βH1*) and adult (*βmajor* and *βminor*) β-globin genes. β-Globin genes are shown as *boxes* and HSs as *spheres*. *HS*, DNase I-hypersensitive site. The *lower bars* indicate H3- and H4-hyperacetylated zones.

Antibodies—Anti-acetylated histone H3 (06-599) and anti-H3-meK4 (07-030) antibodies were obtained from Upstate Biotechnology, Inc. Anti-H3 dimethyl Lys-79 was described previously (26). Preimmune rabbit serum served as a control for the specific antibodies.

RT-PCR Analysis—Total RNA was prepared from the same CB3 and WT cells used for ChIP with Trizol (Invitrogen). cDNA was synthesized by annealing RNA (1 μg) with 250 ng of a 5:1 mixture of random and oligo(dT) primers by heating at 68 °C for 10 min. After denaturation, samples were incubated with reverse transcriptase (Superscript II, Invitrogen) combined with 10 mM dithiothreitol, RNasin (Promega), and 0.5 mM dNTPs for 1 h at 42 °C. The reaction mixture was diluted to a final volume of 150 μl and heat-inactivated at 95–99 °C for 5 min. Quantitative real time RT-PCRs (25 μl) contained 2.5 μl of cDNA, 12.5 μl of SYBR Green (Applied Biosystems), and the appropriate primers. Product accumulation was monitored by SYBR Green fluorescence. Relative expression levels were determined from a standard curve of serial dilutions of MEL cDNA samples. Forward and reverse primers for real time RT-PCR (5′-3′) are as follows: β-globin, CAGCCTCAGTGAGCTCCACTG and GATCATATTGCCCAGGAGCC; and glyceraldehyde-3-phosphate dehydrogenase, GAAGGTACGGAGTCAACGGATTT and GAATTTGACCATGGGTGGAAT.

Protein Analysis—Lysates were prepared by boiling cells for 5 min in SDS sample buffer (50 mM Tris (pH 6.8), 100 mM dithiothreitol, 2% SDS, 0.1% bromphenol blue, 10% glycerol). Extracts from 1×10^5 cells were resolved on SDS-polyacrylamide gels, transferred to Immobilon P membranes (Millipore), and analyzed by Western blotting as described (18). Blots were incubated with anti-p45/NF-E2 antibody (40), followed by protein A-peroxidase (Sigma) and detected via chemiluminescence with an ECL-plus kit (Amersham Biosciences).

Differentiation of Murine Embryonic Stem Cells into Primitive Erythroid Colonies—Murine ES cells were differentiated into EryP as described (41, 42). Briefly, day 4 embryoid bodies were collected, dissociated with trypsin, and replated at 1×10^5/ml in methylcellulose (Sigma) containing 10% plasma-derived serum (Antech, Tyler, TX), 5% protein-free hybridoma medium (PFHM2, Invitrogen), L-glutamine (2 mM), transferrin (300 μg/ml; Roche Molecular Biochemicals), and monothioglycerol (4.5×10^{-4} M), together with 2 units/ml erythropoietin (Amgen, Thousand Oaks, CA). Primitive erythroid colonies were collected 4 days later via digestion with cellulase (1 unit/ml) for 20 min at 37 °C. Cells were resuspended in culture media, cross-linked with 0.4% formaldehyde for 10 min at room temperature, and analyzed by ChIP as described above with the cell lines.

RESULTS AND DISCUSSION

Quantitative Analysis of H3-meK79 Levels in Mammalian Chromatin—A quantitative ChIP assay was developed to measure H3-meK79 levels in mammalian chromatin (Fig. 2). We initially analyzed the distribution of H3-meK79 within the erythroid-specific β-globin locus (Fig. 1) in Me_2SO-induced MEL cells, which express high levels of the adult β-globin gene *βmajor*. The cross-linked chromatin fragments used for ChIP had an average size of 400–500 bp (Fig. 2*A*), and assays were

