Journal of Cellular Biochemistry 95:234–242 (2005)

MLL: How Complex Does It Get?

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Abstract The mixed lineage leukemia (*MLL*) gene encodes a very large nuclear protein homologous to *Drosophila* trithorax (trx). MLL is required for the proper maintenance of *HOX* gene expression during development and hematopoiesis. The exact regulatory mechanism of *HOX* gene expression by MLL is poorly understood, but it is believed that MLL functions at the level of chromatin organization. *MLL* was identified as a common target of chromosomal translocations associated with human acute leukemias. About 50 different MLL fusion partners have been isolated to date, and while similarities exist between groups of partners, there exists no unifying property shared by all the partners. *MLL* gene rearrangements are found in leukemias with both lymphoid and myeloid phenotypes and are often associated with infant and secondary leukemias. The immature phenotype of the leukemic blasts suggests an important role for MLL in the early stages of hematopoietic development. *Mll* homozygous mutant mice are embryonic lethal and exibit deficiencies in yolk sac hematopoiesis. Recently, two different MLL-containing protein complexes have been isolated. These and other gain- and loss-of-function experiments have provided insight into normal MLL function and altered functions of MLL fusion proteins. This article reviews the progress made toward understanding the function of the wild-type MLL protein. While many advances in understanding this multifaceted protein have been made since its discovery, many challenging questions remain to be answered. J. Cell. Biochem. 95: 234–242, 2005.

Key words: MLL; leukemia

The mixed lineage leukemia (*MLL*) gene was isolated as a common target of chromosomal translocations observed in human acute leukemias [Ziemin-van der Poel et al., 1991; Gu et al., 1992; Tkachuk et al., 1992; Thirman et al., 1993]. Cytogenetic analyses have identified more than 50 different loci which translocate to *MLL*, resulting in either acute lymphoid leukemia (ALL) or acute myeloid leukemia (AML) [Rowley, 1998]. Chromosomal translocations involving *MLL* are predominant in infant leukemias and leukemias occuring in patients previously treated with DNA topoisomerase II inhibitors [Tkachuk et al., 1992; Thirman et al., 1993]. In each case, *MLL* is broken within an 8.3 kb breakpoint cluster region (BCR) and exons encoding approximately 1,400 amino-terminal amino acids are fused in-frame to a fusion partner gene. One of the interesting aspects of *MLL*-associated leukemias is the large number and the diversity of the fusion partner genes. In general, *MLL* rearrangement is associated with a poor prognosis regardless of the fusion partner [Ayton and Cleary, 2001]. Numerous studies have proposed different roles for *MLL* and fusion partner genes in leukemogenesis. In this article, we look into some recent advances in determining the normal MLL function and discuss some proposed models of *MLL*-related leukemogenesis.

WILD-TYPE MLL FUNCTION

Sequence comparisons and genetic studies have identified *MLL* as a functional ortholog of the *Drosophila* trithorax (*trx*) gene [Tkachuk et al., 1992; Yu et al., 1995]. Trx, like MLL,

Grant sponsor: National Institutes of Health (HIH)/ National Cancer Institute (NCI); Grant number: CA40046; Grant sponsor: Dr. Ralph and Marian Falk Medical Research Trust.

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Received 21 December 2004; Accepted 22 December 2004
DOI 10.1002/jcb.20430

belongs to the Trithorax Group (trx-G) of proteins, which are responsible for positive maintenance of gene expression during development. Another evolutionarily conserved family of proteins, the Polycomb Group (Pc-G), antagonizes the function of trx-G proteins by acting in a repressive manner on the same target genes. Both groups are believed to regulate gene expression through an epigenetic mechanism [Orlando et al., 1998]. The best-studied downstream targets of MLL and trx function are the homeobox (*HOX*) genes, which control segment specificity and cell fate in the developing embryo [Krumlauf, 1994]. In mammalian cells, *HOX* genes are found in four clusters on different chromosomes. The position of each *HOX* gene within the cluster is conserved across species and corresponds to its anterior-posterior expression pattern during embryogenesis [Krumlauf, 1994].

