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MLL histone methylases in gene expression, hormone signaling and cell cycle

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1. ABSTRACT

Histone methyl-transferases (HMTs) are key enzymes that post-translationally methylate nuclear histone proteins and play critical roles in gene expression, epigenetic regulation and diseases in eukaryotic organisms. Mixed lineage leukemias (MLLs) are human HMTs that specifically methylate histone H3 at lyisine-4 and regulate gene activation. MLLs are also well known to be rearranged often in acute myeloid and lymphoid leukemias. Human encodes several MLLs that have similar enzymatic activities but diverse functions. Herein, we have reviewed the recent advances in understanding the diverse functions of MLL family of HMTs in gene regulation, hormone signaling and cell cycle regulation in human.

2. INTRODUCTION

In eukaryotes, DNA, the blue print of life, is packaged inside the nucleus in the form of complex chromatin (1, 2). The molecular mechanism by which the genetic information is packaged inside the chromatin, transcribed and regulated is poorly understood. Nucleosome, the repeating unit of chromatin, contains four histone proteins (two copies each), H2A, H2B, H3, and H4. These histones undergo various kinds of posttranslational modifications such as acetylation, methylation, phosphorylation, etc and determine the “active” or “silent” states of genes (3-5) (Figure 1 and Table 1). Histone acetylation is generally associated with gene activation. Histone methylation, however based on the nature and

Table 1. Histone lysine methylations and their functions

H3					H4
K4	**K9**	**K27**	**K36**	**K79**	**K20**
MLL1, MLL2, MLL3 MLL4, Set1A, Set1B, Set9	G9A, Su39H1, Su39H2, EU-HMTase1, ESET, SETDB1	ZH2	SETD2, HYPB, NSD1	Dot1L/ KMT4	Pr-SET7 SET8, SUV4-20
Transcriptional activation	Heterochromatin formation, transcriptional silencing	Gene silencing	Transcription elongation, Chromatin maintenance	Telomere silencing, meiotic check point, DNA damage response	Heterochromatin formation

Table 2. Proteins associated with Set1 like HMT complexes in yeast and human

	Yeast	Human					
Complexes	**Set1**	**Set1**	**MLL1**	**MLL2**	**MLL3**	**MLL4**	**MLL5**
Components	Set1	Set1A&B					
	Bre2	Ash2	Ash2	Ash2	Ash2	Ash2	
	Spp1	CGBP	CGBP	CGBP	CGBP		
	SWd1	Rbbp5	Rbbp5	Rbbp5	Rbbp5	Rbbp5	
	Swd2	Swd2					
	Swd3	WDR5	WDR5	WDR5	WDR5	WDR5	
	Sdc1	Dpy30	Dpy30	Dpy30	Dpy30	Dpy30	
			Menin	Menin			
	HCF1	HCF1	HCF1				
			ERα	ERα	ERα	ERα	
				ASC-2	ASC-2	ASC-2	
			MOF				

position of the modification, are associated with both gene activation and silencing (4, 6-12). For example, methylations of histone H3 at lysine-4 (K4) and K36 are found in active genes; while methylations at H3K9, K27, and H4K20 are associated with silent genes (74, 78) (see Table 1 and Figure 1 for different types of histone lysine methylations and their functions). Presence of various histone deacetylase and demethylases in cells makes these modification marks reversible (13-16). Although, various histone modifying enzymes have been discovered, their detail function in gene regulation and disease are largely unknown and are just beginning to be revealed. In this review we discussed the recent advancements in understanding the functions of mixed lineage leukemia (MLL) family of histone methyl-transferases (HMTs) that methylate specifically histone H3K4 and are linked with gene expression, hormone signaling, cell cycle regulation and cancer.

3. MLL, HISTONE METHYLATION AND GENE EXPRESSION

3.1. H3K4 methylation in yeast and its roles on transcription

H3K4 methylation is an evolutionarily conserved mark closely associated with transcriptionally active chromatin (2). The function of H3K4 methylation is well studied in yeast (17). Set1 is the sole H3K4 specific HMT present in yeast (7, 18, 19) and a SET1 null mutation results in a slow-growth phenotype (18, 20, 21). In yeast, Set1 interacts physically with RNA polymerase II (RNAP II) during transcription and regulates gene expression (22-24). Set1 mediated trimethylation of H3K4 is associated with early phase of transcription and have been implicated with transcriptional memory (23). Set1 mediated histone methylation is functionally coupled with histone H2B monoubiquitination, Paf1 complex and other transcription factors involved in transcriptional regulation (22, 23, 25-27). In yeast Set1 is a component of a multi-protein complex (known as COMPASS) containing subunits Set1, Bre2, Spp1, Swd1, Swd2, Swd3, and Sdc1 (Table 2) and its HMT activity is fully active only in the context of a multiprotein-subunit complex. Recently, it has also been demonstrated that Set1 is required for methylation of conserved lysines in a kinetochore protein, Dam1, suggesting its important roles in mitosis (7).