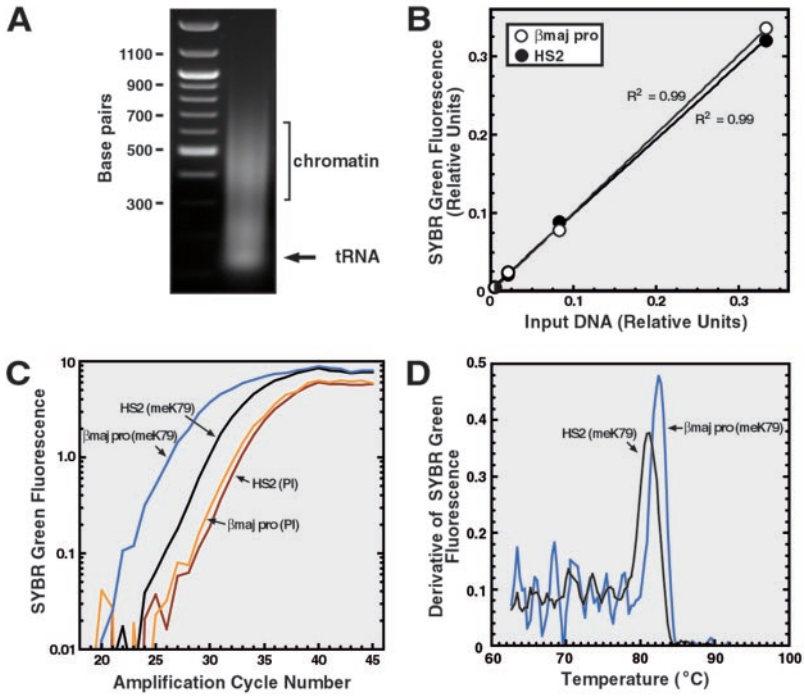

FIG. 2. **Quantitative ChIP analysis of H3-meK79 at the murine β-globin locus.** *A*, agarose gel analysis of sonicated, deproteinized chromatin used in the ChIP assay. Chromatin fragments smaller than 1 kb, averaging ~400–500 bp, were visualized by ethidium bromide staining. *B*, standard curves of SYBR Green fluorescence signals obtained from dilutions of input DNA from induced MEL cells using HS2 and *βmajor* promoter specific primer pairs. *C*, representative amplification plot of H3-meK79 at HS2 and *βmajor* promoter in induced MEL cells; *PI*, preimmune. *D*, dissociation plot obtained from amplicon shown in *C*. The single peak indicates the generation of single amplicon for each primer pair.

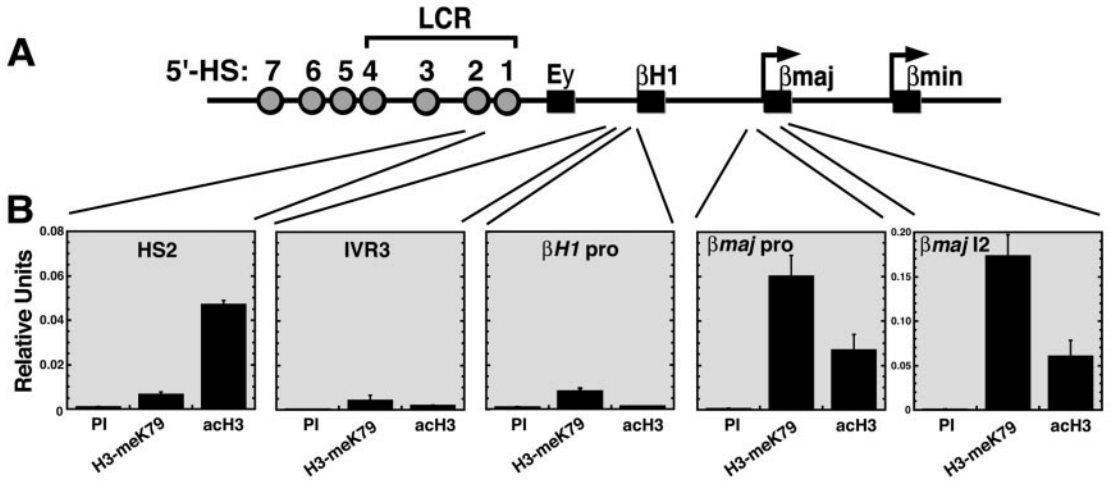

FIG. 3. **Acetylated histone H3 and H3-meK79 have distinct patterns at the β-globin locus of Me_2SO-induced MEL cells.** *A*, organization of the murine β-globin locus. *B*, cross-linked chromatin was isolated from Me_2SO-induced MEL cells. The relative levels of H3-meK79 and H3 acetylation were measured by quantitative ChIP analysis at various sites of the endogenous murine β-globin locus (mean ± S.E., at least three independent experiments). Note that the first four graphs have a common *y* axis with the units indicated on the *left*, and the graph depicting *βmaj* I2 data has a distinct *y* axis. *Pro*, promoter; *I2*, intron 2; *PI*, preimmune; *acH3*, acetylated H3.

