Profile of Histone Lysine Methylation across Transcribed Mammalian Chromatin^{∇†}

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Complex patterns of histone lysine methylation encode distinct functions within chromatin. We previously reported that trimethylation of lysine 9 of histone H3 (H3K9) occurs at both silent heterochromatin and at the transcribed regions of active mammalian genes, suggesting that the extent of histone lysine methylation involved in mammalian gene activation is not completely defined. To identify additional sites of histone methylation that respond to mammalian gene activity, we describe here a comparative assessment of all six known positions of histone lysine methylation and relate them to gene transcription. Using several model loci, we observed high trimethylation of H3K4, H3K9, H3K36, and H3K79 in the transcribed region, consistent with previous findings. We identify H4K20 monomethylation, a modification previously linked with repression, as a mark of transcription elongation in mammalian cells. In contrast, H3K27 monomethylation, a modification enriched at pericentromeric heterochromatin, was observed broadly distributed throughout all euchromatic sites analyzed, with selective depletion in the vicinity of the transcription start sites at active genes. Together, these results underscore that similar to other described methyl-lysine modifications, H4K20 and H3K27 monomethylation are versatile and dynamic with respect to gene activity, suggesting the existence of novel site-specific methyltransferases and demethylases coupled to the transcription cycle.

Covalent modification of histone facilitates the proper functioning of the chromatin fiber, specifying diverse nuclear processes, including gene regulation, heterochromatin formation, and DNA repair (33, 62). Lysine methylation displays the highest degree of complexity among known covalent histone modifications, with each site of methylation regulating the association of different effector molecules (35). On the basis of work with several model systems, methylation of lysines 4, 36, and 79 of histone H3 (H3K4, H3K36, and H3K79) occurs primarily in association with gene activation (1, 3, 48), whereas methylation of H3K9, H3K27, and H4K20 have been described at sites of gene repression and/or heterochromatin (8, 46, 52, 58). Methylation of H3K9, a mark enriched at pericentromeric heterochromatin (34, 51), has recently been found by our lab as well as others to also be present at the transcribed regions of active mammalian genes (7, 53, 64, 69), suggesting that certain methyl marks can have multiple functions in the cell, the outcome of which is determined by context. The versatility of lysine methylation marks is perhaps best exemplified by H3K79 and H4K20 methylation, modifications implicated in transcriptional regulation (24, 44, 70) as well as being required for double-strand break repair in several organisms (20, 55). Identification of the numerous biological functions encoded by histone lysine methylation is a major area of research interest, as these mechanisms are intimately associated with cellular

senescence (6), genomic instability (46), and leukemogenesis (44).

One mechanism by which an individual site of histone methylation can serve multiple biological functions in the cell is through regulation of the processivity (mono-, di-, and trimethylation) of the methyltransferase reaction at different chromosomal regions (56). In mammalian chromatin, H3K4 dimethylation (H3K4me2) is enriched broadly across active or poised chromatin, whereas H3K4 trimethylation (H3K4me3) is enriched exclusively at punctate positions near transcription start sites (3, 32, 56). In *Saccharomyces cerevisiae*, histone H2B ubiquitylation is required for di- and trimethylation of H3K4 and H3K79, but not for monomethylation, demonstrating one mechanism by which methyltransferase processivity can be regulated (61, 62). By microscopic analysis, 4',6'-diamidino-2-phenylindole (DAPI)-dense heterochromatin is enriched for H3K9me3 and H3K27 monomethylation (H3K27me1), but not for H3K9me2, H3K9me1, H3K27me2, or H3K27me3 (45). H4K20me3 is also enriched at DAPI-dense heterochromatin, whereas H4K20me2 and H4K20me1 localize primarily to euchromatin (58). The inactive X chromosome acquires its own distinct pattern of histone lysine methylation with broad staining for H4K20me1 (28, 58), H3K27me3 (47, 63), and H3K9me2 (19, 63). Notably, immunostaining of chromosomes does not rule out the presence of modifications at individual genes, as H3K9me2 and H3K9me3 antibodies do not stain the active X chromosome but the modifications can be detected at transcribed regions by chromatin immunoprecipitation (ChIP) (7, 53).

