REVIEW

The diverse functions of Dot1 and H3K79 methylation

Anh Tram Nguyen \(^1\,^2\) and Yi Zhang \(^1\,^2\,^3\)

\(^1\)Howard Hughes Medical Institute, Chevy Chase, Maryland 20815, USA; \(^2\)Department of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA

**DOT1** (disruptor of telomeric silencing; also called Kmt4) was initially discovered in budding yeast in a genetic screen for genes whose deletion confers defects in telomeric silencing. Since the discovery ~10 years ago that Dot1 and its mammalian homolog, DOT1L (DOT1-Like), possess histone methyltransferase activity toward histone H3 Lys 79, great progress has been made in characterizing their enzymatic activities and the role of Dot1/DOT1L-mediated H3K79 methylation in transcriptional regulation, cell cycle regulation, and the DNA damage response. In addition, gene disruption in mice has revealed that mouse DOT1L plays an essential role in embryonic development, hematopoiesis, cardiac function, and the development of leukemia. The involvement of DOT1L enzymatic activity in leukemogenesis driven by a subset of MLL (mixed-lineage leukemia) fusion proteins raises the possibility of targeting DOT1L for therapeutic intervention.

Eukaryotic DNA is wrapped around a histone octamer (H3/H4 heterotetramer and two H2A/H2B dimers) to form the nucleosome, the fundamental building block of chromatin [Van Holde et al. 1980; Lugner et al. 1997; Kornberg and Lorch 1999; Zhang and Dent 2005]. Histone proteins are subject to reversible post-translational modifications, which play an important role in the establishment and maintenance of gene expression patterns within any given cell type of an organism. Studies in the past decade have established that covalent histone modifications influence chromatin structure and function directly or indirectly through the recruitment of effector proteins to specific chromatin regions [Strahl and Allis 2000; Martin and Zhang 2005; Kouzarides 2007].

One of the covalent histone modifications is methylation, which takes place on both lysine (K) and arginine (R) residues [Zhang and Reinberg 2001; Martin and Zhang 2005]. Lysine methylation exists in mono, di, and tri states, while arginine methylation only occurs in mono and di states. One way that these methyl marks contribute to transcriptional regulation is to serve as a platform for the recruitment of effector proteins. The well-studied lysine methylation residues include K4, K9, K27, K36, and K79 of histone H3, and K20 of histone H4. In general, methylation at H3K9, H3K27, and H4K20 correlates with transcriptional repression, while methylation at H3K4, H3K36, and H3K79 correlates with gene transcription [Kouzarides 2002; Peterson and Laniel 2004; Martin and Zhang 2005]. In addition to its role in transcriptional regulation, methylation has also been linked to X inactivation, cell fate determination, terminal differentiation, and the spatiotemporal patterning of Hox genes [Cavalli 2006; Minard et al. 2009]. Moreover, aberrant histone methylation has also been linked to various human cancers [Feinberg et al. 2002; Handel et al. 2010].

Histone methylation is catalyzed by a group of histone methyltransferases. Based on their catalytic domains, the lysine methyltransferases (KMTs) that have been characterized to date can be divided into two classes. The first class contains an evolutionarily conserved SET {Su(var)3-9, Enhancer of Zeste \([E(Z)]\), and Trithorax (trx)} domain [Jenuwein et al. 1998]. In contrast, the second class does not possess a SET domain and consists of only an evolutionarily conserved protein named Dot1 (disruptor of telomeric silencing; also called Kmt4) [Singer et al. 1998] and its homologs in other organisms [Feng et al. 2002; Lacoste et al. 2002; van Leeuwen et al. 2002]. Dot1 and its homologs contain a catalytic methylase fold resembling that of class I methylases [Ng et al. 2002a; Min et al. 2003; Schubert et al. 2003]. Over the years, great progress has been made in elucidating the function of Dot1 and its associated H3K79 methylation mark in various organisms and cellular processes. Here we summarize these studies with an emphasis on Dot1’s enzymatic activity and its diverse biological functions.

**Dot1/DOT1L (DOT1-Like) enzymatic activity and its regulation**

*Dot1 and its homologs possess H3K79 methyltransferase activity*

Using different approaches, several groups independently discovered that Lys 79 within the globular domain of histone H3 [H3K79] is subject to methylation, and that
the yeast protein Dot1 and its human homolog, DOT1L, are responsible for catalyzing the methylation reaction (Feng et al. 2002; Lacoste et al. 2002; Ng et al. 2002a; van Leeuwen et al. 2002). Both enzymes are capable of catalyzing mono-, di-, and trimethylation in a nonprocessive manner (Min et al. 2003; Frederiks et al. 2008). Dot1 and its homologs appear to be solely responsible for H3K79 methylation, since knockout of Dot1 in yeast, flies, and mice results in complete loss of H3K79 methylation (van Leeuwen et al. 2002; Shanower et al. 2005; Jones et al. 2008).

Dot1 homologs share a conserved region with four sequence motifs—I, post I, II, and III—found in SAM methyltransferases (Fig. 1A). The crystal structures of both yeast Dot1 and human DOT1L also reveal an open \( \alpha/\beta \) structure composed of a seven-stranded \( \beta \) sheet that

![Figure 1](image-url)

**Figure 1.** Dot1 is a conserved class I SAM-dependent methylase. (A) Sequence alignment of the conserved catalytic core region of Dot1 across multiple species that include yeast (NP_010728), worm (NM_058569), fly (AE003675), mouse (BB678539), and human (NP_115871). Secondary structure elements are shown above the alignment. The dashed line indicates disordered regions. Invariant amino acids are shown in white letters against a gray background, while conserved residues are in bold. Amino acids conserved across all five species are in bold against a green background. Every 10 amino acids is marked by a plus sign. The conserved methyltransferase fold motifs—I, post I, II, and III—are labeled and boxed in red. (B) The crystal structure of the substrate-binding pocket of human DOT1L [Protein Data Bank [PDB] code 1NW3] demonstrates that it is more similar to that of rat PRMT1 [PDB code 1ORI], a class I arginine methyltransferase, than to the KMT hNSD1 [PDB code 3OOI].
is characteristic of the class I SAM-dependent methyltransferases [Fig. 1B; Cheng and Roberts 2001; Min et al. 2003; Sawada et al. 2004]. Despite the fact that both human and yeast Dot1 proteins are structurally more similar to arginine methyltransferases [Fig. 1B], members of the Dot1 family proteins catalyze histone lysine methylation rather than arginine methylation [Feng et al. 2002, Lacoste et al. 2002, van Leeuwen et al. 2002; Zhang et al. 2004; Shanower et al. 2005]. Due to the structural similarities between Dot1 and arginine methyltransferases [Fig. 1B], there has been some speculation as to whether Dot1 can also catalyze arginine methylation. However, extensive efforts using reverse-phase high-performance liquid chromatography (HPLC) coupled with nano-liquid chromatography electrospray ionization mass spectrometry (LC-ESMS) and tandem mass spectrometry [MS/MS] have failed to demonstrate that Dot1 has arginine methyltransferase activity [van Leeuwen et al. 2002].

Interplay between H2B ubiquitination and H3K79 methylation

Characterization of DOT1L enzymatic activity indicated that it preferentially methylates H3K79 in the context of nucleosomes rather than core histones or recombinant H3 [Feng et al. 2002]. This observation suggests that DOT1L may recognize other features of the nucleosome. Based on nucleosome structure, K79 lies within loop 1 of histone H3, which is solvent-accessible and lies adjacent to the interface between the H3/H4 tetramer and H2A/H2B dimer [Luger et al. 1997]. This unique position of H3K79 and the preference of DOT1L for nucleosomal substrates suggested that trans-histone cross-talk may play a role in regulating DOT1L enzymatic activity, which indeed turned out to be the case.