conducted under linear conditions (Fig. 2*B*). Representative amplification curves for the detection of H3-meK79 at HS2 of the LCR and at the *βmajor* promoter are shown in Fig. 2*C*. Thermal dissociation of the PCR products revealed a homogenous dissociation curve (Fig. 2*D*), indicative of a single major PCR product for each primer set.

Analysis of H3-meK79 at functionally distinct sites of the β-globin locus (Fig. 3*A*) revealed strong enrichments at the active *βmajor* promoter and within the *βmajor* gene at intron 2 (Fig. 3*B*). By contrast, H3-meK79 levels were low at HS2, at the inactive *βH1* promoter, and at the representative intergenic site IVR3. IVR3 does not contain known functionally relevant sequences. Acetylated H3 was high at HS2, at the *βmajor* promoter, and at *βmajor* intron 2. The fact that H3-meK79 was considerably lower at HS2 *versus* the *βmajor* promoter was surprising, because we showed previously (18–20) that other histone modifications, notably acetylated H3, acetylated H4, and H3-meK4, are strongly enriched at both the HS2 and the *βmajor* promoter. Based on the fact that ~90% of yeast histone H3 is methylated at Lys-79, and based on the SIR localization model (27), we reasoned that H3-meK79 might be uniformly enriched throughout the active β-globin domain. Rather, H3-meK79 had a highly variable distribution, paralleling the distribution of acetylated H3, with the exception that H3-meK79 was low at HS2 (Fig. 3*B*).

To gain a broader perspective of how H3-meK79 is distributed in mammalian chromatin, we measured H3-meK79 at other active and inactive genes (Fig. 4). H3-meK79 levels were high at both the promoter and exon 2 of the broadly expressed *cad* gene. By contrast, upstream sequences, promoters, and exonic sequences from the inactive neuronal-specific *necdin* gene and the muscle-specific *MyoD1* gene had very low levels of H3-meK79. Thus, taken together with the β-globin results of Fig. 3, H3-meK79 levels are highest at active genes.

