Journal of Cellular Biochemistry 95:429–436 (2005)

PROSPECTS

A COMPASS in the Voyage of Defining the Role of Trithorax/MLL-Containing Complexes: Linking Leukemogensis to Covalent Modifications of Chromatin

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Abstract Chromosomal rearrangements and translocations play a major role in the pathogenesis of hematological malignancies. The trithorax-related mixed lineage leukemia (*Mll*) gene located on chromosome 11 is rearranged in a variety of aggressive human B and T lymphoid tumors as well as acute myeloid leukemia (AML) in both children and adults. It was first demonstrated for the yeast MLL homolog complex, Set1/COMPASS, and now for the MLL complex itself, that these complexes are histone methyltransferases capable of methylating the fourth lysine of histone H3. The post-translational modifications of histones by methylation have emerged as a key regulatory mechanism for both repression and activation of gene expression. Studies from several laboratories during the past few years have brought about a watershed of information defining the molecular machinery and factors involved in the recognition and modification of nucleosomal histones by methylation. In this review, we will discuss the recent findings regarding the molecular mechanism and consequences of histone modification by the MLL related protein containing complex COMPASS. J. Cell. Biochem. 95: 429–436, 2005. © 2005 Wiley-Liss, Inc.

Key words: MLL; leukemia; set domain; histone methylation; transcription

Chromosomal rearrangements and translocations are considered one of the major causes in the pathogenesis of hematological malignancies [Rowley, 1998; Daser and Rabbitts, 2004; Hess, 2004]. The mixed lineage leukemia (*Mll*) gene, located on chromosome 11, undergoes a variety of rearrangements resulting in the development of aggressive human B and T lymphoid tumors as well as acute myeloid leukemia (AML) in both children and adults [Rowley, 1998] (Fig. 1). The closest homologs of MLL from other species are the product of the trithorax (*trx*) gene in *Drosophila* and the Set1 protein in the yeast *Saccharomyces cerevisiae* [Miller et al., 2001]. In *Drosophila*, it was originally demonstrated that the *trx* gene product is a putative DNA binding factor that activates expression of homeotic genes [Breen and Harte, 1991; Orlando and Paro, 1995; Simon, 1995]. Furthermore, mutations in *trx* mimic homeotic transformations caused by mutations of homeotic genes such as *antennapedia* and *ultrabithorax*. Therefore, it has been proposed that *trx* is necessary to maintain homeotic gene expression [Breen and Harte, 1991; Orlando and Paro, 1995; Simon, 1995]. The Set1 protein in yeast is one of the several Set1 domain-containing proteins in yeast that was originally characterized based on its involvement in transcriptional regulation and telomeric silencing, and has now been demonstrated to be part of a large macromolecular complex known as COMPASS [Miller et al., 2001; Krogan et al., 2002].

Grant sponsor: NIH; Grant numbers: 2R01CA089455, 1R01GM069905.

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Received 20 December 2004; Accepted 22 December 2004
DOI 10.1002/jcb.20421

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THE MOLECULAR ROLE OF MLL AND ITS TRANSLOCATION PARTNERS IN THE PATHOGENESIS OF LEUKEMIA

The MLL translocations are involved in a variety of hematological malignancies. Chromosomal translocations involving the *MLL* gene