In yeast, histone H2B can be monoubiquitinated at Lys 123 [H2B-K123ub]. This modification is catalyzed by the ubiquitin-conjugating E2 enzyme Rad6 and the ubiquitin E3 ligase Bre1 [Robzyk et al. 2000; Wood et al. 2003a]. Bre1 not only serves as an E3 ligase for H2B ubiquitination, but is also required for the recruitment of Rad6 to chromatin [Wood et al. 2003a]. Interestingly, both H3K79 and H2B-K123 lie in proximity on the same exposed nucleosome surface [Luger et al. 1997], which provides a structural basis for an interplay between the two modifications (Fig. 2A). Consistently, deletion of Rad6 and members of the Paf1 complex not only blocked H2B ubiquitination, but also prevented H3K4 and H3K79 methylation [Briggs et al. 2002, Ng et al. 2002b, Sun and Allis 2002]. Furthermore, mutation of H2B-K123 also prevented H3K79 and H3K4 methylation, indicating that H2B ubiquitination is a prerequisite for H3K79 and H3K4 methylation [Briggs et al. 2002, Ng et al. 2002b, 2003b; Sun and Allis 2002; Krogan et al. 2003; Wood et al. 2003b]. This effect appears to be unidirectional, as deletion of Dot1 does not affect H2B ubiquitination (Sun and Allis 2002). Additionally, the relationship between H2B ubiquitination and histone methylation is residue-dependent, as H3K36 methylation is not affected by Rad6 deletion [Briggs et al. 2002, Ng et al. 2002b].

The critical role of H2B ubiquitination in mediating H3K79 methylation raised the question of how H2B

Figure 2. Ubiquitination of yH2B-K123/hH2B-K120 regulates Dot1-mediated H3K79 methylation. (A) The crystal structure of the human nucleosome [PDB code 3AFA] is shown on the left. Histone H2A is shown in light green, H4 is shown in sand, H2B is shown in hot pink, and H3 is shown in blue. A close-up view of the nucleosome surface is on the right, with the location of H2B-K120 shown as yellow spheres and H3K79 shown as green spheres. Note that H2B-K120 and H3K79 are located in proximity on the same solvent-exposed surface of the nucleosome. (B) Proposed models for trans-histone regulation of Dot1 enzymatic activity by H2B-K123ub. Histone H2B-K123 ubiquitination is mediated by the ubiquitin-conjugating enzyme Rad6 and its E3 ubiquitin ligase, Bre1. Components of the Paf1 complex are required for efficient H2B ubiquitination and di- and trimethylation of H3K4 and H3K79. (Bottom left) The first model suggests that Dot1 can bind directly to H2B-K123ub for intranucleosomal H3K79 methylation. (Bottom right) The second model suggests that other factors, such as the Set1-containing COMPASS, are required to bridge the interaction between Dot1 and the nucleosome. Rtf1 of the Paf1 complex recruits the COMPASS complex to methylate H3K4. The Cps35 subunit of the COMPASS interacts directly with H2B-K123 and Dot1, thus bringing Dot1 to the nucleosome for H3K79 methylation. (Bottom right) In the third model, ubiquitination of H2B causes a conformational change to the nucleosome, making H3K79 more accessible to DOT1.
ubiquitination contributes to H3K79 methylation. Three mutually nonexclusive possibilities have been proposed. They include (1) indirect interaction between Dot1 and H2B-K123ub through a bridging protein, (2) direct interaction between Dot1 and H2B-K123ub, and (3) that ubiquitination of H2B causes chromatin structure changes to facilitate Dot1-mediated H3K79 methylation. Published evidence supports the use of all three mechanisms. For example, H2Bub can recruit Rpt4 and Rpt6 to chromatin to facilitate H3K79 methylation (Ezhkova and Tansey 2004). In addition, Cps35, a component of the Set1 [also called Kmt2]-containing COMPASS complex, interacts directly with both H2B-K123ub and Dot1 and is required for H3K79 trimethylation [Lee et al. 2007]. These studies suggest that ubiquitinated H2B indirectly recruits Dot1 to methylate H3K79 through some bridging factors [Fig. 2B, top right]. On the other hand, Dot1 can interact directly with ubiquitin through a lysine-rich region (amino acids 101–140) within the N terminus of Dot1, which is also required for nucleosome binding, and deletion of this region resulted in reduced di- and trimethylation [Oh et al. 2010]. In addition, chemically engineered ubiquitinated H2B has been shown to stimulate intra-nucleosomal H3K79 methylation by Dot1L without the presence of other bridging factors [Fig. 2B, bottom left; McGinty et al. 2008; Chatterjee et al. 2010]. Finally, in support of the third mechanism, it was shown that ubiquitination of H2B can disrupt chromatin compaction and interchromatin fiber interactions to increase substrate accessibility [Fig. 2B, bottom right; Fierz et al. 2011]. This contradicts a previous study demonstrating that H2B ubiquitination regulates nucleosome stability rather than chromatin conformation (Chandrasekharan et al. 2009). The discrepancy between the two studies may be caused by the use of different substrates. Muir and colleagues [Fierz et al. 2011] used chemically engineered and reconstituted nucleosomal substrates, whereas Sun and colleagues (Chandrasekharan et al. 2009) used modified nucleosomes purified from different mutant yeast strains. In summary, current literature supports the proposal that several mutually nonexclusive mechanisms, illustrated in Figure 2B, are used for the regulation of H3K79 methylation by H2B ubiquitination.

Reversibility of H3K79 methylation

Although the establishment of H3K79 methylation is well characterized and is highly regulated by multiple processes, active demethylation of this mark is less well understood. Thus far, with the exception of H3K79, all other known histone lysine residues subject to methylation can be demethylated by at least one histone demethylase ([Klose et al. 2006; Klose and Zhang 2007; Cloos et al. 2008; Liu et al. 2010; Qi et al. 2010]. However, several lines of evidence suggest that H3K79 methylation might be reversible, as it is subject to dynamic regulation. First, in yeast and human cells, the H3K79me2 methylation level fluctuates with the cell cycle [Feng et al. 2002; Schulze et al. 2009]. Second, H3K79me2 is demethylated during early embryonic development in both mice and flies, suggesting that an inactive demethylase might reside in mature oocytes [Shanower et al. 2005; Ooga et al. 2008]. Third, a study investigating the antagonistic effects of 2-hydroxyglutarate (2-HG) on α-KG (a cofactor for dioxygenases that include the JmJC domain-containing histone demethylases and the TET family of 5-methylcytosine hydroxylases) demonstrated that global levels of H3K79me2 increased in the presence of synthetic 2-HG and mutant isocitrate dehydrogenase (IDH), a NADP-dependent enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-KG. These observations suggest that a dioxygenase may catalyze the removal of H3K79me2 marks in vivo [Xu et al. 2011]. We anticipate that it may not be too long before the identity of this elusive H3K79-specific demethylase is revealed.

Dot1-mediated H3K79 methylation in transcriptional regulation

Dot1 and telomeric silencing in Saccharomyces cerevisiae

Four years prior to the characterization of Dot1’s enzymatic activity, DOT1 was identified in a genetic screen for genes whose overexpression disrupts telomeric silencing. The establishment of telomere and telomere-proximal DNA silencing is achieved through the recruitment and binding of Sir (silent information regulator) proteins [Norris and Boeke 2010]. Interestingly, overexpression and deletion of Dot1, as well as mutation of H3K79, compromise the silencing at telomeres and HML/HMR loci. In all three cases, the level of Sir proteins bound at telomeres is reduced, thus limiting their ability to silence genes. Sir3 interacts with H3K79 through its N-terminal BAH domain, and this interaction is impeded by H3K79 methylation, and, consequently, Sir3 binding and spreading of heterochromatin is affected [Katan-Khaykovich and Struhl 2005; Onishi et al. 2007; van Welsem et al. 2008]. Reciprocally, Sir3 inhibits H3K79 methylation by competing with Dot1 for binding to the same basic patch of histone H4 that is required for Dot1 enzymatic activity [Altaf et al. 2007; Fingerman et al. 2007]. Thus, balancing the amount of Sir protein binding and the level of H3K79 methylation is important for regulating heterochromatin formation at telomeres.