3.2. MLLs are human histone H3 lysine 4 (H3K4) specific methyl-transferases

The Set1 HMT has diverged and became more complex in higher eukaryotes (17, 28-35). In mammals, six different Set1 homologs have been characterized: MLL1, MLL2, MLL3, MLL4, Set1A and Set1B, collectively known as MLL family of HMTs (Table 2) (36, 37)**.** Like yeast Set1, each of these MLL family of HMTs possess H3K4-specific methylation activities and are associated with gene activation (38-42). In spite of their similar enzymatic activity (H3K4-methylation), our sequence analysis demonstrates that these proteins do not share more than 30 % sequence homology. However, each of them contains a conserved SET domain (responsible for HMT activity) and various protein-protein and protein-DNA interaction domains (Figure 2) (28, 34, 43-45). MLL1 and MLL3 contain multiple DNA binding AT hook domains that may be responsible for targeting them into specific gene promoters. MLL1 and MLL4 contain CXXC Zn - finger motifs that may also be involved in DNA binding and in gene targeting. Each of the MLLs (MLL1-4) contains multiple PHD domains (plant homeodomain) that are involved in protein-protein interaction and often found in chromatin associated proteins (Figure 2)**.** The PHD domains present in MLLs have been implicated in

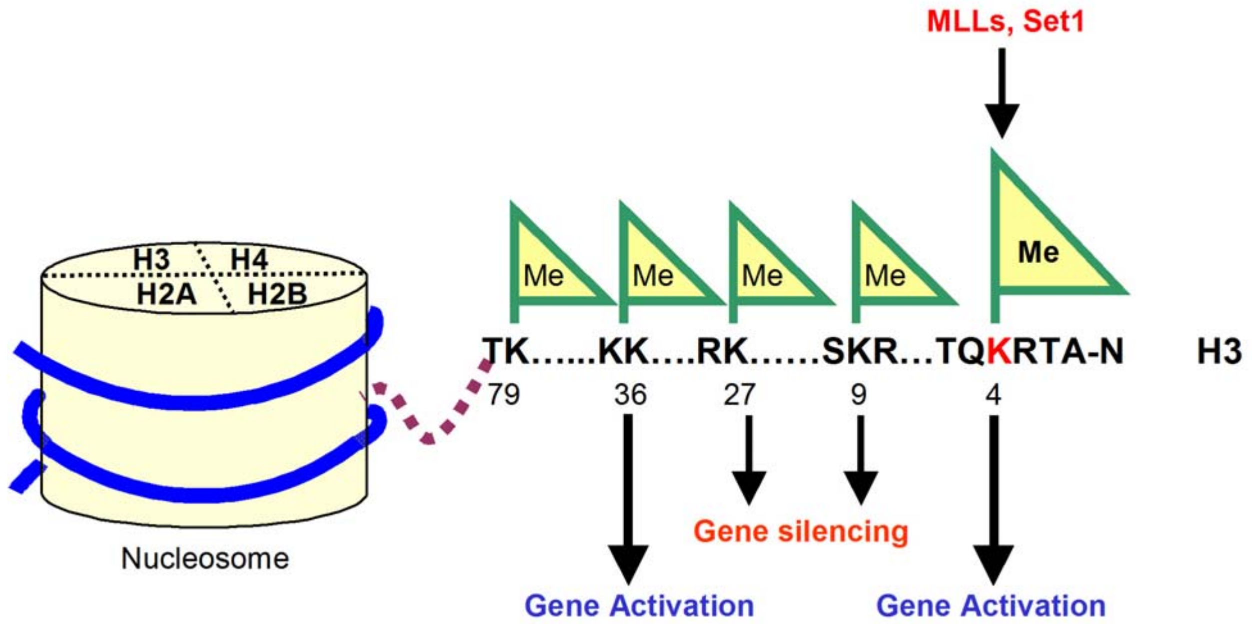

Figure 1. Methylation of Histone H3 at lysine-4 (H3K4) by MLLs and Set1 is linked with gene activation.

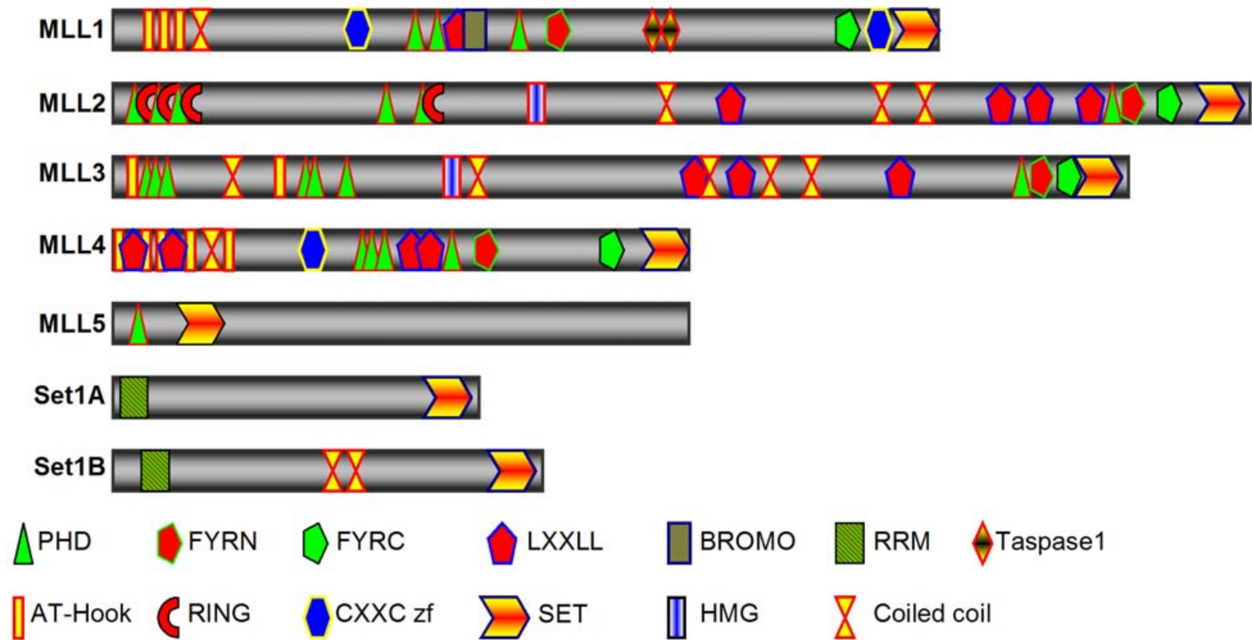

Figure 2. Domain structures of MLLs. PHD (plant homodomain) are usually involved in protein-protein interaction; FYRN and FYRC domains are involved in hetero-dimmerization between MLL^N and MLL^C terminal fragments. LXXLL domain (also known as NR box) are involved in interaction with nuclear receptor (NR). BROMO domains are involved in the recognition of acetylated lysine residues in histone tails. RRM is the RNA recognition motif. Taspase1 site is the proteolytic site for the protease Taspase1. AT-hook is a DNA binding domain. RING fingers are involved in protein-protein interactions. CXXC-zf is a Zn-finger domain that is involved in protein-protein interaction. SET is responsible for histone lysine methylation. HMG domains are involved in binding DNA with low sequence specificity. Coiled coil domains mediate homo-oligomerization.

