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Role of H3K4 mono-methylation in gene regulation

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Abstract

Methylation of histone H3 on the lysine 4 residue (H3K4me) is found throughout the eukaryotic domain, and its initial discovery as a conserved epigenetic mark of active transcription from yeast to mammalian cells has led to the histone code hypothesis. However, recent studies have raised questions on whether the different forms of H3K4me play a direct role in gene regulation or are simply by-products of the transcription process. Here, we review the often-conflicting experimental evidence, focusing on the mono-methylation of lysine 4 (H3K4me1) that has been linked to the transcriptional state of enhancers in metazoans. We suggest that this epigenetic mark acts in a context-dependent manner to directly facilitate the transcriptional output of the genome and the establishment of cellular identity.

INTRODUCTION

Histone proteins are subject to diverse post-translational modifications (PTMs), carried out by the “writer” proteins that deposit histone modifications, or marks, on specific residues of the N-terminus tail of histones [1]. Methylation on the lysine 4 residue of histone H3 (H3K4me) is one of the first types of histone methylation linked to active transcription, a discovery that contributed to the “histone code” hypothesis, which posits that various histone modifications, acting in a combinatorial or sequential fashion on one or more histone residues, specify unique functional output of the genome [2, 3]. Genome-wide profiling of different methylation states of H3K4me, namely mono-, di- and tri-methylation of the lysine, in the mammalian genome further established mono-methylation of H3K4 (H3K4me1) as a marker for poised or active state of enhancers, whereas tri-methylation of H3K4 (H3K4me3) marks the poised or active state of promoters [4, 5]. These studies also showed that di-methylation of H3K4 (H3K4me2) marks both active promoters and enhancers. Strong correlation of H3K4me2/3 with active promoter state has been found in all eukaryotes, and that between H3K4me1/2 and active enhancers in virtually all metazoans, however whether these histone modifications are directly involved in gene regulation or merely a by-product of transcriptional activation has come under heated

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debate recently [6, 7]. While some studies reveal a direct involvement of H3K4me1, H3K4me2, or H3K4me3 in various regulatory steps of transcription [8–10], others suggest that these modifications are dispensable for transcriptional regulation in some contexts [11–17], questioning the very notion of histone code hypothesis. Here, we review the diverse lines of experimental evidence focusing on the role of H3K4me1 in gene regulation, and provide a conceptual framework to help reconcile the sometime contradicting experimental observations.

General approaches to functional characterization of the histone marks *in vivo*

Writer perturbation experiments.

Histone modifications are catalyzed by the histone modifying enzymes that are often referred to as writers. To assess the role of histone modifications, one approach is to ablate the catalytic function of the writers, through deletion of the write genes, targeted mutagenesis of their catalytic domains, degron-mediated acute protein depletion, and other means (Figure 1). A major challenge in delineating the functional role of H3K4me in mammalian cells is that there are at least six histone methyltransferases (HMTs), including SET1A, SET1B, MLL1, MLL2, MLL3 and MLL4 that can deposit methylation marks on H3K4. They are known as the catalytic subunits of the Complex of Proteins Associated with Set1 (COMPASS) [18]. Interpreting phenotypes associated with writer mutations is thus very difficult due to partial redundancy among these writers. Further complicating the data interpretation, growing evidence suggests that some of the HMTs can have non-histone substrates that contribute to gene regulation, and as a result, changes in transcription upon HMT mutation cannot always be attributed to changes in histone methylation profiles [19–21]. Additionally, the H3K4 methyltransferases tend to be large proteins with many functional domains, carrying HMT-independent activities [11, 17, 22]. Finally, most of the histone writers are essential for survival of cells or organisms, making it hard to study the loss-of-function mutants in organismal development. Consequently, interpreting the results from the experiments employing knockout (KO) or catalytically deficient (CD) mutants of SET1 and MLL proteins should use extra caution and scientific rigor.

Histone replacement experiments.

In part to address the difficulties outlined above, researchers have studied the role of histone modifications by replacing the endogenous histone genes with mutants harboring unmodifiable amino acid residues at the site of interest, in experiments known as histone replacement [23] (Figure 1). Although this approach blocks all potential histone modifications on a given residue and does not distinguish different methylation states (-me1, -me2 and -me3) of residues, it can directly examine the functional importance of a certain histone residue. Results from such experiments in yeast and Drosophila suggest that H3K4 methylation is not essential for survival, but is necessary for fine tuning of gene expression. Indeed, H3K4A and H3K4R mutant yeast strains are viable but exhibit growth defects [24]. Histone replacement experiments using H3K4A and H3K4R have also been successfully performed in Drosophila, with the mutant flies showing impaired cell proliferation but

minimal changes in developmental gene expression [25]. Complete histone replacement of H3K4 has not been reported in mammalian systems yet, likely due to technical challenges caused by the existence of multiple copies of H3 genes (for example, the human genome harbors at least 16 genes encoding histone H3 [26]). Specifically, replication-dependent canonical H3 (H3.1 and H3.2) have multiple copies within large histone gene clusters, whereas the histone variant H3.3 can be incorporated into nucleosomes independently of DNA replication and is encoded by two gene copies (*H3f3a* and *H3f3b*), making it accessible to histone replacement albeit some compensatory effect from H3.1 and H3.2 can be expected. Accordingly, Gehre *et al.* generated H3.3K4A mutant mouse embryonic stem cells (mESCs) and reported impaired differentiation and gene expression changes upon H3.3K4A mutation [27]. On a separate note, Jang et al. achieved overexpression of H3.3K4M mutant specifically in the adipose and muscle lineages, and found H3.3K4M mutation destabilizes MLL3/4 proteins and impairs adipose tissue and muscle development [28]. In addition, Zhang *et al.* and Sankar *et al.* have demonstrated the feasibility of generating partial and complete H3K27R mutant ESCs respectively using CRISPR-based genome editing, which suggested that H3K27ac is dispensable for enhancer activity and transcriptional activation [29, 30]. Future studies applying this approach to H3K4 in the mammalian system, as well as comprehensive epigenome and transcriptome analysis on established histone replacement mutants of other organisms, should provide further insights and clarify the role of H3K4me in gene regulation.