While multiple sites of lysine methylation have been linked with gene activation, each modification is distributed to unique positions across the active gene (48). H3K4me3 occurs predominantly at the transcription start sites of active genes,

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whereas H3K36me3 exhibits a more 3' distribution across transcribed regions (1, 3, 48, 49, 57). It has recently been reported that H4K20me1 is enriched at the active major β -globin and *c-myc* genes. At the major β -globin gene, this mark was observed at comparable levels near the promoter and the transcribed portion (67). Whether this particular modification is a general feature of active genes and its precise relationship to transcriptional activity remain to be determined.

Defining spatial distributions for methyl-lysine marks is relevant to the determination of biological function. For example, H3K36 methylation can have a repressive effect on promoter activity (31, 65); however, this modification is distributed in the coding regions of active genes, where it serves to suppress aberrant transcription initiation in the wake of elongating RNA polymerase II (Pol II) (9, 23, 25). Shared distributions of methyl marks can also denote important biological pathways; for example, the shared pattern of H3K4 and H3K27 methylation at repressed developmental genes in embryonic stem cells suggests a role in maintaining pluripotency (4). Extensive analysis of the spatial distribution of lysine methylation in mammalian cells has been reported for only a few methyl-lysines (3, 4, 64). Therefore, the extent to which patterns of lysine methylation are coordinated to encode important functions remains an unanswered question. Coordinated lysine methylation is particularly relevant, as some histone methyltransferases can methylate multiple lysines; e.g., Ash1 has been reported to methylate H3K4, H3K9, and H4K20 (2). In addition, demethylase enzymes can have multiple substrates, e.g., JHDM3A demethylates H3K9 and H3K36 (27, 74).

Here we present a profile of the spatial distributions for each position of histone lysine methylation with respect to transcriptional activity in mammalian chromatin. Using a chromatin immunoprecipitation assay with antibodies against the six known sites of histone lysine methylation performed at the highly active mammalian poly(A) binding protein C1 (PABPC1) gene, we made several novel findings. First, we observe two distinct patterns of lysine methylation. H3K4me3 and H3K79me3 are similarly enriched near the transcription start site, whereas H3K9me3, H3K36me3, and surprisingly H4K20me1 colocalize and are maintained across the entire transcribed region. In addition, we identify H4K20me1 as a true mark of transcription elongation in mammalian chromatin on the basis of its enrichment downstream of several active promoters and its sensitivity to the elongation inhibitor 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB). Also, we observe H3K27me1 present at all euchromatic regions examined; however, this modification is selectively removed in the vicinity of the transcription start sites at active genes. This suggests that H3K27me1 removal may be a general prerequisite for the initiation of high-level transcription. Finally, we observe antagonistic distributions of H3K9 acetylation with H3K9me3 across an active gene, suggesting that histone acetylation near the transcription start site may impose a 5' boundary on H3K9me3 in the transcribed region. Together these data provide new insights into the coordination of histone lysine methylation in transcribed chromatin and further suggest the presence of novel methyltransferases and demethylases within euchromatin that deposit and remove these modifications.

MATERIALS AND METHODS

Cell culture. HeLa cells were cultured in Dulbecco modified Eagle medium with 10% fetal calf serum, 2% penicillin or streptomycin, 1% glutamine, and 1% Na pyruvate. G1E cells were cultured as described previously (72). Cells were treated with 1 μ M tamoxifen or 100 μ M 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB; Sigma) where indicated. Tamoxifen treatment was performed for 21 h.

Antibodies. The following antibodies were used in this study: H3K4me3 (catalog no. 07-473; Upstate), H3K9me3 (catalog no. 07-442; Upstate), H3K27me1 (catalog no. 07-448; Upstate), H3K27me2 (catalog no. 07-452; Upstate), H3K27me3 (catalog no. 07-449; Upstate), H3K36me3 (ab9050; Abcam), H3K79me3 (ab2621; Abcam), H4K20me1 (ab9051 [Abcam] and catalog no. 07-440 [Upstate]), H4K20me2 (ab14092 [Abcam] and catalog nos. 07-367 and 07-031 [Upstate]), H4K20me3 (catalog no. 07-463; Upstate), total RNA polymerase II (sc899; Santa Cruz), LEO1 (BL677; Bethyl), and total histone H3 (ab1791; Abcam).