Targeted homozygous disruption of *Mll* in mice is embryonic lethal and, similar to *trx* mutants in flies, *Hox* gene expression is initiated but not maintained in these animals [Yu et al., 1998]. Lethality at day E10.5 is associated with numerous defects in segmental identity. In these mice, the *Mll* allele was disrupted in exon 3, downstream from the AT-hook region (Fig. 1, ¥) [Yu et al., 1995]. Targeting *Mll* at exons 12–14 (Fig. 1, #), in the BCR, delayed lethality for a couple of days [Yagi et al., 1998]. Yolk sac and fetal liver hematopoiesis is perturbed in both types of *Mll* mutants. Colony forming assays with cells from these tissues show a greatly decreased number, smaller size, and slower proliferation in *Mll* null cells in comparison to

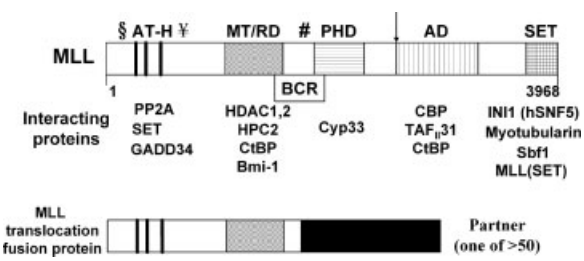

Fig. 1. Schematic of the MLL protein and fusion proteins that result from chromosomal translocations. The MLL protein functional domains and the proteins they interact with are indicated above and below the diagram, respectively. AT-H, AT-hooks; MT/RD, DNA methyltransferase homology/repression domain; BCR, breakpoint cluster region; PHD, plant homeodomain; AD, transcriptional activation domain; SET, histone methyltransferase domain. Arrow is Taspase 1 cleavage site. Symbols represent regions used for *MLL* targeting in different studies. See text for references on these studies.

the wild-type [Hess et al., 1997; Yagi et al., 1998]. Surprisingly, embryonic stem (ES) cells having *Mll* disrupted in the third exon upstream from the AT-hook region showed an increased proliferative capacity in similar assays (Fig. 1, §) [Fidanza et al., 1996]. It is still unclear why these experiments produce vastly different results, and while there is no evidence for Mll expression in these cells, it is plausible that the differences come from the different targeting methods and the portions of Mll protein expressed. Even animals carrying only one normal *Mll* allele show a phenotype that differs from the wild-type mice. *Mll*+/− mice are anemic and show growth retardation with specific segment transformations [Yu et al., 1995]. The dramatic phenotype of the *Mll* heterozygous mice suggest that both copies of the gene are essential during development thus implying haploinsuficiency as one of the possible contributing mechanisms of *MLL*-associated leukemias.

While it is likely that MLL has many downstream target genes, *HOX* genes have been studied the most extensively. Embryonic fibroblasts derived from the *Mll* mutant mice (Fig. 1, ¥) show specific downregulation of more 5′ *Hoxa* and *Hoxc* cluster genes (*Hoxa5, -a7, -a9, -a10, -c4, -c5, -c6, -c8, -c9*) [Hanson et al., 1999]. It is believed that Mll controls *Hox* gene expression by binding to *Hox* regulatory elements and association with other transcriptional regulators. Indeed, MLL binding to the promoters of *HOXA9* and *Hoxc8* was described recently [Milne et al., 2002; Nakamura et al., 2002]. Overexpression of some *Hox* genes, including *Hoxa9*, has been linked to leukemia in mouse models. Furthermore, *HOXA9* and *HOXA10* are overexpressed in patient leukemia cells with *MLL* translocations [Ferrando et al., 2003]. Together with the studies showing the role of certain *HOX* genes during normal hematopoiesis, a case can be made that translocation of *MLL* leads to the aberrant expression of its downstream targets generating the leukemic phenotype [Lawrence et al., 1996]. *HOXA9* is normally downregulated as blood cells differentiate from progenitors to more mature stages of hematopoiesis. Overexpression of these *Hox* genes by some MLL fusion proteins may cause an early arrest in hematopoietic differentiation leading to the immature phenotype associated with MLL leukemias. The MLL–ENL fusion protein was shown to require the presence of

Hoxa7 and *Hoxa9* genes for immortalization of mouse bone marrow progenitor cells [Ayton and Cleary, 2003]. However, this may not be a generality for all MLL fusions because MLL–GAS7 is able to transform hematopoietic progenitors in the absence of *Hoxa7* and *Hoxa9* [So et al., 2004]. An alternative explanation for these discrepant results may be the use of different starting populations of progenitor cells. The MLL–ENL study used 5-fluorouracil-mobilized, lineage-depleted progenitors, whereas the MLL–GAS7 study employed positive selection with the c-Kit marker.