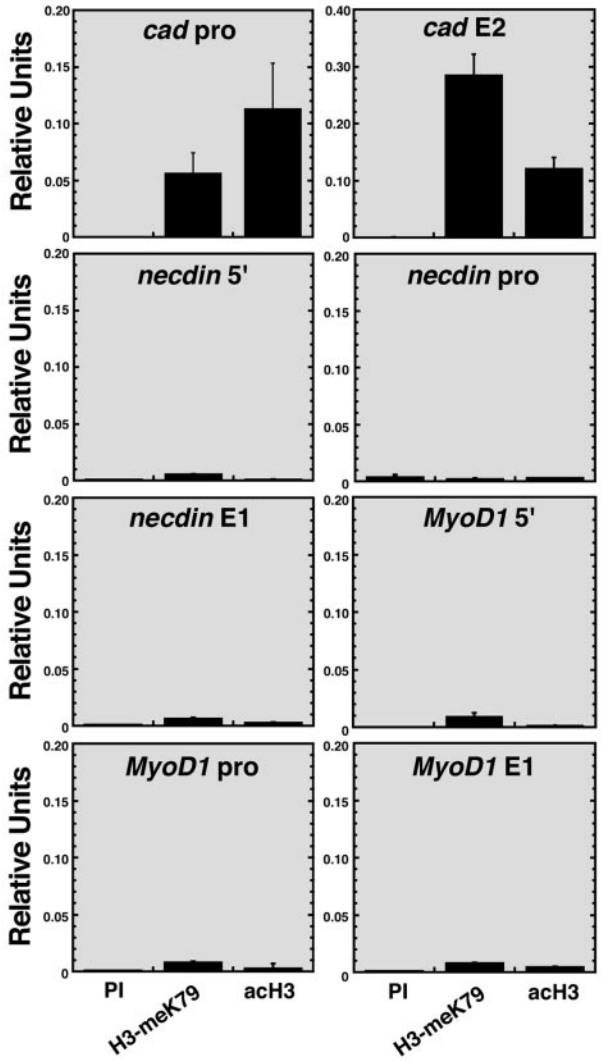

FIG. 4. **H3-meK79 is preferentially enriched at transcriptionally active genes.** Cross-linked chromatin was isolated from Me_2SO-induced MEL cells. The relative levels of H3-meK79 and H3 acetylation were measured by quantitative ChIP analysis at *cad* promoter, *cad* exon 2, upstream from *necdin* (*necdin 5′*), *necdin* promoter, *necdin* exon 1, upstream of *MyoD1* (*MyoD1 5′*), *MyoD1* promoter, and *MyoD1* exon 1. Mean ± S.E. of at least three independent experiments. *Pro*, promoter; *E1*, exon 1; *E2*, exon 2; *5′*, 5′ upstream; *PI*, preimmune; *acH3*, acetylated H3.

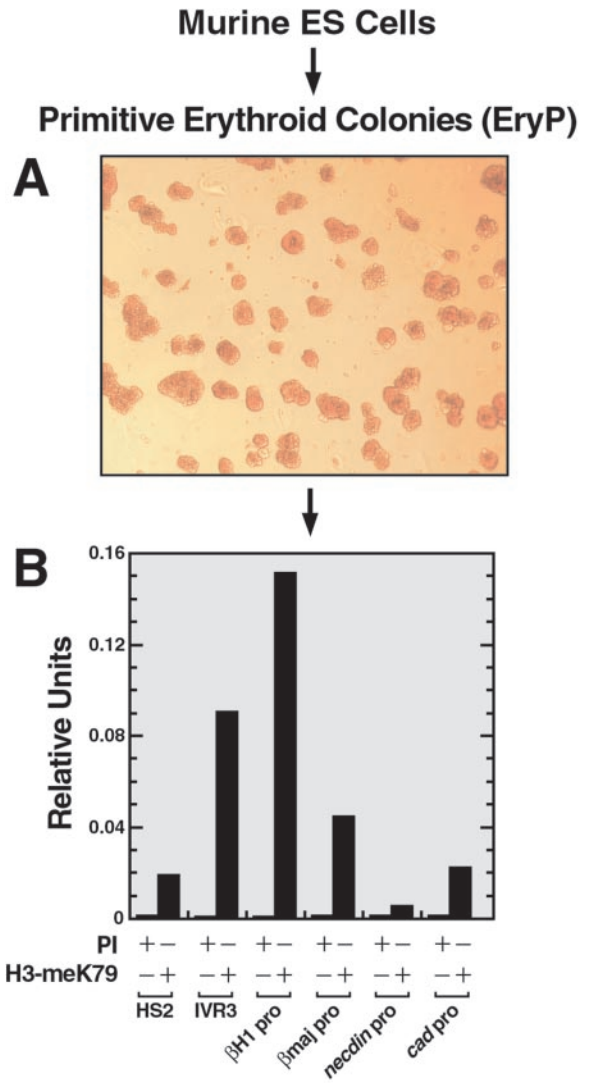

FIG. 5. **In contrast to Me_2SO-induced MEL cells, H3-meK79 is broadly distributed throughout the β-globin domain in primitive erythroid cells derived from murine embryonic stem cells.** *A*, EryP cells were generated via *in vitro* differentiation of murine ES cells. *B*, cross-linked chromatin was isolated from EryP, and the relative level of H3-meK79 was measured by quantitative ChIP analysis (mean ± S.E. of two independent experiments). *Pro*, promoter; *PI*, preimmune.

Dynamic Regulation of H3-meK79 at the Endogenous Murine β-Globin Locus—We showed previously (20) that the central region of the murine β-globin locus, characterized by low level histone acetylation in adult erythroid cells, is highly acetylated in embryonic erythroid cells of a 10.5-day post-conception murine yolk sac. This result indicated that the acetylation pattern of the β-globin locus is developmentally dynamic. Accordingly, we hypothesized that establishment of broad low level acetylation at the central subdomain of the β-globin locus, which contains the embryonic *Ey* and *βH1* genes, was required for silencing of the embryonic β-globin genes. Based on the low level H3-meK79 at the *βH1* promoter and at IVR3, both residing within the central subdomain, we asked whether establishment and/or maintenance of H3-meK79 is developmentally dynamic. Rather than using yolk sac as in our previous analysis, we used primitive erythroid cells derived from murine ES cells via *in vitro* differentiation (Fig. 5*A*). This *in vitro* differentiation system recapitulates regulatory events occurring during normal erythropoiesis (41–44). As large numbers of embryonic erythroid cell colonies, EryP, can be readily differentiated from ES cells, and it is difficult to obtain large numbers of primary embryonic erythroid cells from murine yolk sac, the ES cell system has major advantages for analyzing the chromatin structure of the β-globin locus during embryonic erythropoiesis. No ChIP analyses have been described using specific cell types differentiated from ES cells.