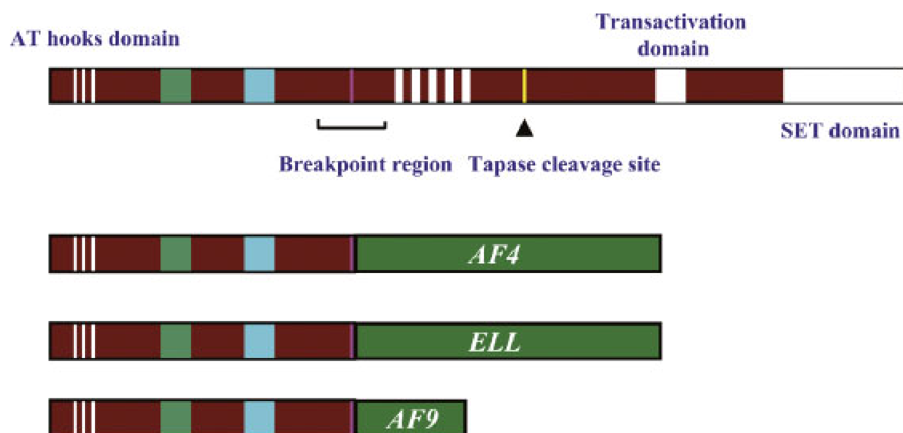

Fig. 1. Schematic representation of the MLL protein and a few of its chimeras found in pathogenesis of leukemia. The human mixed lineage leukemia (*Mll*) gene on chromosome 11 encodes a ~4,000 amino acid containing protein consisting of several domains. A common feature of the MLL family of proteins is the presence of a 130–140 amino acid motif termed the SET domain. The SET domain of MLL has histone methyltransferase activity specific for lysine 4 of histone H3. The *Mll* gene can undergo translocations with other genes such as *AF4*, *ELL*, and *AF9*. Such chromosomal translocations have been demonstrated to result in the pathogenesis of leukemia. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

occur in approximately 80% of infants with AML or acute lymphoblastic leukemia (ALL) and approximately 5% of adult patients with AML and up to 10% with ALL [Rowley, 1998; Daser and Rabbitts, 2004; Hess, 2004]. These translocations result in the fusion of the N-terminal region of the MLL gene product to other cellular genes products to produce chimeric proteins (Fig. 1). To date, over 40 MLL fusion partners have been identified with each partner displaying seemingly limited structural similarity [Gu et al., 1992; Nakamura et al., 1993; Prasad et al., 1993, 1994; Bernard et al., 1994; Rubnitz et al., 1994; Thirman et al., 1994; Hillion et al., 1997; So et al., 1997; Sobulo et al., 1997; Megonigal et al., 1998; Taki et al., 1998; Osaka et al., 1999; Daser and Rabbitts, 2004; Hess, 2004] (Fig. 1).

Compared to its *Drosophila* homolog *trx*, much less is known about the function of the *Mll* gene in mammals during development, and how translocations involving *Mll* result in the pathogenesis of leukemia. Studies during the past decade have demonstrated that *Mll* translocations are oncogenic [Corral et al., 1996; Lavau et al., 1997; DiMartino et al., 2000]. There are several possible models for the role of *Mll* translocations in the pathogenesis of leukemia. *Mll* translocations can result in leukemia for many reasons including the following: (a) the chimeric protein is dominant negative (a gain-of-function effect); (b) the chimeric protein has a new biochemical function that perhaps could not be regulated; (c) there is a loss of function for either the MLL complex or its translocation partner as the result of translocation; (d) *Mll* translocations can result in multimerization of MLL; or (e) since several of the MLL partners exist in macromolecular complexes, it is also tantalizing to hypothesize that one or more of the MLL partner's associated proteins may be shared as a common subunit among several of the MLL partners, thereby contributing to the transformation activity associated with MLLs partners in leukemia. Based on this last model, a consequence of translocations of the *MLL* gene with other partners could be for MLL to form complexes with other proteins that normally interact with its translocation partner. According to this model, the result of the translocation of the MLL partner gene is that such partner-associated proteins could then travel with MLL to promoter sequences regulated by MLL and thus misregulate gene transcription.

The exact molecular mechanism of leukemia development via MLL translocations at this time is, however, unresolved. Given the remarkable number of MLL partners as well as the differences in the latency of leukemia development associated with each partner, it is highly probable that multiple mechanisms contribute to MLL leukemogenesis; however, for several of the MLL partners, there is strong evidence suggesting that the loss of MLL alone is not sufficient for leukemia development. For example, MLL knockout mice do not develop

leukemia, even though development of the hematological compartment and a number of other tissues are compromised [Parry et al., 1994; Rabbitts, 1994; Corral et al., 1996; Rowley, 1998]. Additionally, no mutations of the *MLL* gene have yet been described in the pathogenesis of AML or ALL.

BIOCHEMICAL PROPERTIES OF THE MLL PROTEIN AND ITS MOLECULAR ROLE IN THE REGULATION OF GENE EXPRESSION

The 3,968 amino acid-containing MLL protein consists of an N-terminal A-T hook DNA binding domain, a DNA methyltransferase-like domain with several continuous zinc fingers near the center of the molecule and a conserved SET domain at its C-terminal domain [Rabbitts, 1994; Rowley, 1998; Hess, 2004] (Fig. 1). Based on both genetic and biochemical studies, it has been proposed that both MLL and its *Drosophila* homolog, the *trx* gene product, are involved in activation of gene expression during development. It has also been proposed that the repressive Polycomb group (PcG) gene products function by opposing the activities of MLL and Trx. Expression analysis studies have demonstrated that the MLL protein displays a wide pattern of expression during development and is expressed in most adult tissues, including hematopoetic cells such as myeloid and lymphoid cells [Hess et al., 1997].