MANY PIECES OF THE PUZZLE

MLL translocations lead to the formation of fusion proteins where the amino-terminus of *MLL* is fused in-frame to the carboxyl-terminus of the translocation partner. Many fusion partners contain domains that can function as transcriptional activators in reporter gene assays, whereas others contain oligomerization domains [Ayton and Cleary, 2001]. In both cases it is possible to suggest that a gain-of-function by the fusion proteins may lead to leukemogenesis. However, lack of *Mll* causes defects in embryonic hematopoiesis indicating that the presence of normal Mll is absolutely required for proper hematopoietic development. Chromosomal translocations cause a loss of several MLL functional domains that may be required for its proper function (Fig. 1).

The most conserved region between trx and MLL is the C-terminal SET domain, also present in numerous chromatin-associated proteins. The SET domain was named for *Drosophila* founding members containing a region of high sequence similarity: Su-(var)3–9, enhancer of zeste, and trx. Many SET-containing proteins have been identified and a number of them were shown to possess intrinsic histone methyltransferase activity [Yeates, 2002]. Methylation of the lysine residues on histone tails is part of the proposed histone code in which different histone modifications direct the recruitment of transcriptional regulators, ultimately exerting an influence on gene expression [Jenuwein and Allis, 2001]. While the initial studies failed to confirm such enzymatic function in the MLL SET domain, more recently two groups have shown that mouse and human MLL SET domains are indeed able to methylate lysine residues on histone tails [Milne et al., 2002; Nakamura et al., 2002]. The specificity of the MLL SET domain is for lysine 4 (K4) on histone H3. K4 on histone H3 can be mono-, di-, or tri-methylated and these marks are usually present at transcriptionally active chromatin regions. In yeast, tri-methylation of H3 K4 is associated with actively expressed genes while di-methylation is believed to be present at all accessible genes [Santos-Rosa et al., 2002]. It is possible that MLL regulates its target genes by providing the tri-methylation modification. Since no histone demethylases have been identified to date, tri-methylation of lysine residues could persist over time and present a way for regulating gene expression even once MLL dissociates from the target genes.

Besides binding to the core histones, the SET domain of MLL interacts with several different proteins. One of them is the MLL SET domain itself, showing the ability to homo-dimerize [Rozovskaia et al., 2000]. Another protein that interacts with the carboxyl-terminus of MLL is hSNF5, a member of the SWI/SNF protein complex that functions in chromatin remodeling [Rozenblatt-Rosen et al., 1998]. Binding of MLL to chromatin-associated proteins supports the idea that wild-type MLL regulates target gene expression at the level of chromatin through interactions with other transcriptional regulators.

A region just upstream of the SET domain is also lost in the MLL fusion proteins caused by chromosomal translocations. Using GAL4–MLL chimeras, it was shown that this region of the MLL protein contains a transcriptional activation domain [Zeleznik-Le et al., 1994]. This activation domain binds to the CREB-binding protein (CBP), a known regulator of gene expression [Ernst et al., 2001]. CBP controls gene expression by both mediating recruitment of transcriptional activators, and by its intrinsic histone acetyltransferase (HAT) activity. The binding of MLL and CBP is direct and was shown to be necessary for MLL-mediated transcriptional activation. All MLL mutants unable to bind CBP have a drastic reduction in their ability to activate expression of a reporter gene. Interestingly, CBP is also one of the MLL translocation partners, and the MLL–CBP fusion protein retains the HAT activity believed to be important for the transcriptional activation [Sobulo et al., 1997]. There are many other MLL fusion partners that contain transcriptional activation domains which are retained in

the chimeric proteins. The presence of these activation domains in leukemic cells suggests a possible gain-of-function mechanism for MLL-associated leukemias. The presence of a potent transcriptional activation domain may lead to overexpression of MLL downstream targets, causing differentiation arrest and the leukemogenic phenotype. However, a number of MLL fusion partners are not involved in transcriptional regulation suggesting that there is more to the model than this simple explanation.