H3K4me1's regulatory role during transcriptional activation of genes

Much of what we know about the role of H3K4me1 came from writer perturbation experiments. Among the six H3K4 methyltransferases, the main writer proteins are MLL3 and MLL4, deletion of which results in enhancer-specific reduction of H3K4me1 levels in mammalian cells and in Drosophila [31, 32]. Whether other H3K4 HMTs can catalyze H3K4me1 *in vivo* and compensate for the loss of MLL3/MLL4 remain to be further characterized. MLL3 and MLL4 are partially redundant as H3K4me1 methyltransferases, and MLL3 can compensate for the loss of MLL4 to some extent during adipogenesis [31, 32]. However, Mll3 and Mll4 KO mice die at different developmental stages and exhibit discordant phenotypes, suggesting they are not completely redundant during mouse development [32, 33]. Initially, characterization of mESCs with MLL3/4 genetic deletions showed that while a subset of genes are dependent on MLL3/4-bound, H3K4me1-marked enhancers, transcription of the majority of genes exhibits no significant change upon MLL3/4 ablation and does not depend on MLL3/4-mediated H3K4me1 at these regions [11, 34, 35].

Distal enhancers and their target promoters are functionally connected through long-range chromatin interactions. Our group examined whether this process is dependent on H3K4me1 by dissecting the role of MLL3/4 on the Sox2 super enhancer locus in ESCs [9]. Both MLL3/MLL4 dKO and MLL3-Y4792A/MLL4-Y5477A double catalytically deficient mutant (dCD) cells showed reduced H3K4me1 signal on the Sox2 super enhancer and reduced Sox2 gene expression. In addition, these mutations do not seem to affect the stability of MLL3/4-COMPASS complexes [11]. *In situ* Hi-C revealed that MLL3/MLL4 loss leads to global reduction of chromatin interactions at enhancers in ESCs, including

the Sox2 super enhancer. 4C-seq experiments on MLL3/4 dCD ESCs further demonstrated that histone methyltransferase activities of MLL3/MLL4 are required for enhancer-promoter interactions at Sox2 locus. Additionally, loss of Cohesin binding resulted in reduced chromatin interactions at H3K4me1-marked enhancers. Mechanistically, knockdown of Cohesin complex component Rad21 in WT ESCs showed similar decrease in chromatin interactions at Sox2 locus and Sox2 gene expression albeit no obvious change of H3K4me1 at this locus or genome-wide, suggesting MLL3/MLL4 mediated H3K4me1 at the Sox2 super enhancer acts upstream of the Cohesin complex to regulate chromatin interactions. In addition, an *in vitro* pull-down assay revealed that Cohesin complex bound more strongly to the H3K4me1- and H3K4me2-marked mono-nucleosomes than H3K4me3-marked or unmodified nucleosomes [9]. A follow-up study by Kubo *et al.* further mapped histone modifications, long-range chromatin contacts as well as gene expression in MLL3/4 dCD ESCs undergoing differentiation toward neural precursor cells (NPCs), and revealed the role of MLL3/4 HMT activity on induction of enhancer-promoter contacts and transcriptional activation at a subset of lineage-specific genes during cellular differentiation [36]. Together, these data provide a mechanistic link between H3K4me1 at enhancers and long-range chromatin interactions between enhancers and target gene promoters (Figure 2A). The work by Yan *et al.* [9] also exemplifies the advantage of using a “model locus” coupled with site-specific epigenome editing to establish causal relationships in gene regulation mechanisms, where novel mechanistic insights can be built upon existing knowledge of gene regulation on the selected locus. As many famous genomic loci containing enhancers with physiological roles have been extensively characterized for transcription factor binding, chromatin interactions, and other gene regulatory mechanisms, such as the beta-globin cluster involved in hematopoiesis [37] and the fast myosin locus that is important for skeletal muscle function [38], they could serve as additional “model loci” to characterize the potential regulatory roles of histone modifications using targeted epigenome editing [39].

Another mechanism by which H3K4me1 contributes to gene regulation was suggested by nucleosome pulldown coupled with SILAC and mass spectrometry analysis, which identified a subunit of the BAF (SWI/SNF) complex that can be specifically associated with nucleosomes that contain H3K4me1. Importantly, the same study showed that BAF complex associated with chromatin in an H3K4me1-dependent manner, and its nucleosome-remodeling activities could be enhanced at H3K4me1-containing nucleosomes *in vitro* [8]. This study suggests that H3K4me1 is playing an active role in transcriptional regulation via recruitment of chromatin remodeling complexes (Figure 2B). Future work using site-specific chromatin capture coupled with high-resolution proteomics could further advance our understanding of protein players involved in H3K4me1-mediated enhancer regulation [40, 41].