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation was performed as described previously (15). Cross-linking was performed with 1% formaldehyde. Samples were analyzed in duplicate by real-time PCR using SYBR green dye on either an ABI Prism 7000 or 7500 machine. To allow comparison among primer sets, unprecipitated input samples from each condition were diluted serially and used as the standard for all PCRs. All data shown are the averages of two or more independent experiments. Error bars represent standard deviations. Primer sequences are provided in Table S1 in the supplemental material.

RESULTS AND DISCUSSION

The human PABPC1 gene encompasses a 25-kilobase domain of highly transcribed chromatin. On the basis of genome-wide profiles of H3K4, H3K36, and H3K79 methylation in *S. cerevisiae* (48), each modification acquires a distinct distribution that is similar among most, if not all, active genes. Therefore, to generate a lysine methylation landscape for transcribed mammalian genes, we initially focused our analysis on a single active gene with the expectation that the results would be generalizable. Using published microarray data (73), we identified the widely expressed gene PABPC1 [poly(A) binding protein C1] as having abundant mRNA levels suggestive of highly active transcription. PABPC1 is a large gene [19.2 kb from the transcription start site to the poly(A) sequence] and therefore ideally suited for comparative high-resolution profiling of histone lysine methylation. All ChIP data shown for PABPC1 were generated in HeLa cells. A total of 12 regions of the PABPC1 locus were analyzed ranging from 1.5 kb upstream of the promoter to sequences 5 kb downstream of the poly(A) sequence. The +0.5-kb region within the inactive CD4 gene, which is expressed only in T cells, served as negative control for activation-associated modifications.

To validate that high PABPC1 mRNA levels reflect a high rate of transcription, we performed ChIP experiments with antibodies against total RNA polymerase II and an antibody against LEO1, a subunit of the recently identified human PAF elongation complex (54, 77, 78). As shown in Fig. 1A and B, total RNA polymerase II is present across the active PABPC1 gene. Remarkably, the enrichment of total RNA polymerase II at the PABPC1 promoter is nearly 150-fold relative to the inactive CD4 gene (negative-control region). Such enrichment is more than 10-fold greater than we have observed previously at active erythroid-specific promoters (68), suggesting potent promoter activity for PABPC1. As shown in Fig. 1B, total RNA polymerase II in the transcribed region is far less abundant than at the promoter region (only fivefold enrichment at position +12 relative to CD4), consistent with recruited Pol II in

FIG. 1. The human PABPC1 gene encompasses a 25-kilobase domain of highly transcribed chromatin. A) ChIP of total RNA polymerase II across the PABPC1 locus, at MYT1, and CD4. B) Results of panel A presented on a different scale. C) ChIP using antibodies against LEO1. IgG, immunoglobulin G.

the preinitiation complex having a more stable association with chromatin than Pol II in elongation complexes. In addition, we detect Pol II beyond the poly(A) element of the gene extending an additional 5 kb, suggesting that transcription termination occurs >5 kb downstream of the poly(A) sequence. ChIP performed with antibodies against LEO1, which reflects the distribution of transcription elongation complexes, likewise demonstrates a broad domain (ranging from 5-fold to 20-fold enrichment relative to CD4) of transcribed chromatin (Fig. 1C). Notably, LEO1 occupancy reflects a greater bias for downstream transcribed regions than for the promoter region, which is distinct from total Pol II. In summary, these data verify human PABPC1 as a highly transcribed gene spanning >25 kb of mammalian chromatin, thus affording a clear distinction of the promoter, 5', middle, and 3' transcribed regions by high-resolution ChIP analysis of histone modifications.

To verify that trends in lysine methylation observed at PABPC1 were generalizable, we analyzed a second active gene, RPLP0, in HeLa cells (see Fig. S2 in the supplemental

material). Moreover, the dynamics of histone lysine modifications were studied at several inducible and repressible genes in the murine erythroid cell line G1E (see below).