interactions with histone deacetylases (HDACs), polycomb group proteins (PcGs) and with methylated lysines in histone tails (45, 46). The FYDN and FYDC are two interesting domains that are present in MLL^N and MLL^C terminal regions of MLL1 (47-49). These domains are involved in forming heterodimers between MLL^N and MLL^C terminal fragments produced upon proteolysis of MLL1 by protease Taspase1 (Figure 2) (47-49)**.** LXXLL domain (also known as NR box) present in MLLs make these proteins interesting for nuclear receptor mediated gene activation and hormone signaling (33, 50-52). In fact, MLL2, MLL3, and MLL4 that contain multiple LXXLL domains are shown to interact with nuclear receptors and play important roles in hormone dependent gene activation (33, 50-52)**.** Although, further studies are needed to elucidate the detail function of different domains present in MLLs, their importance have been addressed using deletion mutation experiments in mice models which showed that progressive deletion of MLL functional domains leads to progressively more severe phenotypes (53-55).

The multiplicity of MLL family of HMTs in vertebrate genomes indicates that MLLs may have specialized functions likely in regulating differential expression of specific target genes or in methylation of distinct non-histone proteins for other functions. The distinguishable phenotypes of deletions or truncations in MLL1, MLL2 and MLL3 genes in mice suggests that MLL proteins are not redundant in their function but instead are specialized to deal with the regulatory complexity of vertebrate development (29, 51, 56). Like Drosophila Trx, mammalian MLL1 and MLL2 have a role in long-term maintenance of Hox gene expression patterns that play important role in specifying cell fate during development and hematopoiesis (29, 51, 56).

In addition to the critical roles in histone methylation and gene regulations, rearrangements of the MLL1 is well known in a variety of aggressive human leukemias, including acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) (1,2, 20, 54). These rearrangements take several forms. The most common are balanced translocations in which the genomic sequences encoding the N-terminal portions of MLL1 are fused to sequences encoding the C-terminus of another translocation partner. Notably, chromosomal breakpoints are found in a consistent region of MLL1 (Figure 2) and more than 40 different MLL1 fusions have been described (23, 57-59). When these genes are transcribed, spliced, and translated, results in an in-frame fusion protein with oncogenic activity. The most frequent translocations in MLL1 are the t (4;11) and t (11;19) translocations, which are associated with the expression of MLL-AF4 and MLL-ENL, respectively, and a pro-B cell or mixed lineage phenotype (43, 44). At least 50% of cases of infant ALL harbor the t (4;11) translocation (43, 44). The frequent occurrence of the t (4;11) translocation in infant leukemia suggests that MLL-AF4 is unusually potent and less dependent on secondary genetic 'hits' for leukemia development. MLL2 and MLL3 are also frequently amplified in solid tumors, pancreatic carcinoma and glioblastomas (60, 61).

3.3. MLLs are present as multi protein complexes

Considering the importance in gene expression and disease, various researchers have isolated different MLL family of HMTs from human cells and characterized their protein composition and function (36, 41, 62-64). These studies demonstrated that MLL1, MLL2, MLL3, MLL4, Set1A, and Set1B are present in distinct multi-protein complexes inside the cells and each of these complexes shares several common subunits which includes Ash2, Rbbp5 (retinoblastoma binding protein 5) and Wdr5 (WD40 repeat containing protein) along with several unique components (Table 2). By sequence analysis it is established that Ash2, Rbbp5, Wdr5 and CGBP (human CpG binding protein, also known as CFP1) are human homolog of yeast Set1 complex components Bre2, Swd1, Swd3, Sdc1 and Spp1 respectively (36). Recently, using immuno affinity purification method, we have demonstrated that CGBP interacts with at least four different HMTs MLL1, MLL2, MLL3, and Set1 and is a core component of these functionally active HMTs (65 and unpublished observation). Similarly, Cho *et al.* (2007) showed Dpy30 (a dosage compensation protein) as a common interacting subunit of MLL1, MLL2, MLL3, MLL4, and Set1 complexes (66). Although, these protein complexes have been isolated, the detail functions of MLLs and their interacting components in histone methylation, gene expressions, and diseases still remain elusive.

3.4. MLL interacting proteins play critical roles in histone methylation and MLL target gene expression

The findings that there are at least five core components (Ash2, Rbbp5, Wdr5, Dpy30 and CGBP) shared by several human H3K4 specific HMT complexes and these core components are evolutionarily conserved from yeast to human, suggest that they play important roles in regulating MLL mediated histone methylation and target gene expression (see Figure 3 for scheme showing the roles of MLLs in transcription activation). Recently, Wysocka *et al.* (2005) demonstrated that MLL interacting protein Wdr5 is required for histone H3K4 trimethylation (67). Wdr5 binds preferentially to dimethylated H3K4 and knock down of Wdr5 results in decreased expression of MLL1 target genes HoxA9 and HoxC8 without affecting binding of MLL1 complexes to these target gene promoters (67). Moreover, independent knock down of Rbbp5 and Ash2 also reduced expression of HoxA9 and HoxC8 (67). Dou *et al. (*2005) showed that MLL1 recruitment to either the HoxA9 or HoxC8 locus was not disturbed upon knock down of either Wdr5, Rbbp5, or Ash2 (28, 41). These observations indicate that the core components Wdr5, Rbbp5 and Ash2 are not involved in MLL1 recruitment but regulate MLL1 associated histone methylation activity. Shilatifard and colleagues showed that Ash2 is also pivotal for trimethylation of MLL1 target gene promoters (40). The combined siRNA knock down results demonstrated that all three core components, Wdr5, Rbbp5 and Ash2, are needed for Hox gene expression and this requirement lies in the regulation of H3K4 dimethylation and trimethylation (40).