Functional characterization of H3K4me1 at enhancers through catalytic deficient mutants

While the above findings support an active role of H3K4me1 at enhancers, further studies using catalytic deficiency (CD) mutants painted a more complex picture. Jang *et al.* generated MLL4 catalytically dead mutant ESCs (and knock-in mice) that carry Y5477A/

Y5523A/Y5563A mutations in the enzymatic SET domain of the MLL4 protein, and found this protein to be highly unstable, suggesting H3K4 methyltransferase activity is required for MLL4 protein stability [42]. Dorighi *et al.* generated MLL3-Y4792A; MLL4-Y5477A double catalytically deficient (dCD) mutant ESCs together with MLL3/MLL4 double knockout (dKO) ESCs, both of which resulted in global reduction of H3K4me1 to roughly 50% of wild-type (WT) level as quantified by mass spectrometry. Using these models, they report that while dKO cells exhibit significant loss of H3K27ac (an active chromatin mark) in addition to H3K4me1 loss at enhancers, dCD cells show only partially diminished H3K27ac signals. In addition, RNA-seq analysis revealed that while MLL3/4 target genes are mostly down-regulated in dKO cells, very mild expression changes of the same set of genes can be observed in dCD cells. Considering that dKO and dCD cells have similar bulk H3K4me1 level, these data indicate that non-methyltransferase function of MLL3/MLL4 is required for their co-activator function, while their HMT activity could be dispensable for transcription [11]. Dorighi *et al.* also reported that truncations of MLL3 and MLL4 prior to the C-terminal SET domain resulted in unstable protein products, supporting the conclusion from Jang *et al.* that extensive SET domain mutations can destabilize MLL3/MLL4 [11, 42]. Using a different genome editing strategy, Rickels *et al.* generated several ESC clones harboring deletion of the SET domains of both MLL3 and MLL4 ($MLL3^{\Delta SET}$; $MLL4^{\Delta SET}$). Unlike other SET domain deletion mutants describe above, these clones had comparable protein stability as the WT proteins. $MLL3^{\Delta SET}$; $MLL4^{\Delta SET}$ ESCs exhibit significant reduction of H3K4me1, H3K4me2 and H3K27ac levels. The defects in gene expression of $MLL3^{\Delta SET}$; $MLL4^{\Delta SET}$ ESCs were between those from MLL3/MLL4 dCD cells generated by Dorighi *et al.* and MLL3/MLL4 dKO ESCs which exhibit the most severe defects due to deletion of both genes [12].

Xie *et al.* recently generated new mouse strains and cell lines expressing stable MLL3/4 catalytically deficient mutant proteins to characterize the role of MLL3/4-driven H3K4me1 in gene regulation during mouse development [13]. Notably, $MLL3^{Y4792A}$/$MLL4^{Y5477A}$ (MLL3/4 dCD) mice die at E6.5 due to failure to initiate gastrulation. $MLL3^{KO}$/$MLL4^{Y5477A}$ ESCs can be differentiated into all three germ layers using an embryoid bodies (EBs) differentiation model, but have defects in extraembryonic endoderm (ExEn) gene induction. Furthermore, conditionally ablation of MLL3/4 enzymatic activity in the epiblast lineage but not extraembryonic lineage using Sox2-Cre (Sox2-MLL3/4 dCD) leads to embryonic lethality at mid-gestation stage between E10.5 and E12.5, but the Sox2-MLL3/4 dCD embryos are morphologically indistinguishable from control embryos until E8.5. These data suggest the early-gestation lethality of global MLL3/MLL4 dCD mice is attributable to defects in extraembryonic lineage including ExEn, and MLL3/MLL4 enzymatic activity is important for ExEn lineage during early gestation as well as other lineages during mid-gestation. Mechanistically, Xie *et al.* demonstrated that expression and genomic binding of GATA6, the master transcription factor of ExEn lineage commitment, require MLL3/4 methyltransferase activity (Figure 2C). In contrast, a closer examination of ESC to EB and NPC differentiation revealed that MLL3/4 enzymatic activities are largely dispensable for germ layer specification and neural lineage commitment. In addition, at MLL4-bound enhancers that are induced during ESC to EB differentiation and show reduced H3K4me1 level in MLL3/4 dCD mutant, H3K27ac and chromatin accessibility at these loci as well as

their nearby gene expression show few alterations, suggesting that MLL3/MLL4-mediated H3K4me1 could be largely dispensable for enhancer activation [13]. This is also supported by a previous report that during ESC to EB differentiation, pre-deposition of H3K4me1 mark is not sufficient to induce H3K27ac enhancer mark and recruit p300 for enhancer activation [35].

Together, it can be appreciated that in certain lineages, the HMT activity of MLL3/4 is required for normal development, such as in ExEn lineage during early embryonic development as well as other lineages that contribute to the mid-gestation lethality of Sox2-MLL3/4 dCD mutant mice [13]. However, the above discussed studies also highlight a role of MLL3/4 in gene regulation that could be independent of their histone-methyltransferase activities in certain developmental processes such as ESC to EB and NPC differentiation, raising question on whether H3K4me1 directly contributes to gene regulation or is merely a by-product of MLL3/4's HMT activities at enhancers (Table 1) [11–13, 35, 42].
One possibility to clarify this inconsistency is that mammalian MLL3/MLL4-COMPASS contains a conserved subunit UTX, a histone H3K27 demethylase that removes repressive H3K27me3 mark to allow deposition of active H3K27ac mark [43, 44]. On the other hand, it is also important to note that all the above-mentioned MLL3/MLL4 catalytically deficient mutants continue to exhibit prominent amount of residual H3K4me1 in the genome, as demonstrated by western blotting, mass spectrometry and ChIP-Seq analysis [11–13]. It is plausible that the residual H3K4me1 at enhancers could be due to (i) incomplete ablation of the catalytic activity of MLL3/4 by point mutation of the catalytic tyrosine (Y) residues to alanine (A); or (ii) a compensatory H3K4me1 deposition by other (known or unknown) H3K4 methyltransferases; or a combination of both (i) and (ii). Supporting these hypotheses, it can be recognized from the transcriptome analysis by Rickels *et al.* that the MLL3$^{\Delta SET}$; MLL4$^{\Delta SET}$ mutant cells with the entire catalytic domain of MLL3 and MLL4 deleted showed significant loss of H3K4me1 signal examined by western blotting and a closer resemblance to MLL3/MLL4 global KO cells, whereas MLL3-Y4792A; MLL4-Y5477A dCD cells generated by Dorighi *et al.* showed 50% loss of H3K4me1 level examined by mass spectrometry, and milder gene expression change, suggesting a potential dose-dependent response of transcription to H3K4me1 level at enhancer loci and a role in transcriptional regulation [11, 12]. Thus, these different MLL3/4 catalytically deficient mutants are valuable resource to the field and can be further analyzed to further clarify the differences seen in different models (Table 1). In addition, although H3K4me1 is not completely erased in the various catalytically deficient mutant cell lines and genetic mouse models generated by Xie *et al.*, the authors of this study clearly demonstrated the necessity of MLL3/4 HMT activity in ExEn lineage, as well as other lineages during mid-gestation embryonic development that remains to be further characterized [13]. Xie *et al.* also demonstrates that the requirement of HMT activities of MLL3/4 can be better revealed by comparing various differentiation processes, highlighting the importance of using multiple independent cell-state transition models to characterize the role of histone modification in gene regulation [13]. Indeed, various studies using conditional knockout mouse models have revealed lineage-specific functions of histone modifiers [32, 45–47]. Future work examining the requirement of the enzymatic activity of MLL3/4 (as well as other histone modifying enzymes) could benefit from various *in vitro* differentiation models