Spatial distribution of histone methylation generated by active mammalian gene transcription. When measuring modifications previously linked with gene activation, we focused our analysis on the terminal trimethyl modification (for H3K4, H3K9, H3K36, and H3K79). However, for modifications not previously linked with mammalian gene activation (H3K27 and H4K20), we analyzed all three methyl states.

Previous work has shown that H3K4 and H3K36 methylation adopt characteristic distribution profiles in metazoan and *S. cerevisiae* chromatin (1, 3, 36, 49, 57, 59). H3K4me3 occurs primarily in the vicinity of the transcription start site, whereas H3K36me3 occurs across the entire transcribed region. We observed similar profiles of these two modifications at the PABPC1 gene, with H3K4me3 reaching a maximum at +0.5 kb and being relatively depleted beyond 5 kb (Fig. 2A) and H3K36me3 being broadly maintained from +1 kb to +25 kb

FIG. 2. Histone methylation signature generated by active mammalian gene transcription. ChIP performed across the PABPC1 locus. MYT1 (+0.5-kb region) or CD4 (+0.5-kb region) served as controls. All distances are relative to the site of transcription initiation. Histone lysine methylation was measured using the following antibodies: A) H3K4me3; B) H3K9me3; C) H3K27me1, me2, and me3; D) H3K36me3; E) H3K79me3; F) H4K20me1, me2, and me3. G) Total histone H3. IgG, immunoglobulin G.

FIG. 2—Continued.

(Fig. 2D). Importantly, both modifications are low 1.5 kb upstream and at the promoter region of PABPC1, as well as the inactive CD4 gene, consistent with previous data linking these modifications with transcription elongation (29, 41, 76). The characteristic 5' H3K4me3 and 3' H3K36me3 distributions are consistent with genome-wide analyses of the modifications (3, 48), thus validating PABPC1 as a model gene for subsequent lysine methylation analysis.

H3K79 trimethylation has been profiled genome-wide in *S. cerevisiae* and found to occur both 5' and 3' across the transcribed region (48). H3K79 dimethylation has been examined in metazoan chromatin and found to correlate with transcriptional activity (21, 59); however, the precise spatial distribution of H3K79 trimethylation on active genes has not been examined. We observed a 5' distribution of H3K79 trimethylation on the PABPC1 gene with enrichment between +0.5 and +5 kb (Fig. 2E). Like H3K4me3, this modification is not maintained across the majority of the transcribed region from +5 to +25 kb. This distribution is different from that reported in *S. cerevisiae*, which may reflect differences between metazoan and yeast chromatin.

Our lab and others have found H3K9me3 in the transcribed regions of active genes (7, 64, 69). We profiled H3K9me3 across the PABPC1 gene and observed a 3' distribution, with this modification beginning at +1 kb and being maintained across the entire 25-kilobase transcribed region (Fig. 2B). Interestingly, this modification pattern is similar to that of H3K36me3, which may reflect similarity in function (Fig. 2D). However, H3K9me3 displayed a distinct distribution from that observed for H3K4me3, with only partial overlap at the +1-kb and +2-kb regions (Fig. 2A and B). We previously observed similar profiles of H3K4me3 and H3K9me3 in *cis* along several genes that are less than 5 kb in size (69). Failure to distinguish among the H3K4me3 and H3K9me3 profiles in those experiments may be due to limited resolution across relatively short transcribed regions. In addition, the 5' boundary of H3K9me3 may be influenced by the extent of H3K9 acetylation around the transcription start site (see below).

The variability in sensitivity and specificity of individual

batches of serum for particular modifications can alter ChIP assay results. In our experience, antibodies against H3K9 methylation have been particularly variable in their sensitivity, with each batch having a preferential affinity either for transcribed regions or for heterochromatin (69). The ability of H3K9-methyl antibodies to recognize epitopes appears to be influenced by context; e.g., phosphorylation of H3 serine 10 can block antibodies from binding H3K9-methyl (45). In addition, generation of highly sensitive and specific antibodies for H3K9 methylation required the use of branched peptide antigens to mimic the presentation of histone tails as they may occur in native chromatin (46). Notably, all data shown here have used the Upstate H3K9me3 antibody (catalog no. 07-442; Upstate), which was raised against branched H3K9me3 peptides (45). All considerations that are based on ChIP need to take into account the limitations and variations of the available antibodies.