In contrast to Wdr5, Rbbp5, and Ash2, results from our laboratory demonstrated that antisense mediated knockdown of core component CGBP not only decreased

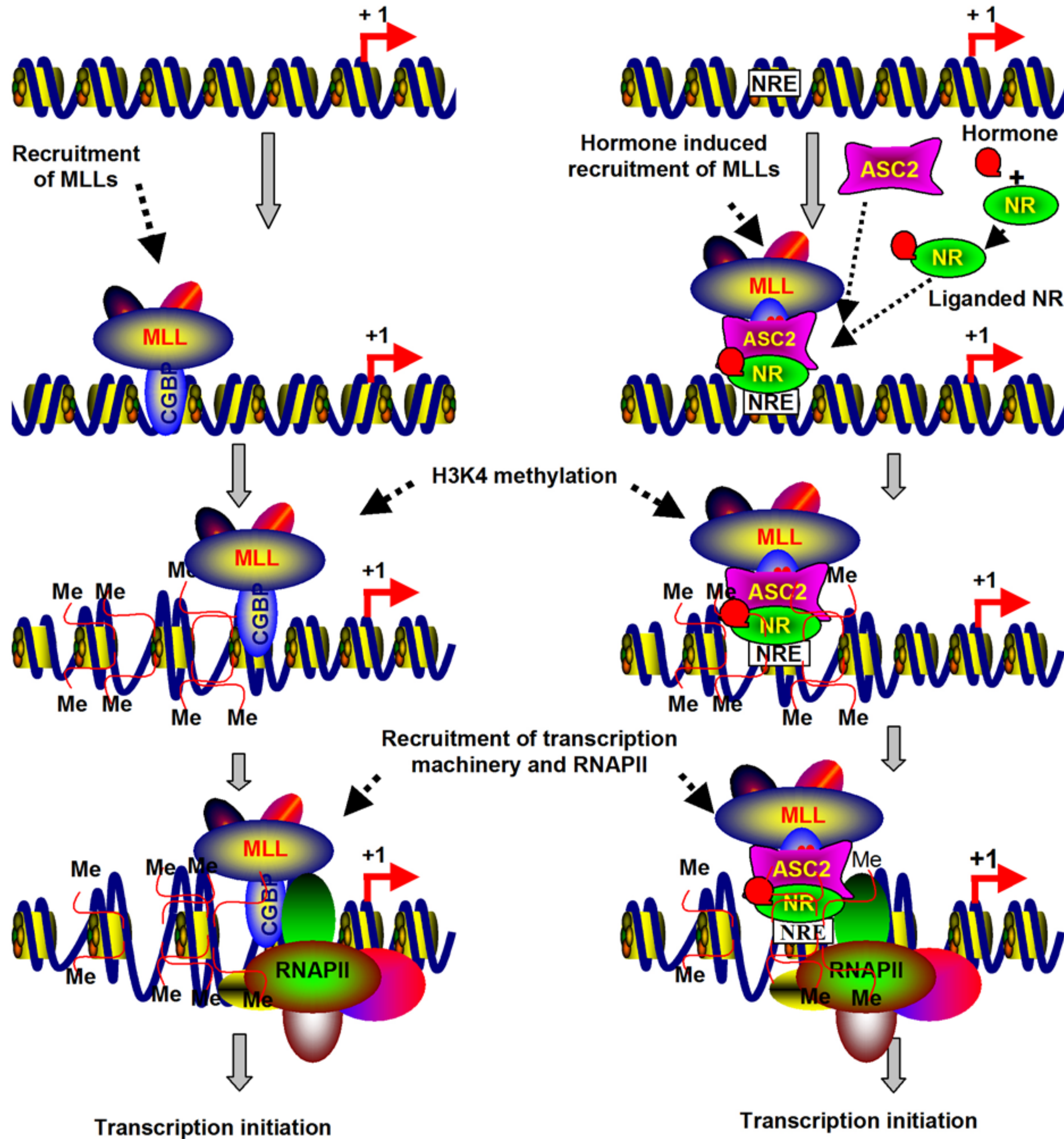

Figure 3. Schematic diagram showing the roles of MLLs in ligand dependent and independent gene activation. Left panels: Roles of MLLs in ligand independent target genes activation. Prior to transcription initiation, MLL complexes are recruited to the promoter of their target genes. The recruitment may be via direct interaction of MLL complexes with the promoter DNA or via interacting component such as CGBP that has DNA binding motifs. Once recruited, MLLs methylate H3K4 that help recruiting transcription machineries including RNA polymerase II leading to transcription initiation. Right panels: Roles of MLLs in trans-activation of hormone dependent genes. In presence of hormones, nuclear hormone receptors (NR) get activated and bind to the nuclear hormone responsive elements (NRE). Activated NRs interact with the MLLs in hormone dependent manner and get recruited to the promoter via interactions with NRs. MLLs may interact directly with NRs via its NRbox (LXXLL motifs) or via MLL interacting proteins such ASC-2 (or Menin, not shown in the figure) that contains NR boxes. Upon recruitments, MLLs may directly act as co-activator leading to trans-activation of the gene or MLL may introduce the H3K4 methylation marks in the promoter leading to gene activation.

the level of promoter H3K4 trimethylation and expression of MLL target HoxA7 gene but also abolished the recruitment of MLL1 into its promoter (65). Notably, CGBP has “AT-hook” and “CXXC znf” DNA binding

motifs and we hypothesize that these motifs play critical role in recruiting the MLL complexes into their specific target gene promoters (Figure 3) (65, 68, 69). Although recent studies have provided important insights into how histone methylation is regulated by bringing together specific HMTs and histone tails via interacting components such as Wdr5, Ash2, CGBP etc, their detail implication in the gene expression, cell differentiation, and development are poorly understood.