We note that, in these previous studies, reporter systems were used to draw general conclusions about Dot1-mediated H3K79 methylation and telomere silencing. Interestingly, two recent studies challenge the role of Dot1 in heterochromatin formation. Employing the URA3 telomere reporter system, Rossmann et al. [2011] demonstrated that the variegated silencing observed in Dot1 mutants is due to a disproportion in ribonucleotide reductase and the promoter of URA3 at telomere VII–L rather than a requirement for Dot1 in general telomere silencing. Takahashi et al. [2011] took an alternative approach by analyzing natural telomere position–effect variegation and show that the role of Dot1 in heterochromatin formation is telomere-specific. Nevertheless, interplay between Sir proteins and Dot1 does serve to
regulate heterochromatin formation at telomeres, but perhaps to a lesser extent than originally thought.

Genome-wide analysis links H3K79me to active transcription

In addition to participating in telomere silencing, genome-wide analysis of H3K79 methylation has linked this modification to transcription. Approximately 10% of the yeast genome is hypomethylated at H3K79 and bound by Sir proteins [Laurenson and Rine 1992, van Leeuwen et al. 2002], which is consistent with the fact that methylated H3K79 inhibits Sir binding [Onishi et al. 2007; van Welsem et al. 2008]. Meanwhile, 90% of the yeast genome bears H3K79 methylation and is actively transcribed [Laurenson and Rine 1992, van Leeuwen et al. 2002], suggesting that H3K79 methylation is a marker of euchromatin. In support of this notion, work by Ng et al. (2003a) showed that H3K79 methylation correlates with euchromatin at sites of active V(D)J recombination, while hypomethylation is found at inactive loci.

The development of high-throughput technology to map specific chromatin modifications on a genome-wide scale has provided an invaluable method to further decipher the role of H3K79 methylation in transcriptional regulation. In Drosophila, chromatin immunoprecipitation (ChIP) coupled with gene expression microarray (ChIP-chip) revealed that histone acetylation, H3K4me2, H3K4me3, and H3K79me2 positively correlated with active gene transcription [Schubeler et al. 2004]. In contrast, another study using ChIP–chip demonstrated in S. cerevisiae that, although H3K79me3 is enriched within transcribed gene regions, there was no correlation between the level of H3K79me3 and transcriptional activity [Pokholok et al. 2005]. This was further supported by Zhao and colleagues [Barts et al. 2007] using ChIP coupled with deep sequencing (ChIP-seq) to map mono-, di-, and trimethyl H3K79 in human CD4+ T cells. They found a slight correlation between H3K79me1 and gene activation. However, H3K79me3 was more closely associated with gene repression, while H3K79me2 did not correlate with transcriptional status [Barts et al. 2007]. These two genome-wide analyses were inconsistent with previous studies linking H3K79 methylation to active gene transcription [Ng et al. 2003a; Schubeler et al. 2004; Okada et al. 2005, 2006; Vakoc et al. 2006].

The discrepancy between different genome-wide profiling studies was later attributed to different methods used for chromatin preparation. The two reports showing no correlation between H3K79 methylation and transcriptional activity performed ChIP in the absence of sodium dodecyl sulfate (SDS), which is required for proper detection of H3K79 methylation in formaldehyde-cross-linked chromatin [Steger et al. 2008]. Accordingly, ChIP–chip using mouse 3T3 cells demonstrates that all H3K79 methylation marks are localized within the body of transcribed genes, and that the amount of enrichment correlates with expression level [Steger et al. 2008]. Subsequently, following the revised ChIP protocol, Zhao and colleagues [Wang et al. 2008] published a second genome-wide study, again using human CD4+ T cells, which confirmed the findings by Steger et al. (2008). Collectively, analyses of yeast, mouse, fly, and human genomes revealed that H3K79 methylation is indeed a marker of active transcription.

Role of Dot1L in transcriptional elongation

The genome-wide localization studies revealed that H3K79 methylation is enriched in gene coding regions [Kouskouti and Talianidis 2005], implicating a potential role for Dot1 in transcriptional elongation. Consistent with this notion, the Paf1 complex, which is associated with elongating RNA polymerase II [Pol II], regulates Dot1 H3K79 methyltransferase activity [Krogan et al. 2003]. In addition, involvement of DOT1L in transcriptional elongation is supported by the purification of various mammalian DOT1L-containing complexes, which are summarized in Table 1.

The first DOT1L-associated complex, termed ENL-associated proteins (EAPs), was purified by immunoprecipitation using antibodies against ENL. In addition to ENL, the complex contains A4F, AF5q31, LAF4, DOT1L, P-TEFb, Ring1, CBX8, and BCoR [Mueller et al. 2007]. A more recent study indicated that the core EAP complex consists of only ENL, AF4, DOT1L, and P-TEFb [Mueller et al. 2009]. P-TEFb is a cyclin-dependent kinase complex that phosphorylates Ser2 within the RNA Pol II C-terminal domain (CTD). This phosphorylation event is required for the transition of RNA Pol II from transcription initiation to the transcription elongation phase. The purified EAP complex possesses both H3K79 methyltransferase

Table 1. Comparison of the composition of the DOT1L-relevant transcriptional elongation complexes

| Complex     | DOT1L | AF4 | AF5q31 | AF9 | AF10 | ENL | P-TEFb | Other                   | Reference                | Reference          |
|-------------|-------|-----|--------|-----|------|-----|--------|-------------------------|-------------------------|---------------------|
| EAP core    | +     |     |        |     |      |     |        |                         |                         | Mueller et al. 2007  |
| Bitoun et al. 2007 | +     |     |        |     |      |     |        |                         |                         | Mueller et al. 2009  |
| DotCom      | +     |     |        |     |      |     |        |                         |                         | Bitoun et al. 2007   |
| SEC         |       |     |        |     |      |     |        |                         |                         | Mohan et al. 2010    |
| AEP         | +     |     |        |     |      |     |        |                         |                         | Lin et al. 2010      |
|             |       |     |        |     |      |     |        |                         |                         | Yokoyama et al. 2010 |

Plus sign indicates that the protein was purified in the listed complex.
and RNA Pol II CTD kinase activities. Importantly, knockdown of ENL reduced genome-wide H3K79me2 as well as global transcriptional elongation activity, suggesting a link between H3K79 methylation and transcriptional elongation [Mueller et al. 2007]. The second DOT1L-containing complex, which is related to EAP, was purified by using an antibody against AF4 [Bitoun et al. 2007]. The complex contains AF4, AF9, AF10, ENL, DOT1L, and P-TEFb. Importantly, overexpression of AF4, AF9, ENL, and AF10 increased H3K79 methylation levels and P-TEFb-dependent transcription elongation [Bitoun et al. 2007]. These studies collectively support a role for DOT1L in transcriptional elongation.

In addition to the two related complexes mentioned above, a recently purified DOT1L-containing complex, DotCom, contains members of the Wnt pathway in addition to DOT1L, AF10, AF17, and AF9 (Mohan et al. 2010). Despite the absence of P-TEFb in this complex, DOT1L was still required for the expression of Wingless target genes, supporting its role in transcriptional activation [Mohan et al. 2010]. Interestingly, another recently purified superelongation complex (SEC) contains multiple transcription elongation factors [ELL1–3, P-TEFb, AFF1/AF4, AFF4, AF9, and ENL] and ELL-associated factors [EAFs], but does not contain DOT1L [Lin et al. 2010]. Similarly, another elongation complex, AEP (which is composed of AF4, AF5q31, ENL, and P-TEFb), also lacks DOT1L [Yokoyama et al. 2010]. It is likely that the variation between different protein complexes purified may result from the different methods and purification procedures used in these studies. It is possible that multiple transcriptional elongation complexes may exist, and that DOT1L may associate with only a subset of elongation complexes. Although DOT1L may not be required for transcription of all genes, it certainly plays a role for transcribing a subset of genes plausibly through facilitating transcriptional elongation. Based on available information, we propose a model that encompasses sequential events (including H2B-K123ub, H3K4me, H3K79me, and RNA Pol II phosphorylation) that lead to transcriptional activation and elongation [Fig. 3].