Recent studies have shown that MLL undergoes proteolytic processing shortly after translation and that this cleavage divides the protein into a larger N-terminal portion (320 kDa) and a smaller C-terminal part (180 kDa) [Yokoyama et al., 2002; Hsieh et al., 2003b]. The enzyme responsible for this processing is Taspase 1, and two cleavage sites are located just upstream of the activation domain [Hsieh et al., 2003a] (Fig. 1). Enzymatic function of Taspase 1 is required for proper *HOX* gene expression implying that MLL processing is absolutely necessary for its proper function. The two fragments generated by the cleavage reassociate noncovalently and this interaction confers stability to the N-terminal portion and correct localization to the C-terminal part [Hsieh et al., 2003b]. Chromosomal translocations prevent this interaction from happening, suggesting that one possible role of the fusion partners may be to provide stability for the MLL N-terminal fragment. However, myc fusion with MLL does not lead to leukemia, indicating that stability of the MLL N-terminal portion is not sufficient for leukemogenesis [Corral et al., 1996].

The region of MLL where the breakpoints occur in patient samples overlaps a group of specific zinc fingers called plant homeodomain (PHD). The PHD fingers are the second most conserved domain between MLL and trx. The PHD fingers are found in a large number of chromatin-associated proteins [Aasland et al., 1995]. MLL has four PHD zinc fingers with a region of homology to bromodomains found between the third and the fourth finger. Proteins containing bromodomains have been implicated in gene transcription through chromatin remodeling as they were shown to bind acetylated lysines on histone tails [Dhalluin et al., 1999]. It is possible that the MLL bromodomain serves a similar function and helps in targeting the protein to regions of DNA enriched in acetylated histones, although this function has not been demonstrated. Because MLL has an atypical bromodomain, it could also have other, so far unknown, functions. MLL PHD fingers have been shown to function in protein–protein interactions. The second PHD finger is required for homodimerization and the third one was shown to bind the cyclophilin Cyp33 [Fair et al., 2001]. Both homodimerization and Cyp33 interaction are conserved in *Drosophila*. Cyp33 binds AU-rich RNA sequence and carries *cis/trans* prolyl-isomerase activity. MLL and Cyp33 co-localize, suggesting that MLL binding may target Cyp33 to specific subnuclear locations. It is interesting that overexpression of Cyp33 results in downregulation of some MLL downstream *HOX* target genes. The same effect is not observed in cells that do not have the MLL PHD zinc finger region intact indicating that Cyp33 affects MLL target genes through its binding to this region. The molecular mechanism of this regulation is not yet fully understood.

The N-terminal region of MLL that is retained in the fusion proteins encompasses the AT-hooks and transcriptional repression domains. MLL contains three AT-hook motifs which have DNA binding activity, specific for AT-rich DNA [Zeleznik-Le et al., 1994]. These domains do not recognize a specific sequence but rather a structure of cruciform or bent DNA. The MLL AT-hook region was shown to interact with SET protein (not to be confused with SET domain), protein phosphatase 2A (PP2A), and the pro-apoptotic protein GADD34 [Adler et al., 1997; Adler et al., 1999]. MLL fusion proteins, but not the wild-type MLL, inhibit GADD34-induced apoptosis suggesting that this may be an important factor in MLL-associated leukemias [Adler et al., 1999]. It is unclear what role the AT-hooks play in MLL gene regulation, but it is possible that they aid in the targeting of MLL to specific genomic region by recognizing these DNA structures. The MLL repression domain contains a region with homology to DNA methyltransferase 1 (DNMT1), also termed the "CXXC" domain. The CXXC domain was shown to bind CpG-rich DNA sequences with a preference for non-methylated DNA [Birke et al., 2002]. Presence of the CXXC domain is required for MLL–ENL immmortalization of myeloid progenitors [Slany et al., 1998]. Recently, the MLL repression domain was shown to interact with histone deacetylases (HDACs) and to recruit polycomb proteins HPC2 and BMI-1, and the co-repressor

protein CtBP [Xia et al., 2003]. Polycomb proteins are known functional antagonists of the trx-G family. Unlike the activation domain in the C-terminus of MLL, the repression domain is retained in the leukemic fusion proteins. Presence of both of these domains in the wild-type protein makes for an attractive hypothesis that the regulated balance between the two transcriptional functions is important for proper MLL function. Thus, loss of the SET domain and the strong activation domain may disturb this balance leading to aberrant MLL functions and eventually leukemic transformation.