3.5. MLLs are key regulators of Hox genes

Hox genes are a group of evolutionarily conserved homeobox containing genes that play crucial roles in embryonic development. Orchestrated expression of Hox genes determines the positional tissue differentiation in higher organisms (70). Hox genes also express throughout postnatal life and play important roles in hematopoietic cell differentiation (71-73). The Hox genes encode a complex network of transcription regulatory proteins whose precise targets remain poorly understood. The overall function of the network appears to be dictated by gene dosage. Human encodes 39 Hox genes located in four different clusters (HoxA: HoxA1-7, 9-13; HoxB: HoxB1-9; HoxC: HoxC4-13 and HoxD: HoxD1, 3, 4, 8-13) on chromosomes at 7p15, 17p21, 12q13 and 2q31, respectively. Several developmental disorders have been traced to mutations in different Hox genes and most of the Hox genes (except that in cluster D) are expressed in hematopoietic stem cells.

Genetic studies in both mice and flies established that MLLs regulate Hox and other homeotic genes expression (56, 74). MLLs control the maintenance but not the initiation of expression of Hox genes during embryogenesis (75). Inactivation of the MLL gene in mice by homologous recombination (knock out) showed that MLLs are not only regulator of hematopoiesis but also are master regulator in embryonic development and hence disruption of MLL expression results in embryonic lethality (43, 55, 76). Studies showed that knock down of MLL results in Hox gene expression abnormalities especially the expression of HoxA7, HoxA9, HoxA10, HoxC4, HoxC8, and HoxC9 in whole embryos, fetal liver or in cultured human cells (43, 55, 65, 76-78). Analysis of the MLL super complex demonstrated direct binding of MLL1 to HoxA7, HoxA9 and HoxC8 promoters (65, 77, 78). Mice heterozygous for MLL-exon3LacZ allele display abnormal homeotic transformations with altered Hox gene expression. Disruption of MLL-exon3LacZ causes haplo-insufficiency with bidirectional homeotic transformations and shift anterior boundaries of several Hox genes (76). Using $MLL1^{-/-}$ deficient embryos and mouse embryonic fibroblasts it is demonstrated that MLL1 is required for the maintenance of selected Hox gene expression (56, 75, 79). Several studies targeting the mouse homologs of genes encoding fusion partners of MLL gave evidence that MLLs are regulators of cell differentiation. Reverse transcriptase PCR (RT-PCR) analysis of MPMP cell lines immortalized by five different MLL fusion proteins revealed a characteristic HoxA gene expression profile and found that all lines expressed HoxA7, HoxA9, HoxA10 and HoxA11 at the 5' end of the HoxA cluster. In contrast, 3' end HoxA genes were variably expressed with periodicity, as evidenced by low levels of HoxA1, higher levels of HoxA3 and HoxA5 and complete absence of HoxA2, HoxA4 and HoxA6 expression (80). Contrary to these findings the Taspase1 RNAi (Taspase1 is essential for cleavage of pre-MLL1 peptide to generate, functional of MLL1 protein) in HeLa cells led to diminished expression of selected Hox genes across the HoxA cluster (47-49, 81). Knockdown of Taspase1 diminished the expression of the 3′ located and “earlier” expressed genes in the HoxA cluster (A1, A3, and A4), but not those genes located towards more 5′ and expressed “later” during embryonic development (A5, A9 and A10) (47-49, 81). This selected attenuation contrasts with the global decrease in expression of most HoxA genes (A1 to A10) in cells with MLL knocked down. These data support the importance of Taspase1 in the correct expression of the early HoxA gene cluster (47-49, 81).

In agreement with genetic studies and mutational analysis, direct interaction of MLL complex with the HoxA7, HoxA9 and HoxC8 promoters indicates important roles of MLL in Hox gene regulation (63, 65, 78, 82). The distinctive role of MLLs in regulating different Hox genes is likely to be mediated through differential histone methylation and effects on chromatin structure (63, 65, 78, 82).

3.6. MLLs interact with nuclear hormone receptors and regulate hormone dependent gene expression

Nuclear receptors (NRs) are large family of transcription factors that are responsible for sensing the presence of hormones and have the ability to access their binding sites in the repressive chromatin (Figure 3) (33, 50-52). Nuclear receptors share a common structural organization, which include a central DNA binding domain (DBD) responsible for targeting the receptor to highly specific DNA sequences comprising a response element (33, 50-52). The ligand binding domain (LBD) is present in the C-terminal half of the receptor and recognizes specific hormonal and non-hormonal ligands directing specificity to the biological response. During ligand binding the conserved C-terminal AF2 (present in LBD) domain undergoes structural changes that are recognized by a helical LXXLL motif (NR box) present in the transcriptional co-activators and functionally convert NRs to a transcriptional activator (33, 50-52) (Figure 3). Numerous co-regulatory proteins (co-activators and co-repressors) have been identified that control transcriptional regulation via interaction with nuclear receptors.