**Role of Dot1 in DNA repair and cell cycle regulation**

**Role of Dot1 in the DNA damage response**

Chromatin structure and nucleosome positioning, which are dynamically regulated by histone modifications, affect transcription by RNA Pol II. In addition, the presence of DNA damage, such as double-strand breaks (DSBs) and lesions, can impede Pol II transcription elongation. Therefore, mechanisms used in the regulation of chromatin conformation and transcription may also be used in repairing DNA damage. Consistent with this notion, studies in yeast and mammalian cells have established a link between Dot1/DOT1L and their associated H3K79 methylation to DNA damage checkpoint control and repair.

The first evidence that suggested a link between DOT1L-mediated H3K79 methylation and DNA damage repair came from the demonstration that the tandem tudor domain of the human 53BP1 protein binds to methylated H3K79 and is recruited to DNA DSBs [Huyen et al. 2004]. Strikingly, both mutation of the tudor domain and blocking H3K79 methylation through either mutating Lys 79 of H3 or knocking down DOT1L inhibited recruitment of 53BP1 to DSBs. The interaction between 53BP1 and methylated H3K79 is evolutionarily conserved,
as Rad9, the yeast ortholog of 53BP1, also binds to methylated H3K79 via its tudor domain [Wysocki et al. 2005]. Since H3K79 methylation levels do not change upon DNA damage, it is believed that DSBs induce changes in the chromatin structure to expose methylated H3K79, which is then recognized by 53BP1 [Huyen et al. 2004]. However, given that Rad9 is still recruited to sites of DSBs in the absence of the chromatin remodeling complexes SWR and INO80 [Javaheri et al. 2006], further studies are needed to prove or disprove this hypothetical model.

Budding yeast cells treated with ionizing radiation [IR] at G1 typically undergo a G1 checkpoint delay. Interestingly, Dot1 mutants are defective in G1- and intra-S-phase checkpoints and progress through the cell cycle at a normal rate even after IR-induced DNA damage [Wysocki et al. 2005]. Moreover, mutations that affect Dot1 enzymatic activity, such as disruption of H2B-K123 ubiquitination or mutation of H3K79, display the same checkpoint defects in response to multiple genotoxic stresses [Giannattasio et al. 2005; Game et al. 2006; Chernikova et al. 2010]. In response to IR damage, the Dot1/Rad6/Bre1 pathway mediates DNA damage repair through G1 homologous recombination [HR] [Game et al. 2006]. Although Dot1 deletion mutants do not display a G2 arrest phenotype [Game et al. 2006], Dot1 is required for Rad9 recruitment to the damage foci and phosphorylation of Rad53 upon IR [Toh et al. 2006]. Dot1-mediated H3K79 methylation is believed to play two distinct roles in Rad9-mediated DNA damage response [Grenon et al. 2007]: the activation of the G1/S checkpoint, and the repair of DNA damage at late G2 phase.

Repair of DSBs can occur via nonhomologous end-joining [NHEJ] or HR. Resection of DSBs is an important step in DNA damage repair by HR, producing 3' ssDNA tail intermediates. It is believed that both H3K79 methylation and Rad9 recruitment play an integral role in regulating resection to limit the amount of ssDNA that is produced [Lazzaro et al. 2008]. HR with the sister chromatid (SCR) in mitosis ensures faithful transmission of the genetic material. A number of proteins, including cohesin, are required for maintaining chromosome structure and for efficient SCR. Interestingly, not only is Dot1 required for the recruitment of Rad9 to the DSB sites and for DNA resection, it also facilitates the recruitment of cohesin and efficient SCR [Conde et al. 2009].

In addition to DSBs, Dot1-mediated H3K79 methylation plays a role in repairing other types of DNA lesions. Ultraviolet radiation [UV] causes the formation of cis-syn cyclobutane pyrimidine and 6-4 photoproduct dimers, which can be repaired by nucleotide excision repair [NER], recombination repair [RR], or post-replication repair [PRR]. Dot1-mediated H3K79 methylation plays a critical role in repairing UV-induced DNA damage [Giannattasio et al. 2005], as loss of H3K79 methylation affects all three repair pathways, resulting in UV hypersensitivity [Bostelman et al. 2007; Lazzaro et al. 2008]. When the base excision repair [BER] pathway fails to repair damages caused by alkylating agents, cell cycle progression and cell survival may be achieved by the translesion synthesis [TLS] pathway that uses error-prone polymerases to bypass DNA lesions. Dot1 negatively regulates TLS, thereby maintaining genome integrity upon DNA damage [Conde and San-Segundo 2008; Conde et al. 2010; Levesque et al. 2010].

Beyond its role in the DNA damage response, Dot1 is also important for meiotic checkpoint control. Diploid parental cells undergo meiotic cell division to generate haploid gametes. Proper segregation of chromosomes during meiosis is ensured by the pachytene checkpoint, which monitors completion of chromosome synopsis and recombination to prevent premature nuclear division. In the event of defective meiotic recombination, the pachytene checkpoint induces arrest at mid-meiotic prophase. In yeast, arrest at pachytene can be observed in dmc1 and zip1 mutants, which are required for proper synopsis formation and repair of DSBs. In a genetic screen for pachytene checkpoint components, dmc1 and zip1 mutants failed to arrest in the absence of Dot1. This failure to arrest is partially due to mislocalization of the nucleolar proteins Pch2 and Sir2, both of which are required for pachytene checkpoint function [San-Segundo and Roeder 2000]. Collectively, an overwhelming amount of data support a vital role for Dot1 in regulating cell cycle progression in yeast upon DNA damage (Fig. 4). Surprisingly, very little work has been done to determine whether Dot1’s role in DNA damage checkpoint control is conserved in mammals.

Cell cycle-regulated H3K79 methylation

Being able to repair damaged DNA is vital for maintaining genome integrity and stability. DNA repair occurs upon checkpoint activation as a cell progresses through the cell cycle phases: G1, S, G2, and M. Consistent with its role in the DNA damage repair pathways, H3K79 methylation levels fluctuate with the cell cycle. In yeast, H3K79me2 levels are low in G1 and S, begin to increase in late S, and are maintained relatively higher in G2 and M phases [Schulze et al. 2009]. Paradoxically, a greater percentage of dot1Δ cells accumulate in G1 [a period with low H3K79me2] compared with wild-type cells. Despite the general correlation between H3K79me2 levels and transcription activity, genes that are expressed specifically in G1 [when H3K79me2 is low] are enriched with H3K79me2 during G2/M [Schulze et al. 2009]. These data suggest that, at least in this subset of genes, H3K79me2 is inversely correlated with gene activity and may have a role in epigenetic memory [Schulze et al. 2009]. However, the cell cycle-dependent variation of H3K27me2 levels in HeLa cells is different from that observed in yeast. In HeLa cells, the H3K79me2 level is high in G1, decreases in S, reaches the lowest in G2, and increases again in M phase [Feng et al. 2002]. Thus, it is apparent that the role of cell cycle-regulated H3K79me2 changes in yeast is not evolutionarily conserved in humans.