as well as mouse embryonic and postnatal development as models, to establish a solid genotype-phenotype relationship with biological relevance in a lineage-specific manner.

A framework to understand the role of H3K4me1 in gene regulation

To date, studies have presented conflicting conclusions on the role of H3K4me1 in gene regulation. While the evolutionary conservation of the H3K4me1 mark on cis-regulatory elements and a conserved writer protein family strongly suggest conserved functionality of H3K4me1 in regulating gene expression, a direct link has yet to be established between altered H3K4me1 status and changes in gene expression, casting doubt on a direct regulatory role of H3K4me1 in gene regulation. Can we reconcile the paradoxical observations with a unified framework?

We propose that H3K4me1 mark on enhancers has context-dependent function in gene regulation that is often masked by redundant mechanisms safeguarding transcription, including redundancies of writers as well as transcription factors (Figure 3). In the case of MLL3/4 ablation, functional redundancy among other SET1/MLL-COMPASS members may allow deposition of H3K4me1 at enhancers to a sufficient degree to allow H3K4me1-dependent transcriptional regulation to occur (Figure 3A). In addition, the regulatory mechanisms dependent upon H3K4me1 may manifest more prominently during cell-state transitions (such as differentiation and response to stimuli) when new gene regulatory networks need to be established, a process that involves global changes of H3K4me1 landscape. During the development of certain cell lineages, where H3K4me1-dependent lineage-determining transcription factors are responsible for triggering the establishment of lineage-specific gene regulatory network, removal of H3K4me1 marks on their enhancers may result in failure of specification of certain lineages such as ExEn (Figure 3B). On the other hand, during establishment of other cell lineages, existence of redundant transcription factors whose binding to enhancers does not depend on H3K4me1 may compensate for the loss of the H3K4me1-dependent lineage-determining transcription factors, and thus secure the unfolding of the regulatory blueprint of transcription for those lineages (Figure 3B).

Although current MLL3/4 ablation studies are not able to directly establish a phenotypical link between H3K4me1 level and gene expression, various studies support a direct role of H3K4me1 in transcriptional control at various regulatory levels. Mediated by its readers, H3K4me1 marks on enhancers can facilitate binding of certain lineage-specific transcription factors as well as other chromatin regulators that enhances transcription [8, 9, 13, 36]. Moving forward, thorough biochemical and phenotypical characterization of H3K4me1 reader proteins is necessary using H3K4me-binding domain loss-of-function mutants, as conducted in a similar fashion for the HMT domains of H3K4me1 writers, to further support a direct role of H3K4me1 in gene regulation. Future studies characterizing the functional impact of ablating the writer and reader activity in various cell state transition models or metazoan development can provide additional mechanistic insights on the requirement of enhancer H3K4me1 in gene regulation.

To clearly delineate the functional requirement of H3K4me1 in gene regulation, we argue that limitations of existing experimental models warrant rigor and caution in data

interpretation, and provide our thoughts on certain research directions that the scientific community can collaboratively work on towards a better understanding of its gene regulation mechanisms. First, the functional redundancy of different H3K4me1 writer proteins needs to be thoroughly investigated. It is still unclear whether the residual H3K4me1 signal in MLL3/4 dCD mutants comes from incomplete ablation of MLL3/4 catalytic activity or functional redundancy from other H3K4 methyltransferases [11–13, 32]. Generation of individual and combinatorial CD mutants of these H3K4 HMTs in the genetic background of MLL3/4 dCD can help further clarify this question. Second, enhancer redundancy likely underlies, at least partially, the mild phenotypes of various MLL3/4 mutant cell lines with residual H3K4me1 on enhancer elements, and serves as a mechanism of safeguarding transcription of essential genes [48]. Chromatin conformation capture of MLL3/4 mutants, coupled with comprehensive epigenome profiling, in the context of various cell state transition events, could further delineate the functional involvement of H3K4me1-marked enhancers in gene regulation [36]. Third, it has been reported that the transcription of genes by distal enhancers in steady state is less reliant on distal enhancers than during dynamic state transitions [49]. Additionally, a new phase separation model of transcriptional control highlights its robustness, and this model would likely tolerate changes in H3K4me1 at enhancers once transcriptional condensates have already been established [50, 51]. On the contrary, during cell-state transition, establishing new gene regulatory networks would require activation of master transcription factors and their target genes. This essential process is dependent on H3K4me1 on certain enhancers with cell-type and temporal specificity to facilitate transcription factor binding for certain cellular state transition processes, such as direct reprogramming and ExEn differentiation [13, 35]. In line with this notion, Garry *et al.* recently identified PHF7, a H3K4me reader, to be a potent activator of direct cardiac reprogramming, where exogeneous cardiac transcription factors are overexpressed in fibroblasts to convert them to cardiac-like myocytes, by cooperating with SWI/SNF chromatin remodeling complex on cardiac enhancers [52]. A recent study by Luo *et al.* also supports this notion and provides experimental evidence and mathematical modeling to suggest that perturbation of many enhancers could result in strong effects during such cell-state transition but negligible effects post-transition [49]. Together, these studies highlight the requirement of enhancer H3K4me1 in gene regulation of various cell state transition processes. Future studies can further clarify which lineages are dependent on H3K4me1 marks at enhancers to allow specification, and which redundant mechanisms, such as via deposition of a different histone mark, allows specification of lineages and switch of gene regulatory network independent of enhancer H3K4me1. Last, we encourage efforts using histone replacement studies in a mammalian system to dissect the role of PTMs on the H3K4 residue in gene regulation.