Earlier studies performed by Korsmeyer et al. indicated that the *Hox* gene cluster, which includes transcription factors that specify segment identity during development, is one of the targets of MLL [Yu et al., 1995]. The expression of *Hox* genes is regulated at the level of transcription during development by the opposing actions of the PcG protein families. These opposing activities ensure proper Hox expression patterns that persist through successive cell divisions. These mechanisms are generally referred to as initiation and maintenance of gene expression. Because heterozygous *Mll* knockout mice show posterior shifts in *Hox* gene expression, it has been proposed that MLL positively regulates *Hox* gene expression during development [Yu et al., 1995].

Several members of both the TrxG and PcG families contain a 130–140 amino acid motif called the Set domain, which is found in a variety of chromatin-associated proteins [Stassen et al., 1995; Jenuwein et al., 1998] (Fig. 1). This domain takes its name from the *Drosophila* proteins *S*u(var)3-9, *e*nhancer of zeste (E(z)), and *t*rithorax (*trx*) [Jones and Gelbart, 1993; Tschiersh et al., 1994]. The closest yeast homolog to MLL is a protein named Set1.

Although the cDNA for MLL was cloned over 15 years ago, until recently, we still knew very little about the biochemical function (activity) of MLL in normal cellular regulation and, more importantly, its biochemical role in the development of human leukemia. We noted several years ago that the Set1 protein from yeast *S. cerevisiae* is highly related to the MLL protein [Miller et al., 2001]. Because of the ease of genetics and biochemistry in yeast, we decided to isolate and characterize the molecular properties of the yeast Set1 containing complex to better understand the functional properties of human MLL. We have now identified this Set1 containing complex, which we have named COMPASS (*com*plex *p*roteins *as*sociated with *S*et1) [Miller et al., 2001]. COMPASS contains the MLL-related Set1 protein and seven other polypeptides, several of which contain WD domains found in other trithorax-related complexes (Fig. 2). We also demonstrated that COMPASS and several other COMPASS components are required for proper histone H3 methylation and telomeric silencing in vivo [Miller et al., 2001; Krogan et al., 2002]. Similar results regarding the Set1 complex were also shown by others following our published study [Roguev et al., 2001; Nagy et al., 2002].

Based on chromatin immunoprecipitation data, it has recently been demonstrated that human MLL can occupy the *Hox* gene promoters. Furthermore, similar to yeast Set1, the human MLL Set domain can methylate the fourth lysine of histone H3 [Milne et al., 2002]. Since MLL is recruited to *Hox* gene promoters, and contains a methyltransferase domain, it was demonstrated that the methyltransferase activity of MLL is required for regulation of *Hox* gene expression. Moreover, we now know that the mammalian MLL, like its yeast counterpart, is found in a COMPASS-like complex that also methylates the fourth lysine of histone H3 (Fig. 2) [Hughes et al., 2004]. The compositional and functional conservation between MLL and Set1 complexes establishes the existence of a highly conserved, ancient molecular machinery for the modification of histone H3 on its fourth lysine by methylation. Since histone H3 modification is required for the proper regulation of