Mouse DOT1L has also been implicated in cell cycle progression of differentiating embryonic stem [ES] cells and erythroid progenitor cells. Although DOT1L-depleted ES cells retain their proliferation and pluripotency
...cycle progression and antagonizing apoptosis in primitive erythroid progenitor cells [Jones et al. 2008]. In contrast to that observed in ES cells, DOT1L knockout in the erythroid lineage results in an accumulation at G0/G1 rather than G2/M [Feng et al. 2010], indicating that the cell cycle effect of DOT1L might be cell type-specific. Further studies are needed to understand why cell cycle regulation by Dot1 and DOT1L are organism and cell type-dependent.

**Biological functions of DOT1L**

**Role of Dot1 in embryonic development**

In addition to the studies performed in cell lines and lower eukaryotes, the role of Dot1-mediated H3K79 methylation was also analyzed in higher eukaryotes, including animals. For example, the cell cycle regulation of H3K79 methylation levels observed in cell lines is also observed during embryogenesis. During fly embryogenesis, methylation of H3K79 is absent in both transcriptionally active somatic nuclei and quiescent pole cell nuclei at the precellular blastoderm. H3K79me2 begins to emerge after gastrulation at the germ band extended embryo stage 8/9 and is restricted to cells undergoing mitosis [Shanower et al. 2005]. Increasing levels of H3K79me2 are then observed in epidermal nuclei during dorsal closure and head involution. The re-establishment of H3K79me2 suggests that this modification plays an important role in regulating transcription of developmental genes [Shanower et al. 2005]. Interestingly, mutation of *Grappa*, the fly ortholog of Dot1, causes phenotypes observed in both Polycomb Group (PcG) and trithorax Group (trxG) mutants [Shanower et al. 2005]. PcG and trxG genes play antagonistic roles by respectively silencing and activating the expression of Antennapedia and Bithorax homeotic genes, which specify anterior-to-posterior parasegmentation identity [Ringrose and Paro 2004]. In addition to a role in parasegmentation, Grappa has been implicated in Wnt/Wingless signaling, which is required for morphogenesis in wing imaginal discs [Mohan et al. 2010]. Using knockdown approaches, Grappa was shown to regulate the active expression of Wingless target genes such as senseless, frizzled 3, and homothorax [Mohan et al. 2010]. Taken together, the data indicate that Grappa may play a dual role in regulating both the active and silenced states of specific chromatin domains during fly development.

Similar to that observed in *Drosophila*, H3K79 methylation is also absent from mouse zygotes and is present at a very low level until the blastocyst stage [Ooga et al. 2008], suggesting that loss of this epigenetic mark is important for early embryogenesis. Although H3K79me2 is detectable from the four-cell to blastocyst stages, H3K79me3 is undetectable even at the blastocyst stage [Ooga et al. 2008]. Germline knockout of *mdot1* results in lethality by embryonic day 10.5 ([E10.5]) during organogenesis of the cardiovascular system [Jones et al. 2008]. Consistently, knockout embryos display cardiovascular defects, including heart enlargement, decreased
vasculature, and anemia (Jones et al. 2008). These studies collectively demonstrate that DOT1L and Grappa play an important role in embryonic development in mice and Drosophila, respectively.

**Role of DOT1L in cardiac function**

The cardiovascular defect observed in mDOT1L knockout embryos (Jones et al. 2008) suggested that mDOT1L may play a role in cardiac development, which is consistent with ex vivo studies demonstrating a correlation between increased H3K79 methylation and induced expression of cardiovascular marker genes during myocardogenesis (Illi et al. 2005). To elucidate the role of mDOT1L in cardiac development and function, we generated a cardiac-specific knockout mouse model by crossing floxed Dot1l mice with α-myosin heavy chain-Cre mice (Nguyen et al. 2011b). Cardiac-specific knockout of mDOT1L caused dilated cardiomyopathy (DCM) (Nguyen et al. 2011b), a disease of the heart muscle manifested by chamber dilation and systolic dysfunction. Mechanistically, mDOT1L-mediated H3K79 methylation directly regulates transcription of dystrophin (Fig. 5). Given that dystrophin is required for forming the dystrophin–glycoprotein complex (DGC) that is involved in signal force transmission and relieving mechanical stress during heart contraction, loss of dystrophin expression causes DGC destabilization, resulting in sarcolemma damage (Kimura 2008). In humans, dystrophin mutations are a known cause of human DCM and muscular dystrophy. Importantly, the DCM phenotype observed in mDOT1L cardiac-specific knockout mice can be rescued by ectopic expression of a functional truncated form of dystrophin (Nguyen et al. 2011b), indicating that dystrophin is a major downstream target that mediates the cardiac function of DOT1L. Interestingly, analysis of DOT1L expression levels revealed that DOT1L is significantly lower in human idiopathic DCM heart tissue compared with normal heart tissue (Nguyen et al. 2011b), raising the possibility that malfunction of DOT1L may be a contributing factor in human DCM pathogenesis.

**Role of DOT1L in erythropoiesis**

In addition to cardiovascular defects, DOT1L knockout embryos display severe anemia by E10.5, suggesting impaired fetal erythropoiesis (Feng et al. 2010). A possible role for mDOT1L in erythropoiesis would be consistent with a previous report demonstrating that H3K79 methylation is enriched at the erythroid-specific β-globin locus (Lin et al. 2003). In support of this, mDOT1L deficiency in yolk sac-derived hematopoietic cells caused a G0/G1 cell cycle arrest and induced apoptosis in erythroid progenitor cells cultured in vitro in the presence of growth stimuli, but had no effect on myeloid lineage growth and differentiation (Feng et al. 2010). RT-qPCR and ChIP analyses indicated that the function of mDOT1L in erythropoiesis was mediated by its role in activation of Gata2. The down-regulation of Gata2 in Dot1l-deficient hematopoietic cells caused derepression of the myeloid transcription factor PU.1 to block erythroid differentiation (Feng et al. 2010).

**Role of DOT1L in leukemia**

It is apparent that DOT1L is vital for normal cellular and biological processes, including DNA damage repair, cell cycle, development, cardiac muscle function, and hematopoiesis. However, transcriptional activation by DOT1L-mediated H3K79 methylation is also exploited in leukemias that stem from chromosomal rearrangements of MLL (mixed-lineage leukemia). This misregulation of DOT1L is achievable because components of the elongation complexes EAP (Mueller et al. 2007), SEC (Lin et al. 2010), and AEP (Yokoyama et al. 2010) interact with DOT1L and are frequent translocation partners of MLL (Ayton and Cleary 2001). Translocation of the MLL gene results in the expression of oncopgenic fusion proteins and is a common cause of acute leukemia (Ayton and Cleary 2001; Hess 2004), accounting for 5%–10% of adult acute leukemias [Daser and Rabbits 2004] and 60%–80% of infant acute leukemias [Ayton and Cleary 2001; Hess 2004; Krivtsov and Armstrong 2007].

![Figure 5](GENES & DEVELOPMENT 1353)
MLL is an H3K4 methyltransferase that maintains the “on” state of Hox genes during embryogenesis and hematopoiesis (Hsieh et al. 2003; Guenther et al. 2005). In leukemia, Hoxa genes remain constitutively active to promote uncontrolled proliferation of leukemic cells. Although MLL fusion proteins lack the catalytic SET domain (Fig. 6A), it is believed that constitutive activation of leukemic oncogenes is mediated by wild-type MLL expressed from the nonmutated allele, as well as the transactivation activity of the MLL fusion partner and other recruited factors such as MENIN (Yokoyama et al. 2005) and LEDGF (Yokoyama and Cleary 2008). Due to the high incidence of MLL-related acute leukemias, a better understanding of the molecular mechanisms used by MLL fusion proteins is vital for the development of effective treatments. However, the heterogeneity of >50 MLL fusion partners makes targeted therapy for MLL-related leukemias difficult.