THE STORY IS TWO COMPLEX

The presence of transcription regulating domains within MLL and their interactions with other transcriptional activators and repressors suggests that many different proteins may be involved in mediating the regulatory role of MLL. Recently two different MLL containing protein complexes were purified [Nakamura et al., 2002; Yokoyama et al., 2004]. The first study identified a very large complex comprised of more than 29 proteins associated with MLL [Nakamura et al., 2002]. The complex formation is not mediated by DNA as intercalation of ethidium bromide did not disrupt the protein interactions. A large number of the 29 proteins belong to two HDAC containing complexes, NuRD and SIN3. Both of these complexes have the same core components (HDAC1/2, RbAp46, and RbAp48) which function in histone binding and deacetylation of lysine residues on histone tails [Ahringer, 2000]. The NuRD and SIN3 complexes diverge in other complex components and complex associating proteins. Mi-2, found in this MLL complex, is another member of the NuRD complex and contains ATP-dependent nucleosome remodeling activity. Other proteins found in the MLL complex also have this chromatin remodeling potential. BRM, BAF170, BAF155, and INI1 associate with MLL and are all components of the SWI/SNF ATP-dependent nucleosome remodeling complex [Wang et al., 1996]. A number of proteins found in this MLL complex are members of the TFIID complex. TFIID is involved in promoter recognition and functions in the recruitment of transcription activators to the RNA polymerase core complex [Lemon and Tjian, 2000]. TFIID is also involved in the recruitment of proteins necessary for RNA processing, some of which are also found associated with MLL. Finally, WDR5 and RBBP5, proteins that contain WD40 protein–protein interaction domains are also found in the MLL complex. WDR5 and RBBP5 are homologs of Swd1 and Swd2 respectively, members of the yeast Set1 protein complex [Yokoyama et al., 2004]. The Set1 protein contains a SET domain, which is highly homologous to the SET domain of MLL. Interestingly, just like the MLL protein, the Set1 complex was shown to methylate lysine 4 on histone H3 [Nagy et al., 2002]. This purified MLL complex is able to perform chromatin remodeling, histone methylation, acetylation, and deacetylation suggesting that all the associated proteins retain their enzymatic activities [Nakamura et al., 2002]. All the complex components bind to the promoter of *HOXA9* only when MLL is present, showing the MLL-dependence of the whole supercomplex being targeted to the target gene region. Also, because the complex formation is not DNA dependent, it is possible that the assembled complex is recruited to the MLL-dependent regulatory elements as a whole.

A second, much smaller, MLL complex was isolated by Yokoyama et al. [2004]. It resembles the above-mentioned yeast and mammalian Set1 complexes as the majority of the Set1 complex components are also found interacting with MLL. The only unifying members of the two different MLL complexes are RBBP5 and WDR5 proteins. RBBP5 and WDR5, along with ASH2L, interact with the SET domain of MLL. This binding is also observed in other Set1 complexes and is required for the histone methyltranferase activity [Nagy et al., 2002]. It is of interest that a close MLL homolog, MLL2, was also shown to interact with ASH2L, RBBP5, and WDR5. MLL2 also interacts with DPY-30 another component of the yeast Set1 complex [Hughes et al., 2004]. HCF, also found in the human but not the yeast Set1 complex, associates with MLL through its Kelch domain. HCF is known to interact with SET1 and SIN3 regulatory complexes, however no recruitment of SIN3 components to this MLL complex was detected [Yokoyama et al., 2004]. The last component of this MLL complex is menin, also found to interact with MLL2 [Hughes et al., 2004]. Menin is a tumor suppressor and mutations in this gene have been implicated in multiple endocrine tumors [Chandrasekharappa et al., 1997]. Similar to the AT-hooks of MLL, menin can bind to various DNA

structures in a sequence-independent manner [La et al., 2004]. Menin interacts with the N-terminus of MLL, which suggests that it has a potential to interact with both the wild-type MLL and the MLL fusion proteins [Yokoyama et al., 2004]. Indeed, menin does interact with the MLL–ENL fusion protein. Interestingly, menin is required for efficient *HOXA9* expression, and it also binds to the *Hoxc8* enhancer and promoter region [Hughes et al., 2004]. Mll binding to the same *Hoxc8* promoter sequence has been described [Milne et al., 2002]. It is still unclear what may be the role for menin in MLL transcriptional regulation or MLL-associated leukemogenesis and more studies are needed to answer this question.