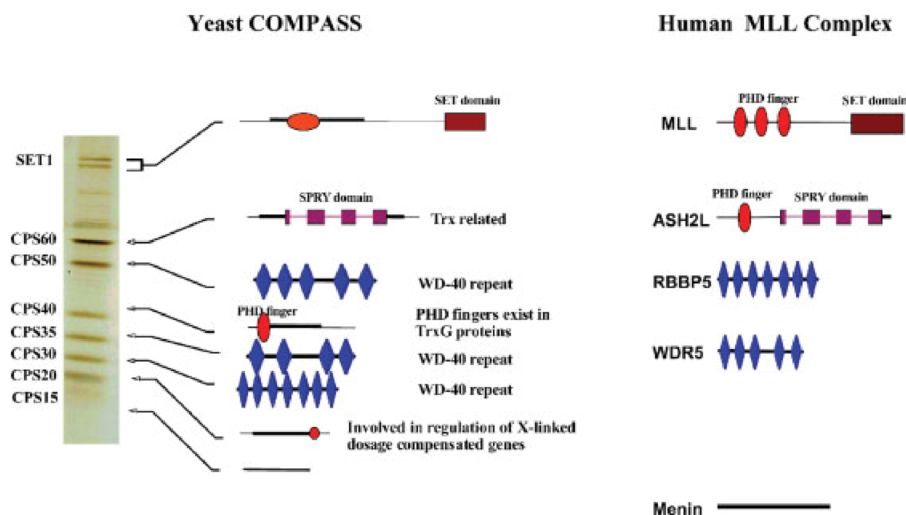

Fig. 2. Comparative analysis of components of the Set1 containing complex COMPASS from yeast to the MLL containing complex, its mammalian homolog. Evolutionarily conserved subunits of the yeast COMPASS and MLL containing complex are shown above. The Menin protein is associated with MLL complex; however, there are no Menin homologs found in yeast genome. SET (SET domain), SPRY (domain present in SP1a and RY anodine receptor), PHD (plant homeodomain), Trx (trithorax).

gene expression in yeast and higher eukaryotic organisms, these studies collectively emphasize the generality and significance of the information obtained from yeast in defining the molecular role of histone methylation by the yeast MLL-like complex COMPASS.

MONOUBIQUITINATION OF HISTONE H2B IS REQUIRED FOR HISTONE H3 METHYLATION BY COMPASS

The process of protein ubiquitination as a mode for targeting proteins for degradation has received much attention during the past decade [Conaway et al., 2002]. Proteins targeted for post-translational modification by ubiquitination are modified by a cascade that is initiated by an E1 ubiquitin activating enzyme. The E1 ubiquitin activating enzyme activates ubiquitin, which is then transferred to one of the large number of E2 ubiquitin conjugating enzymes. The selection of the target protein to be ubiquitinated by the E2 conjugating enzymes is done through a large family of E3 ubiquitin ligases. These E3 ubiquitin ligases function by interacting with both the activated E2 and the substrate. It was previously demonstrated that histone H2B is monoubiquitinated by the E2 conjugating enzyme Rad6 at lysine 123 [Robzyk et al., 2000]. This modification of H2B by monoubiquitination was proposed to function in signaling and not as a degradation mark.

Since we demonstrated that lysine four of histone H3 is methylated by the macromolecular complex COMPASS, we set out to better define the molecular machinery required for the modification of histone H3 by COMPASS. In so doing, we have devised a proteomic approach that we call global proteomic analysis in *S. cerevisiae* (GPS) [Schneider et al., 2003]. In GPS, we test by analyzing extracts of each of the non-essential yeast gene deletion mutants for defects in methylation of histone H3 on its fourth lysine. Employing GPS, we demonstrated that ubiquitination of lysine 123 of histone H2B by Rad6 is essential for the methylation of histone H3 by COMPASS [Dover et al., 2002]. Since histone methylation by COMPASS plays an important role in regulation of gene expression such as telomeric silencing, we have also demonstrated that histone H2B monoubiquitination is also required for proper regulation of telomeric silencing [Krogan et al., 2002]. Similar results regarding the role of histone H2B ubiquitination signaling for histone H3 methylation were also reported by Sun and Allis [2002]. Furthermore, it has also been demonstrated that monoubiquitination of histone H2B is required for histone H3 methylation on lysine 79 by the methyltransferase Dot1p. Collectively, these studies suggest that monoubiquitination of histone H2B plays a regulatory role for either recruitment and/or activation of COMPASS and Dot1p in methylation of histone

H3 and regulation of telomeric silencing [Gerber and Shilatifard, 2003].