Concluding Remarks

H3K4me1 as an enhancer mark and integral component of the “histone code”, might have been engraved in our understanding of epigenetic regulation of gene expression. However, a direct involvement of H3K4me1 in gene regulation has been debated. Recent advances uncover an overwhelming amount of new knowledge and insights that was previously unrecognized, and remind us that there’s still much to be learned in this basic molecular

biology process. New single cell genomic tools are currently being actively developed to systematically identify histone modification landscapes as well as chromatin interactions at single cell resolution [53–56], and advances in super-resolution imaging have enabled spatially resolving gene expression patterns in complex tissues and tracing of chromatin interactions [57–59]. Moving forward, enabled by these new tools to study gene regulation, the field can further benefit from comprehensive characterization of the cis-regulatory elements in the genome in a cell-type-specific manner, across developmental and cell state transition time-series, for a better understanding of how (combinatorial) histone modifications are functionally implicated in transcription.

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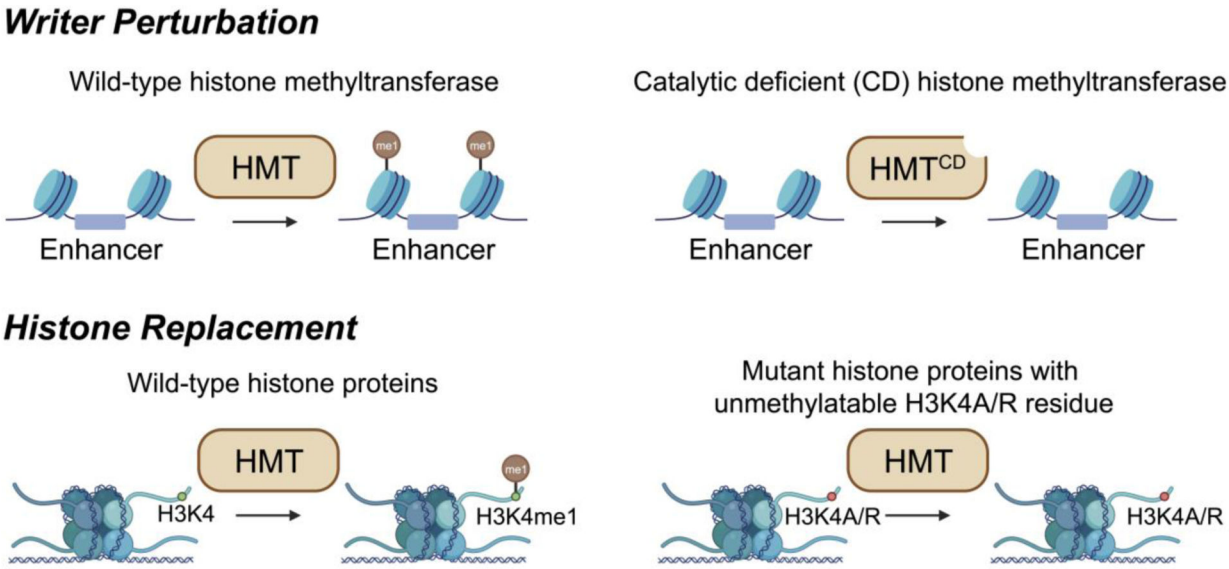

Figure 1. General experimental models to characterize the function of histone marks.

Writer perturbation experiments (upper panel) genetically delete the H3K4me writers or mutate the catalytic residues to generate catalytic deficient (CD) mutants to ablate the histone methyltransferase (HMT) activity of H3K4me writers. Histone replacement experiments (lower panel) mutate the lysine (K) 4 residues of all the histone H3 coding genes into an unmethylatable residue, such as alanine (A) or arginine (R), to erase all potential post-translational modifications on the H3K4 residue.