Activating signal cointegrator-2 (ASC-2) is a co-activator of multiple nuclear receptors including retinoic acid receptors (RAR) and estrogen receptors (ER) (33, 50, 51). ASC-2 is present as a multi-protein complex known as ASCOM (ASC-2 complex) (30). In addition to ASC-2, ASCOM contains histone methyl-tranferases such as MLL3, MLL4 and MLL interacting proteins Rbbp5, Wdr5, Ash2 etc. ASC-2 interacts with RAR via its LXXLL domain and is recruited to the promoter of RAR target genes along with other ASCOM components (30). ASC-2 acts as an adaptor for RAR-dependent recruitment of MLL3 and MLL4 to RAR target genes (51, 52). Using a

mice model Lee *et al.* (2006) showed that the mutation in SET domain or ASC-2-null mutation in embryonic fibroblasts impaired H3K4 trimethylation of RAR target gene RAR-beta-2 (51, 52). Similarly, knock down of both MLL3 and MLL4 impaired H3K4 trimethylation in the RAR-beta-*2* promoter in embryonic fibroblasts (51, 52). Similar roles of ASC-2 have been reported in case of transcriptional regulation of liver X receptors (LRX), where ASC-2 is required for ligand induced recruitment of MLL3 and MLL4 to LXRs target genes promoters. LXR ligand T1317 induces not only expression of LXR-target genes but also their H3K4-trimethylation. Strikingly, both of these ligand effects are ablated in ASC-2-null mutant cells but only partially suppressed in cells expressing an enzymatically inactive mutant MLL3. These observations indicate that ASC-2 is critical for the ligand dependent trans-activation of RAR and LXR target genes **(**51, 52**).**

In addition, the findings that MLL3 and MLL4 are found in distinct ASC-2 containing complexes and are separately recruited to RAR-beta-2 by ASC-2, suggest that MLL3 and MLL4 are present in different ASCOM complexes and ASC-2 confers target gene specificity to MLL3 (ASCOM-MLL3) and MLL4 (ASCOM-MLL4) complexes (51, 52). The ASCOM-MLL3 and ASCOM-MLL4 complexes play redundant but essential roles in ligand-dependent H3K4 trimethylation and expression of NR-target genes, and ASC-2 is likely a key determinant for NR to function through ASCOM. Notably, MLL3 and MLL4 are also found to be present as ASC-2 independent H3K4 HMT complexes, although their detail functions still remain elusive.

In addition to the association of MLLs in ASCOM complex, MLL2 and MLL interacting protein Menin are shown to interact directly with NR (51, 52). MLL2 as well as Menin contain multiple LXXLL motifs through which they interact with estrogen receptors (ER-alpha) and regulate estrogen responsive genes. SiRNA mediated knockdown of MLL2 impaired ER-alpha mediated transactivation of estrogen induced genes such as cathepsin D and to a lesser extent pS2 and inhibited cellular growth (32, 33). Moreover, truncated MLL2 fragment, which only contains the region necessary for binding to ER-alpha, acts as a potent dominant-negative inhibitor of the ER-alpha trans-activation. Similarly, Menin serves as a critical link between activated ER-alpha and H3K4 trimethylation in case of transcription of the estrogen-regulated TFF1 gene (33, 50, 51). Additionally, Menin as an integral component of MLL complex links MLL1/MLL2 complexes to activated nuclear receptors. In addition, as MLL2 contains several N-terminal ring finger domain (Figure 2) that are often associated with ubiquitin ligases, it may also be linked with the degradation of ER-alpha and it is well established that the activation of ER-alpha is coupled with its degradation (83). Notably, histone methylases MLL3 and MLL4 that also contain multiple NR boxes, are implicated in estrogen receptor mediated gene activation (84).

3.7. MLL and cell cycle regulation

The cell cycle is a vital process by which cells divide into daughter cells. The cell cycle consists of four distinct phases: G1 (gap 1), S (synthesis), G2 (gap 2) and M (mitosis) phases. M phase is composed of two tightly coupled processes: mitosis, in which the chromosomes are divided between the two daughter cells and cytokinesis, in which the cytoplasm divides forming distinct cells. Proper maintenance and progression of these stages are critical for normal cellular process (85). Cyclin family of proteins which function as the regulatory subunit of cyclin dependent kinases (CDK) regulate the progression of cell cycle by varying their levels of gene expression. For example, cyclin D shows a continuous expression in all the different phases and thus controls the entry and progression throughout the cell cycle. Cyclin E is required for the transition from G1 to S phase while cyclin A helps the cell to move from S to G2 phase. Cyclin B is responsible for the cell to reach mitosis (85).

3.7.1. MLLs interact with cell cycle regulatory proteins

Numerous evidences indicate that MLLs play crucial role in cell cycle progression (Figure 4) (41, 49, 86, 87). The functional roles of MLLs in cell cycle regulation are supported by the observation that the knock out of Taspase1 results in down-regulation of Cyclin E, A and B and up-regulation of p16 (a S phase inhibitor) (49). Notably, Taspase1, a threonine endopeptidase, cleaves MLL at conserved sites to generate N-terminal 320-kDa (MLL^N) and C-terminal 180 kDa (MLL^C) fragments. Proteolysis of MLL1 by Taspase1 is required to fully activate its HMT activity. Using chromatin Immuno-precipitation (ChIP) experiments, Takeda *et al.* (2006) demonstrated that MLL1 is occupied in the promoters of cyclin E1 and E2 genes and there was a marked reduction in H3K4 trimethylation level as well as the MLL occupancy at the cyclin E1 and cyclin E2 promoters in Taspase1 negative cells (49). Taspase1 deficiency also resulted in upregulation of S-phase inhibitor protein p16 (49).