The first evidence that suggested a role for DOT1L in leukemia came from studies looking for functional partners of DOT1L. A yeast two-hybrid screen identified two MLL fusion partners—AF10 and ENL—as DOT1L-interacting proteins (Okada et al. 2005). Further characteriza-

![Figure 6](image-url)

**Figure 6.** MLL fusion proteins recruit DOT1L for transcriptional activation and leukemogenesis. (A, top) MLL is a SET domain-containing H3K4 methyltransferase with multiple functional domains. (AT-hook and CxxC) DNA binding; (SNL) signal nuclear localization; (PHD) protein interactions; (TAD) transactivation domain; (SET) methyltransferase domain. Frequent DSBs occur within the MLL locus at a breakpoint cluster region (BCR). (Bottom) The N terminus of MLL undergoes chromosomal rearrangements and fuses in-frame to >50 different genes, resulting in the expression of leukemogenic MLL fusion proteins. (B) Constitutive activation of a leukemic transcriptional program, including Hoxa and Meis1 genes, is achieved through mistargeting of DOT1L and its associated proteins through the interaction of DOT1L with MLL fusion partners. DOT1L-mediated H3K79 methylation and the subsequent activation of the target genes result in leukemic transformation.

In addition to MLL-AF10 and CALM-AF10, DOT1L is involved in transformation by other MLL fusion proteins, such as MLL-ENL (Mueller et al. 2007), MLL-AF4 (Krivtsov et al. 2008), and MLL-AF9 (Chang et al. 2010; Nguyen et al. 2011a). Consistent with the fact that DOT1L is involved in MLL-related transformation, DOT1L associates with a number of MLL fusion proteins and participates in the activation of a leukemic transcriptional program (Bitoun et al. 2007; Mueller et al. 2007, 2009). The exact mechanism regarding how DOT1L contributes to the gene activation process requires further elucidation; however, it is clear that DOT1L is broadly involved in leukemogenesis, particularly those mediated by MLL fusion proteins such as AF4, AF9, AF10, and ENL, which account for two-thirds of all MLL-associated leukemias (Ayton and Cleary 2001).

The above studies collectively establish a broad role for DOT1L in transformation, and suggest a general mechanism by which DOT1L contributes to the leukemogenesis process [Fig. 6B]. Interaction between DOT1L and MLL fusion partners leads to mistargeting of DOT1L to the gene targets of MLL fusion proteins, such as the Hoxa cluster and Meis1. Aberrant hypermethylation of H3K79 leads to constitutive transcriptional activation of these genes, which in turn results in leukemic transformation. Inhibition of DOT1L enzymatic activity or disruption of the interaction between DOT1L and the MLL fusion proteins may provide a promising therapeutic strategy for the treatment of MLL fusion-related leukemias. However, given the role of DOT1L in normal hematopoiesis [Feng et al. 2010; Jo et al. 2011], the possible side effects of such drugs have to be carefully evaluated before they can be used in the treatment of leukemia patients.
Concluding remarks

Dot1 is unique not only in its structure, but in its substrate specificity. Although its catalytic domain resembles that of class I arginine methylases, Dot1 methylates histone H3 at Lys 79. H3K79 is also located within the surface-exposed globular domain of H3 rather than its NH₂ tail, where all other known methylation residues reside. Paradoxically, Dot1-mediated H3K79 methylation is capable of regulating transcription at both heterochromatin and euchromatin. Globally, H3K79 methylation is enriched in actively transcribing genes. Consistently, H3K79 methylation is required for the expression of Wnt target genes in Drosophila imaginal discs, dystrophin in cardiac and myoblast cells, and the Hoxa cluster in leukemia cells.

Intriguingly, Dot1 is the sole H3K79 methylase capable of catalyzing mono-, di-, and trimethylation in a non-processive manner. Thus, in the absence of any other mediator proteins, the likelihood of an H3K79me1 mark becoming H3K79me2 and an H3K79me2 mark becoming H3K79me3 have the same probability. If this were the case, it could be inferred that they all play redundant roles in regulating transcription, and brings into question the need for different H3K79 methylation states [Frederik et al. 2008]. Nevertheless, studies suggest that the various H3K79 methylation states have different functions in transcriptional regulation and/or other processes. For example, in yeast, only H3K79me2 fluctuates with the cell cycle, whereas H3K79me3 levels remain constant. Additionally, in early mouse embryogenesis, H3K79me2 and H3K79me3 emerge at distinct stages of development. How the same enzyme achieves specificity to temporally regulate its catalytic activity at specific chromatin domains remains to be determined. One possibility is that its enzymatic activity might be regulated by other proteins, such as Rad6/Bre1 and the Paf1 complex in yeast and other associated proteins identified in mammals. Another possibility is that the steady-state levels of H3K79 methylation might be regulated by a putative H3K79 demethylase.

In addition to transcription, Dot1 plays an important role in cell cycle regulation and the DNA damage response. Furthermore, mammalian DOT1L is an essential gene that is required for embryogenesis, hematopoiesis, and cardiac function. Finally, DOT1L plays a pivotal role in MLL-related leukemogenesis. Given the importance of its enzymatic activity in these processes, DOT1L emerges as a promising target for therapeutic treatments. It is apparent that DOT1L and its associated H3K79 methylation activity are extremely complex. As such, the regulation of DOT1L enzymatic activity and the functional consequences need to be further elucidated before efficient treatments can be developed. We expect that future studies will reveal details regarding how its enzymatic activity is regulated, and how H3K79 methylation leads to transcriptional activation. We also anticipate a revelation of DOT1L functions outside the cardiovascular and hematopoietic systems. Identification and characterization of DOT1L inhibitors and evaluation of their efficacy in leukemia treatment will provide another example where initial inquiry into the function of a basic histone modification leads to a treatment for a deadly disease.

Acknowledgments

We thank Susan Wu for critical reading of the manuscript. We apologize to colleagues whose work cannot be cited due to space constraints. The DOT1L work in the Zhang laboratory is supported by NIH (CA119133) and HHMI. Y.Z. is an Investigator of the Howard Hughes Medical Institute.

References

Altaf M, Utley RT, Lacoste N, Tan S, Briggs SD, Cote J. 2007. Interplay of chromatin modifiers on a short basic patch of histone H4 tail defines the boundary of telomeric heterochromatin. Mol Cell 28: 1002–1014.

Aytong PM, Cleary ML. 2001. Molecular mechanisms of leukemia mediated by MLL fusion proteins. Oncogene 20: 5695–5707.

Barry ER, Krueger W, Jakuba CM, Veilleux E, Ambrosi DJ, Nelson CE, Rasmussen TP. 2009. ES cell cycle progression and differentiation require the action of the histone methyltransferase Dot1L. Stem Cells 27: 1538–1547.

Barski A, Cuddapah S, Cui K, Roh TY, Shones DE, Wang Z, Wei G, Chepelev I, Zhao K. 2007. High-resolution profiling of histone methylation states in the human genome. Cell 129: 823–837.

Bitoun E, Oliver PL, Davies KE. 2007. The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. Hum Mol Genet 16: 92–106.

Bostelman LJ, Keller AM, Albrecht AM, Arat A, Thompson JS. 2007. Methylation of histone H3 lysine-79 by Dot1p plays multiple roles in the response to UV damage in Saccharomyces cerevisiae. DNA Repair (Amst) 6: 383–395.

Briggs SD, Xiao T, Sun ZW, Caldwell JA, Shabanowitz J, Hunt DF, Allis CD, Strahl BD. 2002. Gene silencing: trans-histone regulatory pathway in chromatin. Nature 418: 498.

Cavalli G. 2006. Chromatin and epigenetics in development: blending cellular memory with cell fate plasticity. Development 133: 2089–2094.

Chandrasekharan MB, Huang F, Sun ZW. 2009. Ubiquitination of histone H2B regulates chromatin dynamics by enhancing nucleosome stability. Proc Natl Acad Sci 106: 16686–16691.