PROSPECTS

Transcriptional regulation by MLL is proving to be a very complex phenomenon. To fully understand the role of wild-type MLL in development and hematopoiesis, a number of experimental systems will need to be analyzed. While disruption of MLL function inhibits growth and differentiation of hematopoietic cells in most systems, the presence of oncogenic MLL fusion proteins increases their proliferative capacity. Because MLL is believed to function at the level of chromatin organization and its SET domain possesses intrinsic histone methyltransferase activity specific for H3 K4, it is important to determine if and how different the chromatin structure of the target genes is in the presence of fusion proteins. The MLL fusion proteins lack the SET domain which should prevent methylation of H3 K4, a marker associated with active gene transcription. It is still unclear how the levels of di- and tri-methylation of this residue influence gene expression and if trimethylation is absolutely required for transcription. The majority of *HOX* genes are downregulated as hematopoietic cells progress from progenitors to a fully differentiated stage. It is possible that, first the increase, and then the decrease in gene expression are both mediated by MLL through interactions with alternate complexes. In *Drosophila*, trx was shown to bind the regulatory elements of its downstream target genes even when they are not expressed. The same may be true for MLL, and binding of transcriptional activators and repressors may function in shifting the balance between gene expression and gene repression. The interaction of MLL with proteins such as CBP and HDACs suggest an additional potential mechanism by which MLL function could be regulated. A number of transcription factors are regulated through posttranslational modifications such as phosphorylation or acetylation. It is possible that the same type of modifications on specific MLL residues are important determinants of its function. Similarly, Cyp33 may alter MLL structure/function through its *cis*/*trans* prolyl-isomerase enzymatic activity. While these protein interactions may influence normal MLL function, it is still not understood how MLL is targeted to the regulatory elements of its *HOX* gene targets. It is likely that MLL recruitment involves interactions with specific DNA structures as well as interactions with DNA-associated proteins. One such protein may be menin, a tumor suppressor which, similar to the AT-hook region of MLL, was shown to bind cruciform DNA. Menin binds to the N-terminus of MLL and through this interaction would be able to recruit the wild-type or the oncogenic fusion protein. Interestingly, menin is required for *Hox* gene expression adding further support to the idea that this protein helps in the recruitment of MLL. MLL may also bind to non-methylated CpG sequences in regulatory regions of its target genes. The CpG DNA binding activity of the MLL repression domain may mediate this in vivo, although only in vitro experiments have been done to date.

Purification of two different MLL complexes raises a number of interesting questions. Both complexes were isolated from the K562 cell line, therefore differences between the two complexes cannot likely be attributed to different cell types. However, since MLL is expressed in the majority of tissues, the question arises whether all cell types have both of these complexes or whether different cell types have their own versions of either complex. Because a number of proteins in the larger MLL complex either belong to a family of transcriptional repressors or co-activators, this complex could have both activating and repressive functions with one functional outcome dominating on a particular target gene in a given cell type at a given time. The smaller Set1-like complex would likely be associated with transcriptional activation because in yeast Set1 is found at transcriptionaly active loci [Ng et al., 2003]. It is unknown if the two protein complexes function on the same or different target genes. In either case, they