H4K20 monomethylation has recently been reported to increase at the major β -globin gene upon activation in erythroid cells (67). However, the generality of this finding was uncertain, as loss H4K20me1 was not observed upon repression of the *c-myc* gene (67). In addition, the spatial distribution was not measured at high resolution with respect to the transcription cycle. To clarify the role of H4K20 methylation in mammalian gene activation, we measured mono-, di-, and trimethylation across the PABPC1 gene (Fig. 2F). As in previous reports (67, 69), we failed to detect H4K20me3. This finding is likely to be a true-negative result, as the same antibody gives pronounced enrichment at major satellite repeat elements by ChIP (69). In addition, we failed to detect H4K20 dimethylation at PABPC1. In the absence of a suitable positive control for this modification, we sought to confirm this finding by using a total of three different antisera against H4K20me2 and obtained the same result (data not shown), which suggests that this modification is truly absent in transcribed chromatin. In contrast, antibodies against H4K20me1 (ab9051; Abcam), revealed substantial enrichment across the entire transcribed region (Fig. 2F). While the association of H4K20me1 with gene activity is similar to previous findings (67), its distribution is distinct. The absence of H4K20me1 at an upstream region and at the promoter region together with its enrichment throughout the transcribed region suggests this modification may be deposited by transcription elongation complexes (see below). In addition, the presence of this modification throughout the entire transcribed region is similar to the profiles of H3K9me3 and H3K36me3. The result was verified with an independently derived H4K20me1 antibody (catalog no. 07-440; Upstate), albeit with much lower sensitivity (see Fig. S3 in the supplemental material).

In summary, we have profiled five methyl-histone modifications linked with gene activation (H3K27 methylation is discussed separately below) and observed two distinct categories of distribution. H3K4me3 and H3K79me3 are highest at regions in the immediate vicinity of the transcription start site and fail to persist beyond 5 kilobases into the transcribed region. In contrast, H3K9me3, H3K36me3, and H4K20me1 stretch across the entire transcribed region. Similar findings were obtained at the RPLP0 gene, with a similar breakdown into two basic groups Fig. (see Fig. S2 in the supplemental material). The landscape categories may be relevant to the

biological function of these modifications. It is interesting to note that H3K4 and H3K79 methylation have each been found to positively regulate gene activity in mammalian cells (11, 44, 75, 79), which may explain their proximity to promoter elements. In striking contrast, H3K9, H3K36, and H4K20 methylation have all been shown to exert repressive effects on promoter activity (24, 31, 42, 60, 65), which may explain their relative distribution 3' at active genes, away from the active site of transcription initiation. H3K36 methylation has been shown in yeasts to prevent cryptic promoter elements from becoming aberrantly activated following passage of Pol II (9, 23, 25). It is tempting to speculate that mammalian chromatin has evolved an additional repertoire of H3K9 and H4K20 methylation in the transcribed region to further maintain genomic stability in the wake of elongating Pol II. It is also possible that these marks alone or in concert regulate other aspects of transcription elongation or facilitate transcript processing.

For a control, we profiled total histone H3 density across the PABPC1 locus and at the CD4 gene (Fig. 2G). Except for the promoter region, histone density at the PABPC1 locus was similar to that found at CD4, confirming that enrichment of all five methyl marks at transcribed regions is not due to differences in the total amount of histones. The promoter of PABPC1 is depleted of total histone H3, which may be a function of robust promoter activity leading to eviction of nucleosomes (5) (Fig. 1A). The depletion of histone at the promoter region may account for the low levels of 5' modifications (H3K4me3 and H3K79me3) at this region (Fig. 2A and E). Normalizing histone lysine methylation data to histone density provides generally the same basic profile (see Fig. S1 in the supplemental material).