Chang MJ, Wu H, Achille NJ, Reisenaur MR, Chou CW, Zeleznik-Le NJ, Hemenway CS, Zhang W. 2010. Histone H3 lysine 79 methyltransferase Dot1 is required for immortalization by MLL oncogenes. Cancer Res 70: 10234–10242.

Chatterjee C, McGinty RK, Fierz B, Muir TW. 2010. Disulfide-directed histone ubiquitinylation reveals plasticity in hDot1L activation. Nat Chem Biol 6: 267–269.

Cheng X, Roberts RJ. 2001. AdoMet-dependent methyltransferase, DNA methyltransferases and base flipping. Nucleic Acids Res 29: 3784–3795.

Chernikova SB, Dorth JA, Razorenova OV, Game JC, Brown JM. 2010. Deficiency in Bre1 impairs homologous recombination repair and cell cycle checkpoint response to radiation damage in mammalian cells. Radiat Res 174: 558–565.

Cloos PA, Christensen J, Agger K, Helin K. 2008. Erasing the methyl mark: histone demethylases at the center of cellular differentiation and disease. Genes Dev 22: 1115–1140.

Conde F, San-Segundo PA. 2008. Role of Dot1 in the response to alkylating DNA damage in Saccharomyces cerevisiae: regulation of DNA damage tolerance by the error-prone polymerases Polzeta/Rev1. Genetics 179: 1197–1210.
Conde F, Refolio E, Cordon-Preciado V, Cortes-Ledesma F, Aragon L, Aguilera A, San-Segundo PA. 2009. The Dot1 histone methyltransferase and the Rad9 checkpoint adaptor contribute to cohesin-dependent double-strand break repair by sister chromatid recombination in Saccharomyces cerevisiae. Genetics 182: 437–446.

Conde F, Ontoso D, Acosta I, Gallego-Sanchez A, Bueno A, San-Segundo PA. 2010. Regulation of tolerance to DNA alkylating damage by Dot1 and Rad53 in Saccharomyces cerevisiae. DNA Repair (Amst) 9: 1038–1049.

Daser A, Rabbits TH. 2004. Extending the repertoire of the mixed-lineage leukemia gene MLL in leukemogenesis. Genes Dev 18: 965–974.

Ezhkova E, Tansey WP. 2004. Proteasomal ATPases link ubiquitylation of histone H2B to methylation of histone H3. Mol Cell 13: 435–442.

Feinberg AP, Oshimura M, Barrett JC. 2002. Epigenetic mechanisms in human disease. Cancer Res 62: 6784–6787.

Feng Q, Wang H, Ng HH, Erdjument-Bromage H, Tempst P, Struhl K, Zhang Y. 2002. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol 12: 1052–1058.

Feng Y, Yang Y, Ortega MM, Copeland JN, Zhang M, Jacob JB, Fields TA, Vivian JL, Fields PE. 2010. Early mammalian erythropoiesis requires the Dot1L methyltransferase. Blood 116: 4483–4491.

Fierz B, Chatterjee C, McGinty RK, Bar-Dagan M, Ralph DP, Muir TW. 2011. Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. Nat Chem Biol 7: 113–119.

Fingerman IM, Li HC, Briggs SD. 2007. A charge-based interaction between histone H4 and Dot1 is required for H3K79 methylation and telomere silencing: identification of a new trans-histone pathway. Genes Dev 21: 2018–2029.

Frederiks F, Tzouros M, Oudgenoeg G, van Welsem T, Fornerod M, Krijgsveld J, van Leeuwen F. 2008. Nonprocessive methylation of histone H3 by Dot1L methyltransferase. Proc Natl Acad Sci USA 105: 20000–20005.

Fujita N, Harada T, Makita Z. 2009. Mammalian Dot1L is essential for alkyltransferase activity and methyltransferase activity of H3K79 methyltransferase Dot1L. Proc Natl Acad Sci USA 106: 20827–20832.

Fujita N, Harada T, Makita Z. 2009. Mammalian Dot1L is essential for DNA methyltransferase activity. Proc Natl Acad Sci USA 106: 20827–20832.

Fujita N, Harada T, Makita Z. 2009. Mammalian Dot1L is essential for DNA methyltransferase activity. Proc Natl Acad Sci USA 106: 20827–20832.

Game JC, Williamson MS, Spicakova T, Brown JM. 2006. The DNA damage checkpoint requires histone H2B ubiquitylation and higher-order chromatin compaction. Nat Chem Biol 7: 113–119.

Fingerman IM, Li HC, Briggs SD. 2007. A charge-based interaction between histone H4 and Dot1 is required for H3K79 methylation and telomere silencing: identification of a new trans-histone pathway. Genes Dev 21: 2018–2029.

Frederiks F, Tzouros M, Oudgenoeg G, van Welsem T, Fornerod M, Krijgsveld J, van Leeuwen F. 2008. Nonprocessive methylation of histone H3 by Dot1L methyltransferase. Proc Natl Acad Sci USA 105: 20000–20005.

Fujita N, Harada T, Makita Z. 2009. Mammalian Dot1L is essential for alkyltransferase activity and methyltransferase activity of H3K79 methyltransferase Dot1L. Proc Natl Acad Sci USA 106: 20827–20832.

Fujita N, Harada T, Makita Z. 2009. Mammalian Dot1L is essential for DNA methyltransferase activity. Proc Natl Acad Sci USA 106: 20827–20832.
methyltransferase Dot1l and Rad9 inhibit single-stranded DNA accumulation at DSBs and uncapped telomeres. *EMBO J* 27: 1502–1512.

Lee JS, Shukla A, Schneider J, Swanson SK, Washburn MP, Florens L, Bhaumik SR, Shilatifard A. 2007. Histone cross-talk between H2B monoubiquitination and H3 methylation mediated by COMPASS. *Cell* 131: 1084–1096.

Levesque N, Leung GP, Fok AK, Schmidt TI, Kobor MS. 2010. Loss of H3 K79 trimethylation leads to suppression of Rtt107-dependent DNA damage sensitivity through the translesion synthesis pathway. *J Biol Chem* 285: 35113–35122.

Lin C, Smith ER, Takahashi H, Lai KC, Martin-Brown S, Florens L, Washburn MP, Conaway JW, Conaway RC, Shilatifard A. 2010. AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Mol Cell* 37: 429–437.

Liu W, Tanasa B, Turrina OV, Zhou TY, Gassmann R, Liu WT, Ohgi KA, Benner C, Garcia-Bassets I, Aggarwal AK, et al. 2010. PHF8 mediates histone H4 lysine 20 demethylation events involved in cell cycle progression. *Nature* 466: 508–512.

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 389: 251–260.

Martin C, Zhang Y. 2005. The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol* 6: 838–848.

McIntyre RK, Kim J, Chatterjee C, Roeder RG, Muir TW. 2008. Chemically ubiquitylated histone H2B stimulates hDot1L-mediated intranucleosomal methylation. *Nature* 453: 812–816.

Min J, Feng Q, Li Z, Zhang Y, Xu RM. 2003. Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell* 112: 711–723.

Minard ME, Jain AK, Barton MC. 2009. Analysis of epigenetic alterations to chromatin during development. *Genes Dev* 23: 559–572.

Mohan M, Herz HM, Takahashi YH, Lin C, Lai KC, Zhang Y, Washburn MP, Florens L, Shilatifard A. 2010. Linking H3K79 trimethylation to Wnt signaling through a novel Dot1L-containing complex (DotCom). *Genes Dev* 24: 574–589.

Mueller D, Bach C, Zeisig D, Garcia-Cuellar MP, Monroe S, Sreekumar A, Zhou R, Neviszhskii A, Chinnaiyan A, Hess JL, et al. 2007. A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. *Blood* 110: 4445–4454.