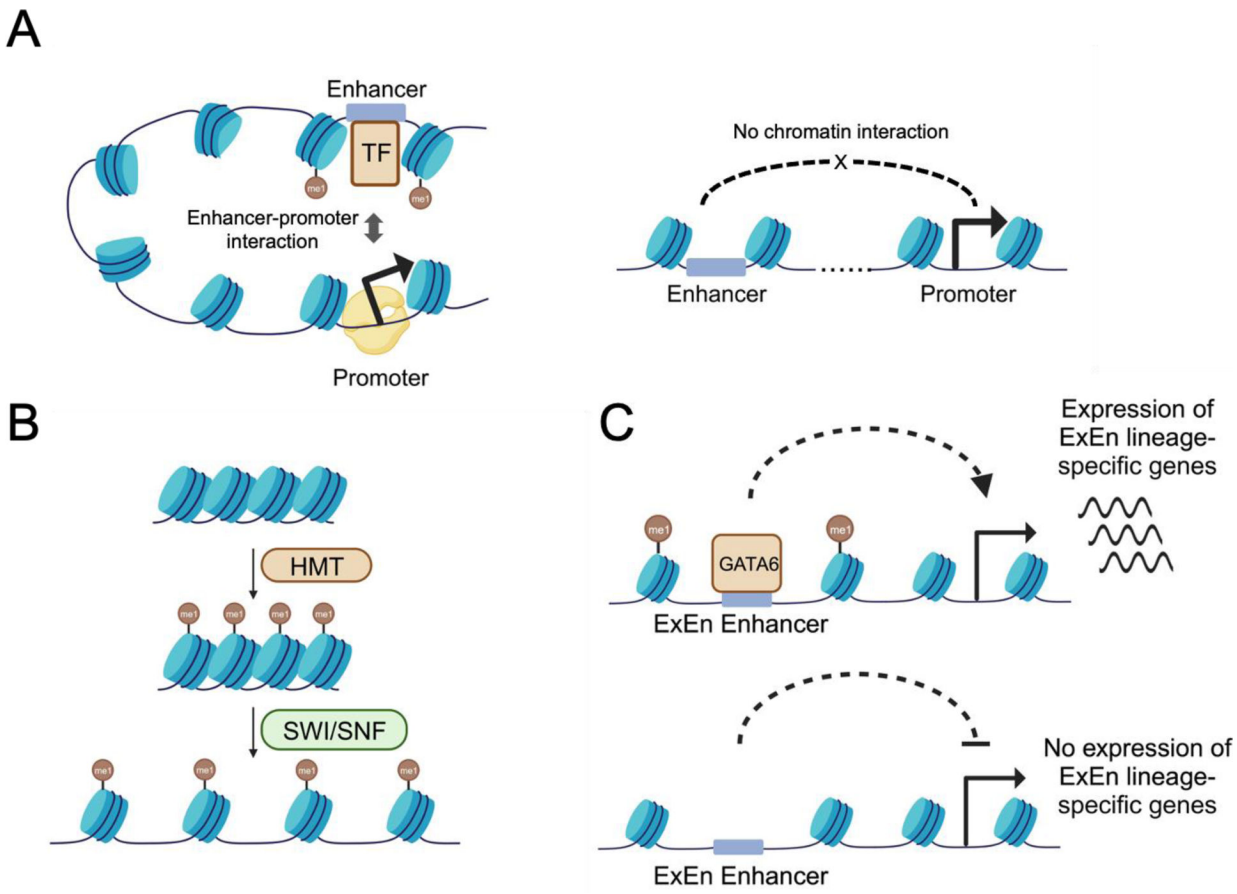

Figure 2. Models on the roles of H3K4me1 in gene regulation.

(A) H3K4me1 at enhancers can facilitate enhancer-promoter interactions via chromatin looping [9]. (B) Nucleosomes with H3K4me1 marks have higher affinity for SWI/SNF chromatin remodeling complexes and are more efficiently remodeled to allow transcriptional activation [8]. (C) Certain lineage-determining transcription factors, such as extraembryonic endodermal (ExEn) transcription factor GATA6, requires H3K4me1 for enhancer binding and activating gene expression. Loss of H3K4me1 at enhancers results in impaired binding of GATA6 and failure in induction of ExEn-lineage specific genes [13].