H3K27 monomethylation is selectively depleted near transcription start sites. H3K27 methylation has been linked with promoter repression by Polycomb complexes (8, 10, 30, 39). To determine whether changes in H3K27 methylation accompany mammalian gene transcription, we measured methylation of this site across the PABPC1 gene. We failed to detect H3K27me2 or H3K27me3 throughout the PABPC1 gene, with MYT1 serving as a positive control for H3K27me3 (26) (note the different y axes) and CD4 as a positive control for H3K27me2. In contrast, H3K27me1 was detected across the entire PABPC1 locus, with selective depletion in the vicinity of the transcription start site (Fig. 2C). In contrast, the transcription start site of CD4 retained H3K27me1, suggesting depletion of this modification is unique to active genes. MYT1, a Polycomb target gene (26), also displays low H3K27me1; however, this is likely due to this modification being shunted to the di/trimethyl state. The depletion of H3K27me1 cannot be accounted for by histone density, as the promoter region alone is depleted of histone whereas the region depleted of H3K27me1 extends to +2 kb within the transcribed region (Fig. 2C). Normalizing the data to histone density likewise demonstrates H3K27me1 depletion around the transcription start site (see Fig. S1 in the supplemental material). A similar profile H3K27me1 was obtained at the RPLP0 gene (see Fig. S2 in the supplemental material).

The steady-state depletion of H3K27me1 in the vicinity of the transcription start site of the PABPC1 gene suggested that levels of this modification might undergo dynamic changes upon induction or repression of transcription. To examine

H3K27me1 levels in relation to transcriptional activity, we performed ChIP experiments in the erythroid cell line G1E (71) that stably expresses a GATA-1–estrogen receptor (ER) fusion protein. Addition of tamoxifen (4-hydroxy-tamoxifen [OHT]) to the growth medium activates the GATA-1–ER to directly induce transcriptional activation of the major β -globin gene as well as repression of the GATA-2 and *c-kii* genes (72). Primers that amplify the upstream regions, promoters, and the early transcribed regions of these genes were used. At the inactive β -major gene prior to transcription induction, we observed equal levels of H3K27me1 across the gene (Fig. 3A). Following OHT-induced activation of major β -globin transcription, H3K27me1 decreased at the promoter and at +0.8 kb within the transcribed region (Fig. 3A) consistent with results obtained at the PABPC1 gene. In contrast, H3K27me1 at –1 kb upstream of the major β -globin gene remains present and unchanged. We measured total histone H3 in parallel and found nucleosome loss at the promoter but no significant change upstream or at the +0.8-kb transcribed region (Fig. 3C). This indicates that the loss of H3K27me1 cannot be explained solely by histone eviction. Moreover, reduction of H3K27me1 is not due to conversion into H3K27me2 or H3K27me3, as neither of these modifications were detected at the major β -globin gene (data not shown), suggesting the existence of an active removal mechanism for H3K27 monomethylation.

At the actively transcribed GATA-2 gene, we observed a similar localized depletion of H3K27me1 around the transcription start site, including the promoter and proximal transcribed region but not at flanking regions positioned at –4.9 kb and +13.7 kb relative to the promoter (Fig. 3B). Upon repression of the GATA-2 gene after OHT treatment, H3K27me1 levels were restored at the promoter and +0.5 regions, becoming evenly distributed across the locus (Fig. 3B). Again, histone density does not account for the observed changes in H3K27me1 (Fig. 3D). In concert, these findings indicate that H3K27me1 is a locally dynamic mark whose levels correlate inversely with transcriptional activity and suggest that removal of this modification may be required for activated transcription.

Moreover, our data suggest that H3K27 monomethylation is broadly distributed throughout euchromatin, consistent with nuclear staining (45). We speculate that acquisition of H3K27me1 is the default state of chromatin, possibly acquired during assembly of chromatin following DNA replication. This is supported by our observation of H3K27me1 present at nearly all tested euchromatic sites, including the inactive embryonic β -globin-like genes (data not shown), and upstream, genic, and downstream regions of all active genes examined. Two independent mechanisms can be considered by which H3K27me1 can be selectively depleted at certain genomic regions. First, the transcription start sites of active genes are depleted of H3K27me1 through an active removal process. Second, stable recruitment of Polycomb PRC2 complexes can convert H3K27 monomethylation to trimethylation, as observed at the repressed MYT1 gene (26). Importantly, loss of H3K27me1 at active genes is not due to conversion to H3K27me2 or H3K27me3, as these modifications are not detected at these regions. It is interesting to note that the distribution of H3K27me1 is a perfect inverse of H3K4me3, as the