Similar to the β-globin locus of induced MEL cells (Fig. 3), H3-meK79 was enriched at the *βmajor* promoter in EryP cells (Fig. 5*B*). Despite the fact that these cells are embryonic, EryP express low levels of *βmajor* (data not shown). By contrast to the results with induced MEL cells (Fig. 3), H3-meK79 was high at IVR3 and the active *βH1* promoter. Only low levels of H3-meK79 were apparent at the inactive *necdin* promoter, whereas H3-meK79 was enriched at the active *cad* promoter, comparable to induced MEL cells. Thus, H3-meK79 was developmentally dynamic at IVR3 and the βH1 promoter, being high in EryP (embryonic erythroid cells) and low in induced MEL cells (adult erythroid cells). The developmentally specific H3-meK79 patterns suggest an important regulatory role for H3-meK79 in controlling β-globin expression during erythropoie-

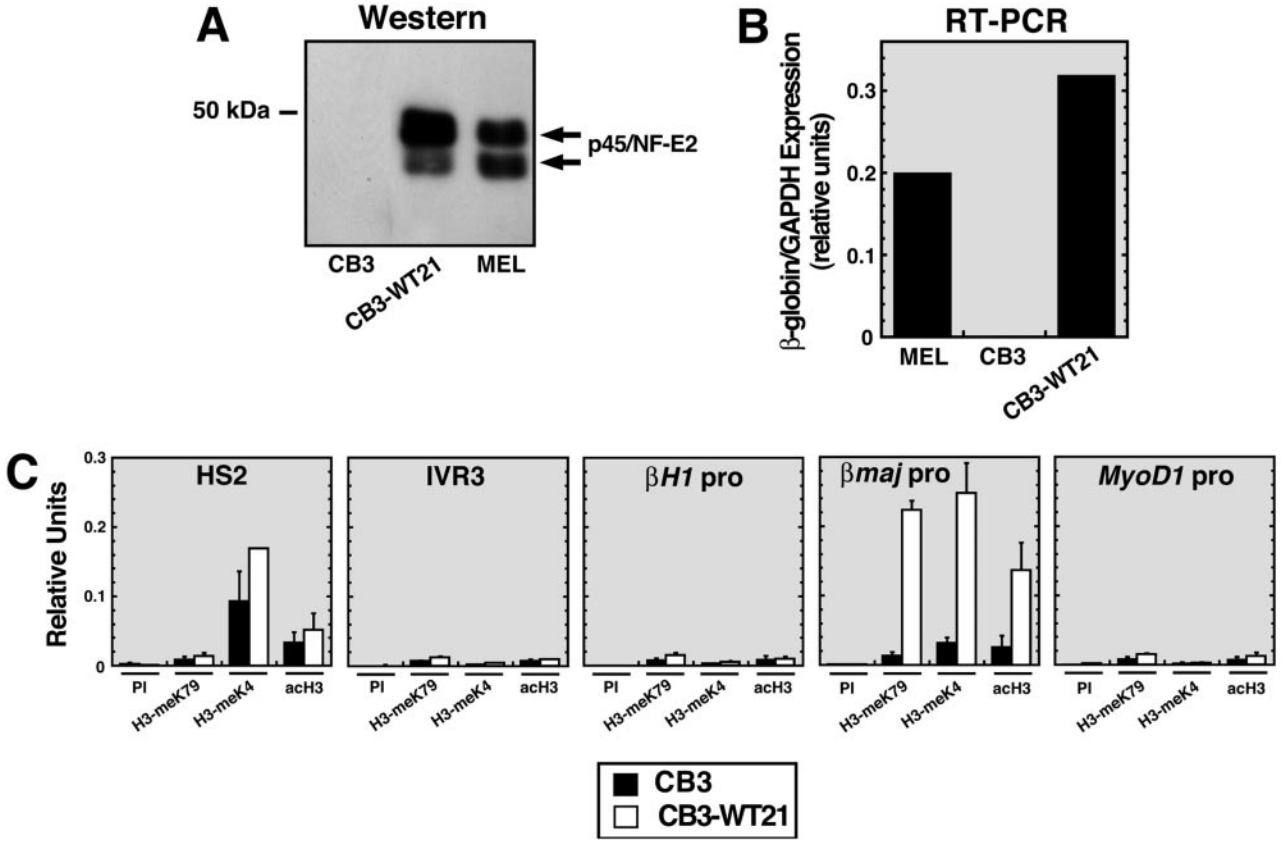

FIG. 6. **p45/NF-E2-dependent establishment of H3-meK79 at the *βmajor* promoter but not at HS2.** *A*, Western blot analysis of p45/NF-E2 expression in total cell lysates from CB3, CB3-WT21, and Me_2SO-induced MEL cells. The *arrows* depict two p45/NF-E2 isoforms, which result from usage of distinct translation start sites. No functional differences have been ascribed to the two isoforms. *B*, real time RT-PCR analysis of β-globin and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) RNA expression in CB3 and CB3-WT21 cells. *C*, relative levels of H3-meK79, H3-meK4, and H3 acetylation were measured by quantitative ChIP analysis at HS2, IVR3, *βH1* promoter, *βmajor* promoter, and *MyoD1* promoter in CB3 and CB3-WT21 (mean ± S.E. of three independent experiments, except for H3-meK4 levels at HS2 in CB3-WT21, which represent duplicate values). *Pro*, promoter; *PI*, preimmune; *acH3*, acetylated H3.

sis. H3-meK79 levels at HS2 were 2.8-fold greater in EryP *versus* induced MEL cells. However, H3-meK79 at HS2 in EryP was much lower than at the active *βH1* promoter, analogous to the result in induced MEL cells, in which H3-meK79 was much lower at HS2 *versus* the active *βmajor* promoter.