In addition to its role in transcription, the E2 ubiquitin-conjugating enzyme Rad6 has been demonstrated to be involved in the ubiquitination of diverse substrates involved in various pathways. These include pathways such as DNA repair, DNA damage-induced mutagenesis, meiosis, and transposition of retrotransposons. Due to its multifunctional ubiquitin-conjugating enzyme activities involved in several different biological pathways, it was always predicted that there would be a specific E3-ligase used to direct Rad6 to monoubiquitylate H2B; therefore, many laboratories were searching for several years for the E3 ligase specific to the role of Rad6 in transcription. Employing GPS, we identified the ring finger-containing protein Bre1 as the E3 ligase required for ubiquitination of histone H2B by Rad6 [Wood et al., 2003a]. We demonstrated that Bre1 can biochemically and genetically interact with Rad6 and is required for direct recruitment of Rad6 to gene promoters. Since Bre1 function is required for histone methylation by COMPASS and Dot1p, we predicted that cells lacking this E3 ligase would be defective in telomeric silencing. Our studies have demonstrated that the E3 ligase Bre1 is required for proper regulation of telomeric silencing [Wood et al., 2003a]. Based on the loss of Bre1 resulting in the loss of all of the detectable monoubiquitinated histone H2B and other supporting data from ours and other laboratories, it can be concluded that Bre1 is the major E3 ligase required for the monoubiquitination of histone H2B by Rad6, which, in turn, sends the signal for histone methylation by COMPASS and Dot1p, as summarized in Figure 3.

THE ROLE OF THE PAF1 COMPLEX IN HISTONE UBIQUITINATION AND METHYLATION

The messenger RNA synthesis by eukaryotic RNA polymerase II proceeds through multiple stages, known as preinitiation, initiation, and elongation [Shilatifard et al., 2003; Shilatifard, 2004]. Studies during the past decade demonstrated that the elongation stage of transcription is a highly regulated process that requires the concerted action of a large number of proteins. The Paf1 complex consists of five polypeptides known as Rtf1, Paf1, Cdc73, Leo1, and Ctr9. Studies in yeast have demonstrated that this complex is associates with the elongating RNA polymerase II [Gerber and Shilatifard, 2003]. Although the subunits of the Paf1 complex are not required for viability in yeast, yeast strains lacking one or more subunits of the Paf1 complex are defective for growth when in the presence of the drug 6-AU. This observation, along with the finding that

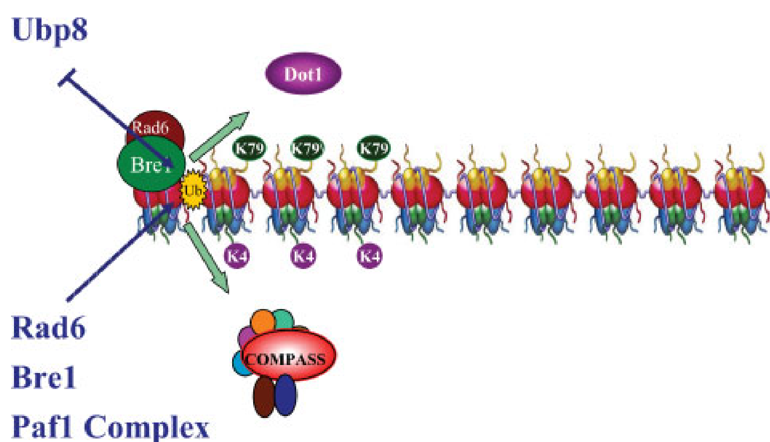

Fig. 3. Schematic representation of the molecular machinery required for the regulation of gene expression by methylation of lysines 4 and 79 of histone H3 uncovered by our biochemical screen global proteomic analysis in *S. cerevisiae* (GPS). Based on our work, as well as results from other laboratories, we know that COMPASS and the MLL complex are required for methylation of lysine 4 of the histone H3 tail. Our GPS biochemical screen in yeast has uncovered a role for the Rad6/Bre1 complex in ubiquitination of histone H2B at promoters, which signals the methylation of histone H3 on lysines 4 and 79 by COMPASS and Dot1p, respectively. We have also demonstrated via GPS that an elongation factor, the Paf1 complex, is required for proper histone H2B ubiquitination and H3 methylation. The ubiquitinated histone H2B is deubiquitinated by the action of deubiquitinating enzymes, such as Ubp8. This enzymatic removal of ubiqutin from the monoubiquitinated histone H2B negatively regulates histone H3 methylation by COMPASS and Dot1p.

the Paf1 complex is associated with elongating RNA polymerase II, resulted in the hypothesis that the Paf1 complex may function as an RNA polymerase II transcription elongation factor; however, its exact molecular role in transcription was not clear.