FIG. 3. H3K27 monomethylation is dynamically removed in the vicinity of the transcription start site upon gene activation and is restored upon gene repression. ChIP experiments performed in G1E cells before or after tamoxifen (OHT) treatment. A) H3K27me1 at the major β -globin (β -major) gene. B) H3K27me1 at the GATA-2 gene. C) Histone H3 density at the major β -globin gene. D) Histone H3 density at the GATA-2 gene. IgG, immunoglobulin G.

enzymes that perform these modifications in *Drosophila*, Polycomb and Trithorax, respectively, are classic antagonists of one another in their regulation of the homeotic gene cluster (16).

H4K20 monomethylation is a mark of transcription elongation in mammalian chromatin. In contrast to a previous report (67), we observe H4K20me1 uniquely present in the transcribed region and not at the active promoter (Fig. 2F), suggesting that H4K20me1 might be a mark of transcription elongation. To test this hypothesis, we determined whether H4K20me1 levels depend on ongoing transcription at dynamically regulated genes in GATA-1-ER-expressing G1E cells. Following induction of major β -globin transcription, H4K20me1 levels markedly increased within the transcribed region with no significant change at the promoter (Fig. 4A). At the active *c-kit* gene in untreated cells, we also observed H4K20me1 at the +18-kb transcribed region but not at the promoter region. Upon repression of *c-kit* after OHT treatment, we found significant depletion of this modification within the transcribed region (Fig. 4B). This basic pattern is similar to that found for H3K36me3 (Fig. 4C and D) and H3K79me3 (see Fig. S4 in the supplemental material), methyl marks previously associated with transcription elongation. The observed changes in methylation cannot be accounted for by changes in histone density, as H3 levels remain the same in the transcribed regions of the major β -globin and *c-kit* genes upon induction or repression, respectively. These findings demonstrate that H4K20me1 is a dynamic modification that spatially

and temporally correlates with transcription elongation, similar to H3K36me3.

To further verify that H4K20me1 is maintained by the elongation stage of the transcription cycle, we treated HeLa cells with the transcription elongation inhibitor DRB and measured methylation by ChIP at the +5-kb transcribed region of PABPC1. Following 3 hours of DRB treatment, the presence of Pol II was markedly reduced in the transcribed region (Fig. 5) but not at the promoter (data not shown), consistent with elongation blockage. H4K20me1 levels decline to ~50% following 3 h of DRB treatment (Fig. 5) and further diminish to ~25% at 6 h (data not shown). The sensitivity of H4K20me1 to elongation inhibition was similar to that obtained for the H3K36me3 elongation mark (Fig. 5). As a control, histone density did not change appreciably following DRB treatment (Fig. 5). H3K27me1 was also unaffected by elongation inhibition at the +5-kb transcribed region, consistent with the dynamics of this modification being limited to the vicinity of the transcription start site. To summarize, these data all support H4K20me1 being a mark of transcription elongation in mammalian cells.

Antagonistic distributions between H3K9 acetylation and H3K9 trimethylation at an active gene. Acetylation and methylation of H3K9 are antagonistic to each other in vitro (51). Experiments in *Schizosaccharomyces pombe* have shown that histone deacetylation is required to maintain H3K9 methylation at heterochromatin in vivo (40), suggesting that compe-

FIG. 4. H4K20 monomethylation is a mark of transcription elongation. ChIP experiments performed in GATA-1-ER-expressing G1E cells before or after tamoxifen (OHT) treatment. A) H4K20me1 at the major β -globin (β -major) gene. B) H4K20me1 at the *c-kit* gene. C) H3K36me3 at the major β -globin gene. D) H3K36me3 at the *c-kit* gene. E) Histone H3 density at the major β -globin gene. F) Histone H3 density at the *c-kit* gene. IgG, immunoglobulin G.