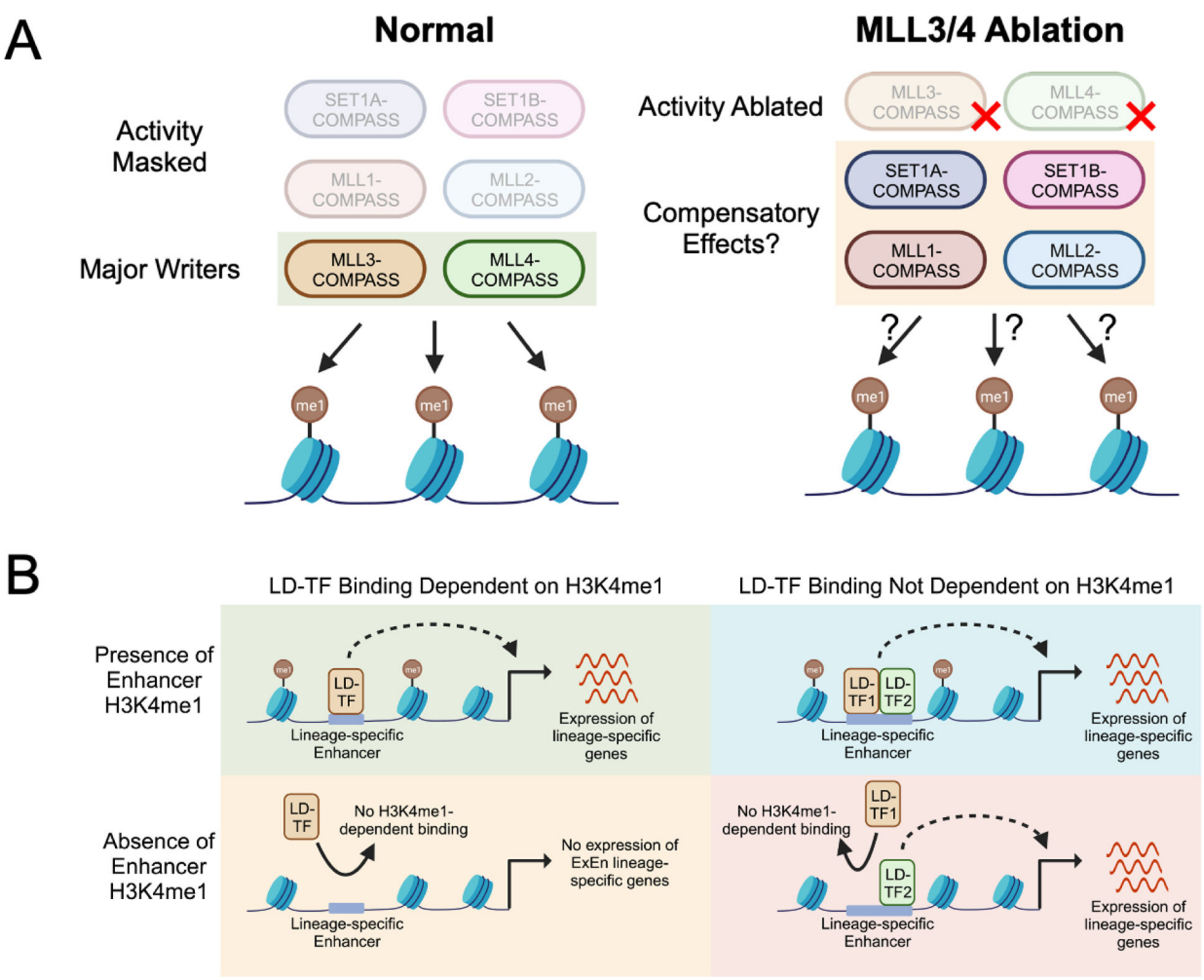

Figure 3. Redundant mechanisms safeguarding transcription in MLL3/4 mutants.

(A) In normal conditions, MLL3/4-COMPASS serve as the major writers for H3K4me1. When MLL3/4 are ablated, other SET1/MLL-COMPASS members may compensate and serve as redundant H3K4me1 writers to maintain H3K4me1 level at enhancers. (B) For a lineage-determining transcription factor (LD-TF) whose binding is dependent on enhancer H3K4me1, loss of H3K4me1 results in failure of LD-TF binding at lineage-specific enhancers and expression of lineage-specific genes (left). However, if a redundant LD-TF is present whose binding is not dependent on H3K4me1 (LD-TF2), in the event of enhancer H3K4me1 loss, it can compensate for the role of LD-TF1 that requires H3K4me1 for enhancer binding activity and drive the expression of lineage-specific genes to support normal lineage specification.

Table 1.

Summary of MLL3/4 mutant lines available

Cell lines					
Genotype	**Effect**	**Mutant MLL3/4 stability**	**H3K4me1 level**	**Phenotypes**	**Reference**
$Mll3^{-/-}$; $Mll4^{f/f}$ brown preadipocyte isolated from E18.5 $Mll3^{-/-}$; $Mll4^{f/f}$ embryos	Upon infection with adenoviral Cre, Mll4 is genetically ablated, creating MLL3/4 dKO	NA	Upon adeno-Cre infection, H3K4me1 level is significantly reduced examined by western blotting (not quantified)	- Upon adeno-Cre infection, - MLL3/4 dKO preadipocytes showed severe adipogenesis defects; upon retroviral-MyoD and adeno-Cre infection, MLL3/4 dKO preadipocytes showed severe myogenesis defects	Lee et al. [32]
$MLL3^{\Delta set}/4^{\Delta set}$ HCT116 cells	HCT116 cells have a homozygous frameshift mutation before the SET domain of MLL3; MLL4 was targeted before the SET domain to introduce a premature stop codon, leading to no detectable protein	NA	Significantly reduced as examined by western blotting (not quantified) and ChIP-Seq; residual H3K4me1 signal can be detected with ChIP-Seq	- Loss of H3K4me1 at the majority of enhancers - A subset of H3K4me1-bound regions (putative enhancers) are not dependent on MLL3/MLL4	$MLL3^{\Delta set}/4^{\Delta set}$ HCT116 cells are generated by Guo et al. [60] and further characterized by Hu et al. [31]
MLL3/4 dKO mESCs derived from $Mll3^{-/-}$; $Mll4^{f/f}$ blastocysts	$Mll3^{-/-}$; $Mll4^{f/f}$ ESCs were derived from blastocytes as reported in Lee et al. [32]; these ESCs were then transfected with a Cre-expressing plasmid to achieve MLL3/4 dKO	NA	Approximately two-fold reduction of bulk H3K4me1 level determined by western blotting	- Minor difference in gene expression profile and self-renewal compared with control ESCs - MLL4 is required for ESC differentiation and p300-mediated enhancer activation	Wang et al. [35]
				- Chromatin interactions show global loss, mainly short-range chromatin contacts within frequently interacting regions (FIREs) - H3K4me1 mark on Sox2 enhancer is greatly decreased - Chromatin interaction between Sox2 promoter and Sox2 enhancer is greatly reduced - Sox2 gene expression is significantly downregulated	Yan et al. [9]
				- MLL3/4 is dispensable for transcriptional activation of much of the gene program during naïve to formative ESC transition - Enhancer activation can happen independently of MLL3/4	Boileau et al. [34]
MLL3/MLL4 dKO mESC	Deletion of early exons of MLL3 and MLL4, resulting in frameshift and complete loss of MLL3/4 proteins determined by western blotting with a custom antibody	NA	Two-fold reduction of bulk H3K4me1 level determined by mass spectrometry	- Strong depletion of enhancer H3K27ac - Genes with nearby MLL3/4-bound enhancers downregulated - Enhancer RNA polymerase II loading and transcription reduced	Dorighi et al. [11]
MLL3/MLL4 dCD mESC	Point mutation of MLL3 (Y4792A) and MLL4 (Y5477A)	Stable	Approximately 2-fold reduction of bulk H3K4me1 level determined by mass spectrometry	- Partial depletion of enhancer H3K27ac - Very minor change in gene expression - No effect on enhancer RNA polymerase II loading or enhancer RNA transcription	Dorighi et al. [11]