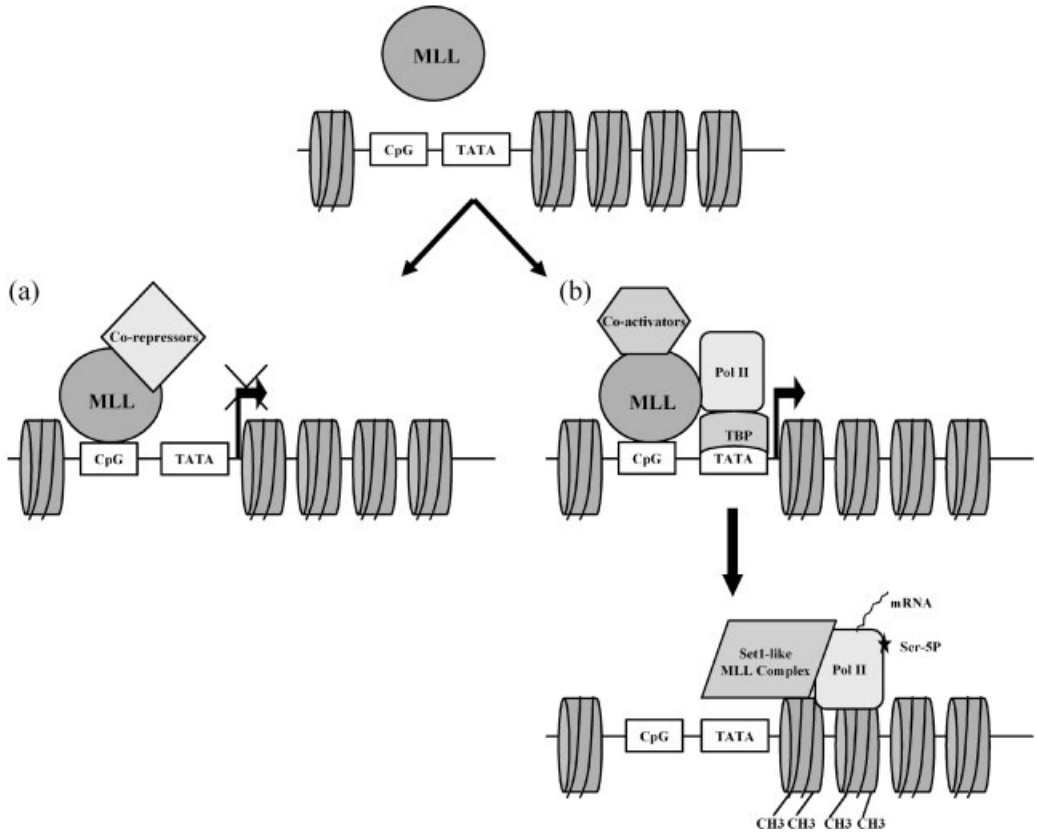

Fig. 2. Model for transcriptional regulation mediated by MLL-containing complexes. Two MLL-containing protein complexes have been identified. The larger supercomplex contains members of co-repressor and co-activator protein families. The effect on downstream targets may be silencing (**a**) or activating (**b**), depending which members of the supercomplex are present at a given time. The MLL Set1-like complex (b-bottom diagram) may be involved in the elongation process and may only be recruited following phosphorylation of RNA polymerase II. This complex facilitates methylation of K4 on histone H3 in the ORF, leaving a mark of transcriptionally active genes.

may or may not be present in the cells at the same time. It is possible that the two complexes function at two different stages of transcriptional regulation. In this model (Fig. 2), the large MLL complex would be recruited to the promoters of MLL downstream target genes by some as yet unknown mechanism. One outcome could include MLL recruitment of HDACs and repressor complexes, causing silencing of the downstream targets. This would suggest, similar to trx in *Drosophila*, that MLL is bound to the regulatory elements of its target genes even when these genes are not expressed. On the other hand, under the appropriate conditions, the SWI/SNF components of the larger complex could alter the chromatin structure in a way that allows for binding of transcriptional co-activators to the RNA polymerase core complex, ultimately leading to gene expression. Once RNA transcription is initiated, the Set1-like MLL complex could be recruited allowing tri-methylation of lysine 4 on histone H3 in the open reading frame (ORF). This is similar to the yeast Set1 complex which is targeted to the 5′ coding regions of transcriptionally active genes where it catalyzes methylation of the same histone H3 residue. In yeast, Set1 recruitment depends on phosphorylation of the RNA polymerase II CTD at serine 5. Phosphorylation of PolII at this residue is a mark for transition from transcriptional initiation to elongation. Comparable to yeast, the MLL Set1-like complex may be required for the elongation phase of gene expression. If expression of *HOX* genes depends on the recruitment of MLL complexes and methylation of histone residues, it is not well understood how the majority of MLL fusion proteins, which lack the histone methyltransferase SET domain, accelerate *HOX* gene expression. In this case, an active HAT activity recruited by the fusion may be able to prevent silencing of the target genes. It is also possible

that partners provide the ability to recruit elongation function, either via methylation activity or by some other means.

In summary, much work has been done in an effort to understand transcriptional regulation by MLL. The regulation of *HOX* genes by MLL presents a great model for unraveling the complexities of how gene expression is controlled through changes in chromatin structure. Understanding how normal MLL functions will also facilitate comprehending MLL-associated leukemias in order to develop successful MLL-directed therapies.

ACKNOWLEDGMENTS

The authors apologize to colleagues whose work we failed to recognize due to space limitations.

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