tion exists between H3K9 acetylation and H3K9 methylation at certain genomic sites to influence transcription activity (22). Since H3K9 acetylation is a mark of active genomic regions, how can this be reconciled with the presence of H3K9 methylation at active genes (7, 53, 64, 69)? To help address this issue, we profiled H3K9 acetylation across the active PABPC1 gene and compared this to H3K9me3 (Fig. 6). Similar to previous reports, H3K9 acetylation was specifically enriched around the transcription start site (from +0.5 kb to +5 kb) with very little acetylation found across the majority of the transcribed region (+5 kb to +25 kb). This distribution is a near inverse of that observed for H3K9me3, which is limited

primarily to the deacetylated region of the gene (+5 to +25 kb), with some overlap at the +2 region. If the levels of acetylation observed at the +0.5 and +1-kb region reach saturating levels, this finding would support H3K9 acetylation imposing a 5' boundary on H3K9 methylation. In addition, histone deacetylase activity may be recruited to the +5- to +25-kb region, which would fit well with the recent demonstration that H3K36me3 recruits histone deacetylase complexes to the coding region of active genes in yeasts (9, 23, 25). These findings further establish that histone modifications that positively regulate promoter activity (in this case H3K9 acetylation) are not maintained across entire transcribed regions, but instead be-

FIG. 5. H4K20 monomethylation is dependent on elongating Pol II. ChIP was performed with the indicated antibodies. Cells were not treated or treated with DRB (+DRB) for 3 hours.

come replaced by modifications that have been linked with repression of promoter activity.

We have described here a complete profile of the landscape of lysine methylation that accompanies mammalian gene transcription. While several previous studies have examined individual modifications in metazoan cells (1, 3, 21, 49, 57), our work describes the first attempt to examine all six known positions of lysine methylation in parallel, revealing novel categories of spatial distribution for activation-associated methyl marks. In addition, by examining all three processive states of H3K27 and H4K20 methylation, we have clarified an association between H4K20 monomethylation and transcription elongation and discovered a novel form of H3K27me1 depletion that occurs at sites of highly active transcription. The dynamic regulation of H3K27me1 and H4K20me1 is illustrated by ac-

tivation or repression of gene transcription in G1E cells. Consistent with genome-wide analysis of histone methylation in *S. cerevisiae* (48), all findings at the PABPC1 gene were recapitulated at all other active mammalian genes examined (RPLP0, major β -globin, GATA-2, and *c-kit*), consistent with patterns of lysine methylation being shared by most, if not all, active mammalian genes. Whether exceptions exist to our findings can be addressed in the future by applying genome-wide ChIP arrays to the six methylated lysines in parallel across euchromatin.

The mammalian histone methyltransferases that associate with transcribed chromatin to coordinate lysine methylation are largely unidentified. MLL1, a mammalian homologue of *S. cerevisiae* Set1p, has been found to occupy the 5' transcribed region of active genes, suggesting this enzyme may be involved in maintaining H3K4 trimethylation at these sites (17, 37). Enzymes that perform H3K9, H3K36, and H3K79 methylation in mammals have been identified; however, a specific association with transcribed chromatin has not been determined (14, 18, 50, 51, 60, 66). On the basis of our findings of dynamic changes in H3K27 and H4K20 monomethylation associated with gene induction and repression, we hypothesize the existence of additional methyltransferase and demethylase enzymes specific for these modifications whose activities are coupled to the transcription apparatus. To date, PR-SET7/SET8 is the only described H4K20 monomethylase known (13, 43); therefore, one might expect the association of this enzyme with elongating RNA polymerase II, although additional unidentified enzymes that perform this modification may exist as well. Also, the rapid removal of H4K20me1 upon cessation of transcription (Fig. 4 and 5) suggests the existence of an unidentified demethylase that may be responsible for removing this modification. The mechanism for global H3K27me1 maintenance in mammalian cells is undetermined. Perhaps a subset of Polycomb proteins may be involved (12, 38). Our data are

FIG. 6. Inverse correlation between H3K9 acetylation and H3K9 trimethylation at the PABPC1 gene. ChIP was performed with antibodies against acetyl-H3K9 and H3K9me3. Note that the acetyl-H3K9 antibody gives stronger signals and that the y axes are different for the two antibodies. IgG, immunoglobulin G.

highly suggestive of an active removal process for this modification, whether by an unidentified demethylase enzyme or replacement with variant histones coupled to transcription activation.

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