				- H3K4me1 mark on Sox2 enhancer is greatly decreased - Chromatin interaction between Sox2 promoter and Sox2 enhancer is greatly reduced - Sox2 gene expression is significantly downregulated	Yan et al. [9]
				- MLL3/4 catalytically activity is required for newly formed enhancer-promoter contacts upon NPC differentiation - Loss of MLL3/4 catalytic activity delays NPC differentiation and impairs activation of a small number of genes	Kubo et al. [36]
MLL3/MLL4 dSET mESC	Truncation of MLL3 and MLL4 prior to the C'-terminal SET domain	Unstable	Not examined; expected to be similar as MLL3/4 dKO	Not examined; expected to be similar as MLL3/4 dKO	Dorighi et al. [11]
$MLL3^{\Delta SET}$; $MLL4^{\Delta SET}$ mESC	Deletion of catalytic SET domain from MLL3 and MLL4; C'-terminal ends of MLL3 and MLL4 are preserved	Stable	Significantly reduced as examined by western blotting (not quantified) and ChIP-Seq; residual H3K4me1 signal can be detected with ChIP-Seq	- Genes with nearby MLL3/4-bound enhancers downregulated, more significant than MLL3/MLL4 dCD but less significant than MLL3/MLL4 dKO mESC from Dorighi et al.	Rickels et al. [12]
$MLL4^{Y5477A/Y5523A/Y5563A}$ mESCs	Point mutation of three catalytically important residues to generate catalytic-dead MLL4 proteins	Unstable	Significantly reduced as examined by western blotting (not quantified); residual H3K4me1 signal can be detected with western blotting	- The three residues mutated in this cell line are important for MLL4 protein stability.	Jang et al. [42]
$Mll3^{-/-}$; $MLL4^{Y5477A/Y5477A}$ mESCs	Mll3 is genetically ablated; Mll4 is expressed only in the catalytically deficient form	MLL3: NA MLL4: Stable	Approximately two-fold reduction of bulk H3K4me1 level determined by western blotting	- Grow in monolayer with higher proliferation rate - Can be differentiated into all three germ layers - Defects in extraembryonic endoderm gene induction and extraembryonic differentiation - During EB differentiation, MLL3/4 bound regions in mutant cells showed little difference in H3K27ac deposition, chromatin accessibility, and target gene expression	Xie et al. [13]
Mouse Lines					
Genotype	**Effect**	**Mutant MLL3/4 stability**	**H3K4me1 level**	**Phenotypes**	**Reference**
Mll3 KO	Premature stop introduced by gene trap	NA	NA	- Lethality around birth - No obvious morphological abnormalities in embryonic development	Lee et al. [32]
Mll4 KO	Premature stop introduced by gene trap	NA	NA	- Early embryonic lethality around E9.5	Lee et al. [32]
$Mll4^{f/f}$; Myf5-Cre	Conditional deletion of MLL4 in brown adipose tissue and skeletal muscle lineages	NA	NA	- Lethality immediately after birth due to breathing malfunction - Back muscle developmental defect	Lee et al. [32]
$Mll3^{-/-}$; $Mll4^{f/f}$	Cross of Mll3 KO and $Mll4^{f/f}$ lines	NA	NA	- Functionally equivalent to Mll3 KO	Lee et al. [32]

Mll3f/f; Mll4SETf/f; Myf5-Cre	Exons 57 and 58 encoding critical amino acids of MLL3 SET domain are flanked by loxP sites; Exons 50 and 51 encoding the entire SET domain of MLL4 are flanked by loxP sites; Cre-mediated deletion of these exons would result in catalytic-dead MLL3/4	Unstable	Significant reduction of H3K4me1 level as determined by western blotting (examined in a brown preadipocyte culture system, not quantified)	- Lethality upon birth due to failure to breathe - Adipose tissue and muscle development defect	Jang et al. [28]
$Mll4^{Y5477A/Y5523A/Y5563A}$	Point mutation of three catalytically important residues to generate catalytic-dead MLL4 proteins	Unstable	Expected to be similar as $MLL4^{Y5477A/Y5523A/Y5563A}$ mESCs	- Growth retardation - Embryonic lethality at around E9.5 to E10.5	Jang et al. [42]
Mll3 KO	Gene targeting between exons 48 and 50 of Mll3, after recombination results in deletion of exon 49	NA	NA	- Lethality around birth due to failure to breathe - No obvious morphological abnormalities in embryonic development	Ashokkumar et al. [33]
Mll4 KO	Gene targeting in intron 1 of Mll4, resulting in premature stop of transcription	NA	No observable change	- Homozygous embryos are defective before gastrulation and show growth retardation starting at E6.5 - Anterior-posterior patterning defects - Embryonic lethality at around E9.5 - Heterozygous embryos show neural fold defects with incompletely penetrance - Adult heterozygous mice show phenotypes resembling Kabuki syndrome	Ashokkumar et al. [33]
$Mll4^{Y5477A/Y5477A}$	Point mutation of the catalytic residue in the SET domain of MLL4	Stable	NA	- Lethality at birth with defective saccular structures in the lung	Xie et al. [13]
$Mll3^{Y4792A/Y4792A}$; $Mll4^{Y5477A/Y5477A}$ (MLL3/4 dCD)	Point mutation of the catalytic residues in the SET domain of MLL3/4, resulting in global MLL3/4 double catalytic deficiency	Stable	Expected to be similar as $MLL3^{Y4792A/Y4792A}$; $MLL4^{Y5477A/Y5477A}$ mESCs	- Growth retardation and severe developmental defect at E6.5 and E7.5 - Can only be found as partially resorbed embryonic remnant at E8.5	Xie et al. [13]
$Mll3^{f/Y4792A}$; $Mll4^{f/Y5477A}$; Sox2-Cre (Sox2-MLL3/4 dCD)	Point mutation of the catalytic residues in the SET domain of MLL3/4 in one allele, conditional knockout of the other alleles of MLL3/4 in the epiblast lineage using Sox2-Cre, resulting in global MLL3/4 double catalytic deficiency in Sox2-marked epiblast lineage and its derivatives	Stable	Significant reduction of H3K4me1 level as determined by western blotting (not quantified)	- Morphologically indistinguishable at E8.5 - Developmental defects and growth retardation at E9.5 - Embryonic lethality between E10.5 to E12.5	Xie et al. [13]