We have shown that acetylated H3, H3-meK4, and to a lesser extent, acetylated H4 are induced by p45/NF-E2 at the *βmajor* promoter in CB3 erythroleukemia cells, which lack p45/NF-E2 (18, 45). Given the developmental modulation of H3-meK79, we tested whether establishment of H3-meK79 at the *βmajor* promoter is p45/NF-E2-dependent (Fig. 6). H3-meK79 levels were compared in CB3 cells lacking or stably expressing physiological levels of the two p45/NF-E2 isoforms (Fig. 6*A*). Expression of p45/NF-E2 in CB3 cells strongly activated β-globin transcription as expected (Fig. 6*B*). ChIP analysis revealed that H3-meK79 was only high at the *βmajor* promoter when p45/NF-E2 was expressed (Fig. 6*C*). Similarly, establishment of H3-meK4 and acetylated H3 at the promoter was p45/NF-E2-dependent, consistent with our previous results (18, 45).

Because H3-meK79 was present at HS2 in induced MEL cells, albeit at low levels (Fig. 3), we also tested whether p45/NF-E2 is important for establishing H3-meK79 at HS2. However, H3-meK79 at HS2 was insensitive to p45/NF-E2. Similarly, p45/NF-E2 only slightly affected the levels of H3-meK4 and acetylated H3 at HS2, consistent with our previous results (18, 45). Taken together with the results of Fig. 5, H3-meK79 levels are highly regulated at the β-globin locus via developmental signals and by the hematopoietic-specific activator p45/NF-E2.

Mechanistic Insights Arising from the H3-meK79 Pattern in Mammalian Chromatin—The distribution of H3-meK79 in mammalian chromatin had not been measured previously. Our results on the distribution of H3-meK79 at functionally distinct sites of mammalian chromatin are summarized in Fig. 7. Results described herein support a model in which H3-meK79 is highly enriched in mammalian chromatin at active genes. Conversely, inactive genes are characterized by low level or no detectable H3-meK79. Because our results demonstrate that H3-meK79 is dynamically regulated, resembling the other functionally important modifications H3-meK4 and acetylated H3, we propose that H3-meK79 and, importantly, enzymes that establish H3-meK79, regulate β-globin expression. As multiple histone modifications of the β-globin locus are insensitive to transcriptional inhibition by the elongation inhibitor 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole (data not shown), it is highly unlikely that dynamic changes in these modifications result from altered transcriptional activity. Rather, changes in histone modifications at the β-globin locus are likely to regulate transcription.