Employing GPS, we recently demonstrated that several subunits of the Paf 1 complex are required for proper methylation of lysine 4 and lysine 79 of histone H3 by COMPASS and Dot1p [Krogan et al., 2003]. Our study further demonstrated that the components of the Paf1 complex are required for the localization of COMPASS with the early elongating polymerase as well as that the Paf1 complex and COMPASS genetically and physically interact with each other [Krogan et al., 2003; Ng et al., 2003a]. As predicted from our previous studies, several of the subunits of the Paf1 complex are required for telomeric associated gene silencing.

To further delineate the molecular role of the Paf1 complex in the process of histone modification, our laboratory and Struhl's laboratory demonstrated that several of the components of the Paf1 complex are required for proper ubiquitination of histone H2B by the Rad6/Bre1 complex [Ng et al., 2003b; Wood et al., 2003b]. It appears that the Rad6/Bre1 complex is recruited to promoters in the absence of the Paf1 complex; however, Rad6/Bre1 is not enzymatically functional at promoters in the absence of the Paf1 complex [Wood et al., 2003b]. Through an unknown mechanism, the Paf1 complex can activate the enzymatic activity of Rad6/Bre1, which results in monoubiquitination of histone H2B at lysine 123 [Wood et al., 2003b].

SUMMARY AND FUTURE DIRECTIONS

Studies during the past several years have demonstrated that the Set1 protein (the yeast homolog of mammalian MLL) is found in a macromolecular complex referred to as COMPASS. COMPASS is the sole histone H3 lysine four methyltransferase in yeast. Furthermore, histone methylation by COMPASS is required for transcriptional regulation such as telomere-associated gene silencing, linking transcriptional regulation to histone tail methylation [Gerber and Shilatifard, 2003]. We now know that mammalian MLL, like its yeast counterpart, is also found in a COMPASS-like complex [Hughes et al., 2004]. There are several common subunits between the MLL-complex and COMPASS. One subunit, Menin, is found with the human complex, however, it does not exist in the yeast genome. Furthermore, similar to COMPASS, MLL complex is also capable of methylating the fourth lysine of histone H3 on chromatin. Such compositional and functional conservation between MLL and COMPASS establishes the existence of a highly conserved molecular machinery for the modification of histone H3 on its fourth lysine by methylation, which is required for the proper regulation of gene expression. These findings emphasize the generality and significance of the information obtained from yeast in defining the molecular role of histone methylation by the yeast MLL-like complex COMPASS.

From yeast studies, we now know that the ubiquitination of histone H2B by the Rad6/Bre1 complex is required for histone methylation by COMPASS. Furthermore, we have identified several of the components of the transcription elongation factor Paf1 complex required for functional activation of Rad6/Bre1. In fact, the phenotype of the cells lacking several components of the Paf1 complex is very similar to cells lacking Bre1. Given the fact that almost all of the detectable monoubiquitinated histone H2B is lost in the absence of Bre1, and that the loss of Bre1 results in the loss of histone H3 di- and tri-methylation, it can be concluded that Bre1 is the sole E3 ligase functioning with Rad6 in ubiquitination of histone H2B.

Based on the compositional and functional conservation between human MLL complex and COMPASS, several fundamental questions have now been raised regarding the role of histone H2B ubiquitination in regulation of histone H3 methylation by the MLL complex. It is not clear whether histone ubiquitination and methylation could play a role during the pathogenesis of leukemia. Also, we do not know whether histone ubiquitination at promoters of genes in mammalian cells is required for the functional activation of MLL. Global sequencing of numerous genomes including *S. cerevisiae*, *Drosophila melanogaster*, mouse, and human has revealed the presence of several Bre1 homologs in higher eukaryotic organisms; however, we do not know whether these proteins can function in ubiquitination of histone H2B. The same is also true of the conserved components of the Paf1 complex. More importantly, it is not clear at this time whether histones are the sole substrates for these enzymes. There is no doubt

that future investigations defining the role of histone modification by mammalian MLL, Bre1, and the Paf1 complex will be instrumental in defining the role of such histone modifications during normal development and the possible role the histone modifications may play during the pathogenesis of leukemia.

ACKNOWLEDGMENTS

Due to reference limitations, we apologize for not citing many of the original research articles relevant to the topics discussed. Studies in the Shilatifard laboratory were supported by NIH grants. A. Shilatifard is a scholar of the Leukemia and Lymphoma Society.

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