Similar to cyclins, E2F family proteins (E2F1-E2F6) are key transcription factors responsible for activation of cell cycle related genes including cyclins (41, 49, 86, 87). Using immuno-precipitation experiments, Takeda *et al.* (2006) demonstrated that MLL1 interacts with several E2Fs (such as E2F2, E2F4 and E2F6), while MLL2 interacts with a different subset of E2Fs such as E2F2, E2F3, E2F5 and E2F6 (49). In an independent experiment, Dou *et al. (*2005) performed the immuno-purification of Wdr5 associated HMTs from human cells and demonstrated that Wdr5 interacts with MLLs along with E2F6 (41). Distinct interactions between E2Fs and MLLs suggest potential roles of MLL proteins in the cell cycle regulation (41). In addition, over expression of MLL5 induced cell cycle arrest in G1 phase and knock down of MLL5 resulted in cell cycle arrest both at G2/M and G1 phases (88, 89). These studies indicate that MLL families of HMTs are critical for cell cycle regulation.

In addition to MLLs, MLL interacting proteins are also shown to be linked with cell cycle regulation. Milne *et al* (2004) showed that Menin, in a cooperative interaction with MLLs, regulates the expression of CDK inhibitors like p27 and p18 (90). Menin activates

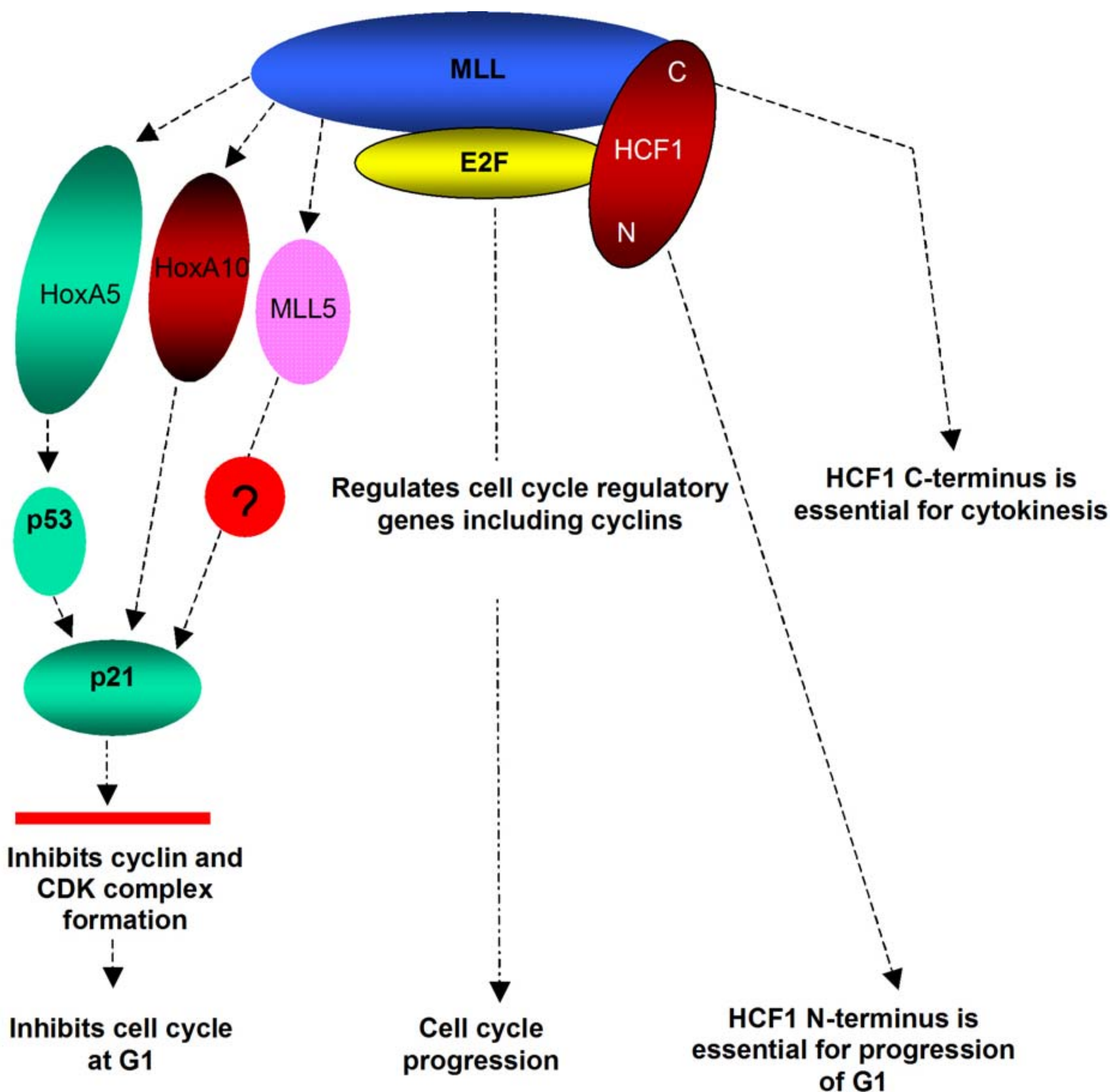

Figure 4. Roles of MLLs in cell cycle regulation. MLL target genes HoxA5 and HoxA10 inhibit cell cycle at G1 phase through upregulation of CDK inhibitor p21. MLL5 inhibits cyclin-CDK complex possibly involving p21. MLLs interact with E2Fs and regulate differential expression of cyclins that regulate proper cell cycle progression. HCF1, a MLL interacting protein, is involved in progression of G1. The C-terminal of HCF1 is essential for proper cytokinesis.

transcription of p27 and p18 by recruiting MLL1 to their promoters and knock down of Menin resulted in down regulation of p27 and p18 expression leading to deregulated cell growth and tumorigenesis (90). HCF-1 (host cell factor 1) is another critical protein that interacts with MLLs and Set1 (64). Knock down of HCF-1 results in dinucleated cells and inhibits cell divisions by arresting the cells in G1 phase (87). HCF1 c-terminal domain has been linked with this dinucleated cells implicating its roles in cytokinesis. Furthermore, using ChIP experiments Tyagi *et al.* (2007) demonstrated that HCF-1 recruits MLL1 and Set1 to E2F responsive promoters during G1 to S phase transition and induces histone methylation and transcriptional activation (87). Association of HCF-1 and E2F are versatile and binding to E2F responsive promoters are cell cycle selective (87). HCF-1 binds both to activator E2Fs like E2F1 and E2F3a and repressor E2Fs like E2F4. Thus, HCF-1 plays dual role both in induction and suppression of vital cell cycle regulatory proteins. HCF-1 selectively associates with activator H3K4 HMT complexes, when bound to E2F1 to activate transcription. It acts as a repressor in association with Sin3 HDAC complex when bound to E2F4 (87).