Mueller D, Garcia-Cuellar MP, Bach C, Buhl S, Maethner E, Slany RK. 2009. Misguided transcriptional elongation causes mixed lineage leukemia. *PLoS Biol* 7: e1000249. doi: 10.1371/journal.pbio.1000249.

Ng HH, Feng Q, Wang H, Erdjument-Bromage H, Tempst P, Zhang Y, Struhl K. 2002a. Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev* 16: 1518–1527.

Ng HH, Xu RM, Zhang Y, Struhl K. 2002b. Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J Biol Chem* 277: 34655–34657.

Ng HH, Ciccone DN, Morshed KB, Oettinger MA, Struhl K. 2003a. Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation. *Proc Natl Acad Sci* 100: 1820–1825.

Ng HH, Dole S, Struhl K. 2003b. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J Biol Chem* 278: 33625–33628.

Nguyen AT, Taranova O, He J, Zhang Y. 2011a. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9 leukemiaogenesis. *Blood* doi: 10.1182/blood-2011-02-334369.

Nguyen AT, Xiao B, Neppel RL, Kallin EM, Li J, Chen T, Wang DZ, Xiao X, Zhang Y. 2011b. DOT1L regulates dystrophin expression and is critical for cardiac function. *Genes Dev* 25: 263–274.

Norris A, Bocke JD. 2010. Silent information regulator 3: the Goldilocks of the silencing complex. *Genes Dev* 24: 115–122.

Oh S, Jeong K, Kim H, Kwon CS, Lee D. 2010. A lysine-rich region in Dot1p is crucial for direct interaction with H2B ubiquitination and high level methylation of H3K79. *Biochem Biophys Res Commun* 399: 512–517.

Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, Su L, Xu G, Zhang Y. 2005. H3K79 methylation by the Polycomb and Trithorax group proteins. *Biochim Biophys Acta* 1749: 1017–1024.

Onishi M, Liou GG, Buchberger JR, Walz T, Moazed D. 2007. Role of the conserved Sir3-BAH domain in nucleosome binding and silent chromatin assembly. *Cell* 128: 1015–1028.

Ooga M, Inoue A, Kageyama S, Akiyama T, Nagata M, Aoki F. 2008. Changes in H3K79 methylation during preimplantation development in mice. *Biol Reprod* 78: 413–424.

Peterson CL, Laniel MA. 2004. Histones and histone modifications. *Curr Biol* 14: R546–R551. doi: 10.1016/j.cub.2004.07.007.

Pokholyok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, Bell GW, Walker K, Rolle PA, Herboldsheimer E, et al. 2005. Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* 122: 517–527.

Qi HH, Sarkissian M, Hu GQ, Wang Z, Bhattacharjee A, Gordon DB, Gonzales M, Lan F, Ongusaha PP, Huarte M, et al. 2010. Histone H4K20/H3K9 demethylase PHF8 regulates zebrafish brain and craniofacial development. *Nature* 466: 503–507.

Ringrose L, Paro R. 2004. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu Rev Genet* 38: 413–443.

Robzyk K, Recht J, Osley MA. 2000. Rad6-dependent ubiquitination of histone H2B in yeast. *Science* 287: 501–504.

Rossmann MP, Luo W, Tsaponina O, Chabes A, Stillman B. 2011. A common telomeric gene silencing assay is affected by nucleotide metabolism. *Mol Cell* 42: 127–136.

San-Segundo PA, Roeder GS. 2000. Role for the silencing protein Dot1 in meiotic checkpoint control. *Mol Cell Biol* 11: 3601–3615.

Sawada K, Yang Z, Horton JR, Collins RE, Zhang X, Cheng X. 2004. Structure of the conserved core of the yeast Dot1p, a nucleosomal histone H3 lysine 79 methyltransferase. *J Biol Chem* 279: 43296–43306.

Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F, Gottschling DE, O'Neill LP, Turner BM, Delrow J, et al. 2004. The histone modification pattern of a higher eukaryote. *Genes Dev* 18: 1263–1271.

Schubert HL, Blumenthal RM, Cheng X. 2003. Many paths to methyltransfer: a chronicle of convergence. *Trends Biochem Sci* 28: 329–335.

Schulze JM, Jackson J, Nakashima S, Gardner JM, Henrich T, Haug J, Johnston M, Jaspersen SL, Kobor MS, Shilatifard A. 2009. Linking cell cycle to histone modifications: SBF and H2B monoubiquitination machinery and cell-cycle regulation of H3K79 dimethylation. *Mol Cell* 35: 626–641.
Shanower GA, Muller M, Blanton JL, Honti V, Gyurkovics H, Schedl P. 2005. Characterization of the grappa gene, the Drosophila histone H3 lysine 79 methyltransferase. *Genetics* 169: 173–184.

Singer MS, Kahana A, Wolf AJ, Meisinger LL, Peterson SE, Goggin C, Mahowald M, Gottschling DE. 1998. Identification of high-copy disruptors of telomeric silencing in *Saccharomyces cerevisiae*. *Genetics* 150: 613–632.

Steger DJ, Lefterova MI, Ying L, Stonestrom AJ, Schupp M, Zhuo D,Vakoc AL, Kim JE, Chen J, Lazar MA, et al. 2008. DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol Cell Biol* 28: 2825–2839.

Strahl BD, Allis CD. 2000. The language of covalent histone modifications. *Nature* 403: 41–45.

Sun ZW, Allis CD. 2002. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* 418: 104–108.

Takahashi YH, Schulze JM, Jackson J, Hentrich T, Seidel C, Jaspersen SL, Kobor MS, Shilatifard A. 2011. Dot1 and histone H3K79 methylation in natural telomeric and HM silencing. *Mol Cell* 42: 118–126.

Toh GW, O’Shaughnessy AM, Jimeno S, Dobbie IM, Grenon M, Maffini S, O’Rorke A, Lowndes NF. 2006. Histone H2A phosphorylation and H3 methylation are required for a novel Rad9 DSB repair function following checkpoint activation. *DNA Repair (Amst)* 5: 693–703.

Vakoc CR, Sachdeva MM, Wang H, Blobel GA. 2006. Profile of histone lysine methylation across transcribed mammalian chromatin. *Mol Cell Biol* 26: 9185–9195.

Van Holde KE, Allen JR, Tatchell K, Weischet WO, Lohr D. 1980. DNA–histone interactions in nucleosomes. *Biophys J* 32: 271–282.

Van Leeuwen F, Gafken PR, Gottschling DE. 2002. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* 109: 745–756.

Van Welsem T, Frederiks F, Verzijlbergen KE, Faber AW, Nelson ZW, Egan DA, Gottschling DE, van Leeuwen F. 2008. Synthetic lethal screens identify gene silencing processes in yeast and implicate the acetylated amino terminus of Sir3 in recognition of the nucleosome core. *Mol Cell Biol* 28: 3861–3872.

Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, et al. 2008. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* 40: 897–903.

Wood A, Krogan NJ, Dover J, Schneider J, Heidt J, Boateng MA, Dean K, Golshani A, Zhang Y, Greenblatt JF, et al. 2003a. Brc1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol Cell* 11: 267–274.

Wood A, Schneider J, Dover J, Johnston M, Shilatifard A. 2003b. The Pafl complex is essential for histone mono-ubiquitination by the Rad6–Brc1 complex, which signals for histone methylation by COMPASS and Dot1p. *J Biol Chem* 278: 34739–34742.

Wysocki R, Javaheri A, Allard S, Sha F, Cote J, Kron SJ. 2005. Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. *Mol Cell Biol* 25: 8430–8443.

Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Xiao MT, Liu LX, et al. 2011. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* 19: 17–30.

Yokoyama A, Cleary ML. 2008. Menin critically links MLL proteins with LEDGF on cancer-associated target genes. *Cancer Cell* 14: 36–46.