It is instructive to relate our results to the current model for the function of H3-meK79 derived from studies in yeast (27). Genetic and biochemical analysis provided evidence that DOT1 methylates H3 at Lys-79 and is required for telomeric silencing (26–28). As summarized in the Introduction, it was proposed that H3-meK79 excludes SIR silencing complexes, which are critical for heterochromatin formation (27). Our results showing that inactive genes have low or undetectable levels of H3-meK79 are consistent with this model. However, the variable

	HS2	IVR3	βH1 pro	βmaj pro	βmaj I2	*necdin* pro	*necdin* E1	*MyoD1* pro	*MyoD1* E1	*cad* pro
MEL	–	–	–	+++	+++	–	–	–	–	++
EryP	+	+++	+++	++	++	–	N.D.	N.D.	N.D.	+
CB3	–	–	–	–	N.D.	N.D.	N.D.	–	N.D.	N.D.
CB3-WT21	–	–	–	+++	N.D.	N.D.	N.D.	–	N.D.	N.D.

–	< 0.016
+	0.016 - 0.03
++	0.03 - 0.06
+++	> 0.06

FIG. 7. **Summary of relative levels of H3-meK79 at selected loci in Me_2SO-induced MEL, EryP, CB3, and CB3-WT21 cells.** Averaged data obtained from quantitative ChIP analysis in Me_2SO-induced MEL, EryP, CB3, and CB3-WT21. Values were assigned units of −, +, ++, and +++ based on the scale shown on the *bottom. N.D.,* not determined.

distribution of H3-meK79 throughout the mammalian β-globin locus in adult erythroid cells, being almost entirely absent from sites within the central subdomain and at HS2, was not predictable from the yeast model.

Why would the central subdomain and HS2 have such low levels of H3-meK79 in induced MEL cells? The central subdomain also has low levels of acetylated histones H3 and H4 (19, 20) and H3-meK4 (18), is inaccessible to restriction endonucleases, and contains sites highly modified by CpG dinucleotide methylation (19). The histone acetylation pattern of MEL cells is physiologically relevant, because it is similar to that of normal adult erythroid cells of the 14.5-day post-conception murine fetal liver (20). It is unknown whether SIR proteins are components of the repressive chromatin structure of the central subdomain in erythroid cells. The low level H3-meK79 at the central subdomain might allow SIR proteins to localize to the central subdomain, thereby facilitating assembly of repressive complexes to establish and maintain the silent state of the embryonic β-globin genes. Alternatively, other factors might preclude the need to utilize H3-meK79 to exclude SIR proteins from the central subdomain.

Based on the recent cloning of human DOT1L, which catalyzes methylation of histone H3 at Lys-79 (29), and the dynamic regulation of H3-meK79 at the β-globin locus, we propose that mDOT1L is an important regulator of β-globin gene transcription. Whereas acetylated H3 and H4 and H3-meK4, which are hallmarks of active chromatin, are highly enriched at the LCR and the active promoters (18, 20), H3-meK79 is uniquely high at active genes in induced MEL cells (Fig. 3). The variable distribution of H3-meK79 within the β-globin locus in MEL cells is consistent with an important regulatory function of H3-meK79 locally at the active adult β-globin genes. By contrast, H3-meK79 had a broader distribution throughout the locus in EryP, and notably the intergenic site IVR3 of the central subdomain had high levels of H3-meK79. The EryP results indicate that H3-meK79 does not reside exclusively at active promoters and genes.

In summary, we have described the first analysis of the distribution of H3-meK79 in mammalian chromatin. This analysis in adult and embryonic erythroid cells yielded patterns that were not predictable from prior studies in yeast. H3-meK79 was considerably more enriched at the *βmajor* promoter *versus* HS2, and H3-meK79 represents the only histone modification detected to date that is preferentially enriched at *βmajor*. Based on these findings, it will be important to determine how mDOT1L expression and activity are regulated during erythropoiesis and whether hematopoietic specific activators differentially recruit mDOT1L to the β-globin locus in embryonic and adult erythroid cells.

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