Our recent studies demonstrated a defined dynamics of MLL histone methylases during cell cycle.

MLL1 which is normally associated with transcriptionally active chromatins (in G1 phase), dissociated from condensed mitotic chromatin, migrated away to the cytoplasm and returned back at the end of telophase when nucleus starts to relax (unpublished observation). However, the global level of MLL1 does not get affected. In contrast, Liu *et al.* (2008) demonstrated a biphasic expression of MLL1 conferred by defined windows of degradation mediated by specialized cell cycle specific E3 ligases (48). They found that MLL1 is marked by SCFSkp2 and APCCdc20 for degradation at S phase and late M phase respectively. They also found that abolished peak expression of MLL1 incurs corresponding defects in G1/S transition and M-phase progression while over expression of MLL1 blocks S-phase progression (48). Their data indicated that a post-translational regulation of MLL1 by the cell cycle ubiquitin/proteasome system (UPS) assures the temporal necessity of MLL1 in coordinating cell cycle.

3.7.2. MLL target Hox genes in cell cycle regulation

The role of MLLs in cell cycle is further emphasized by the involvement of MLL regulated Hox genes in cell cycle. Recent studies demonstrated that HoxA5 activates p53, which is known to regulate the expression of p21, an inhibitor of CDK enzymes that is critical for cell cycle progression (91, 92). Furthermore, Bromleigh and Freedman (2000) showed that Hox A10 directly upregulates the expression of p21 leading to cell cycle arrest at the G1 phase in both monocytic and fibroblast cell lines (92). It is interesting to note that both p21 and p53 plays vital roles in cell cycle regulation. p21 is a CDK inhibitor which inhibits the protein kinase activity of cylin-CDK2 and cyclin-CDK4 complexes. Thus, p21 functions as a regulator of cell cycle progression at G1 phase. p53 is responsible for holding cells at G1/S transition by regulating the expression of p21 and activating the DNA repair proteins when DNA damage is recognized in the cell. It is also responsible for initiating apoptosis if the DNA damage is found to be irreparable. Thus, we hypothesize that HoxA5, like HoxA10, regulates cell cycle through p53 and p21 channel. Also recently, Qian *et al.* (2005) showed that HoxA10 is responsible for differentiation of human endometrial stromal cells under progesterone treatment by regulating the expression of another G1 inhibitor p57 (93). They observed a lower expression of HoxA10 when the level of p57 is high under progesterone application. Lu *et. al. (*2008) further reported that a higher percentage of cells arrested at the G2/M phase of cell cycle under the application of siRNA against HoxA10. While the level of apoptosis was insignificant, the number of cells arrested in G1 stage was considerably lower than the number of cells arrested in G2/M phase (94).

In our laboratory we synchronized cells in G1/S phase by the application of double thymidine treatment procedure and analyzed the expression pattern of HoxA10 at different stages of cell cycle and found that the level of expression for HoxA10 is very high in the S phase which goes significantly down in the G2/M phase and completely absent in G1 phase (unpublished observations). Most importantly down regulation of selected MLLs resulted in cell cycle arrest and inhibits cellular growth (unpublished observation). Although, the detail roles of MLLs and their interacting proteins in cell cycle regulation is still not clear, multiple lines of evidence indicate that MLLs play critical role in regulation of cell cycle and cell cycle regulatory genes (Figure 4).

4. DISCUSSION

Understanding the functions of histone H3K4 methylation is crucial to uncover the complex gene regulatory network and underlying molecular mechanism of various human diseases. H3K4 methylation marks are well recognized as marks for transcriptionally active chromatins. The role of H3K4 methylation is well studied in yeast, but the same is not very clear in higher eukaryotes (95). Set1 is the sole H3K4 specific HMT present in yeast. In contrast, human encodes several yeast Set1 homologs (such as MLL1, MLL2, MLL3, MLL4, Set1A and Set1B) suggesting increased complexity of these enzymes and their functions in regulating gene expression (36, 39, 67). Although, recent discoveries of MLL associated HMT activities and their interacting proteins shed significant light in the complex function of MLL in gene regulation, the distinct functions of different MLLs are still not clear. Increasing amount of evidence indicate that different MLLs form distinct interactions with proteins involved in nuclear receptor mediated gene activation, cell cycle regulation or with chromatin remodeling (Figures 3 and 4). These findings suggest that different MLLs are involved in different specialized functions, although further investigations are need to fully elucidate their mechanisms (Figures 3 and 4).

Although, at this point, significant efforts are being invested in understanding the functions of MLL proteins in target gene regulation and disease, little is known about their own regulation in normal/cancer cells or under stress. Recent studies from our laboratory suggest that MLLs are miss-regulated under toxic and carcinogenic stress establishing a novel link between MLL, stress response and carcinogenesis (unpublished observation). With the present pace of investigations by different laboratories, we hope that within next decade or so, we will have lot more understanding about the complex function of MLLs in gene regulation, stress response and disease that will help in developing novel therapy.

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