Structural basis of recognition and destabilization of the histone H2B ubiquitinated nucleosome by the DOT1L histone H3 Lys79 methyltransferase

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DOT1L is a histone H3 Lys79 methyltransferase whose activity is stimulated by histone H2B Lys120 ubiquitination, suggesting cross-talk between histone H3 methylation and H2B ubiquitination. Here, we present cryo-EM structures of DOT1L complexes with unmodified or H2B ubiquitinated nucleosomes, showing that DOT1L recognizes H2B ubiquitin and the H2A/H2B acidic patch through a C-terminal hydrophobic helix and an arginine anchor in DOT1L, respectively. Furthermore, the structures combined with single-molecule FRET experiments show that H2B ubiquitination enhances a noncatalytic function of the DOT1L-destabilizing nucleosome. These results establish the molecular basis of the cross-talk between H2B ubiquitination and H3 Lys79 methylation as well as nucleosome destabilization by DOT1L.

Supplemental material is available for this article.

Received December 30, 2018; revised version accepted March 8, 2019.

The fundamental unit of chromatin is the nucleosome, a structure composed of a histone octamer and 146-bp DNA (Luger et al. 1997). The structure of the nucleosome is altered by ATP-dependent chromatin remodelers and histone chaperones. A recent cryo-EM work showed that the nucleosome has structural flexibility, as DNA breathing leads to conformational changes in the histones (Bilokapic et al. 2018). The histones in the nucleosome are covalently modified with many moieties, including methyl, acetyl, phosphor, and ubiquitin (Gurard-Levin et al. 2014; Lawrence et al. 2016; Clapier et al. 2017). These histone covalent modifications are key mechanisms that modulate epigenetic gene expression. Among these, histone methylation is very complex, as the locations and levels of histone methylations lead to different consequences in gene expression. For example, histone H3 Lys4 methylation by the MLL methyltransferase is a hallmark for gene activation, while histone H3 Lys27 methylation by the PRC2 methyltransferase is a marker for gene repression (Lawrence et al. 2016). While most histone methylations occur in the N-terminal flexible tails in histones, histone H3 Lys 79 is located on the body of the nucleosome. Histone H3 Lys79 methylation is highly correlated with actively transcribing genes and occurs by the methyltransferase yeast Dot1 and human DOT1L, enzymes that have non-SET methyltransferase domains (Min et al. 2003; Sawada et al. 2004; Nguyen and Zhang 2011). Histone methylations show another complexity, as some methylations are regulated by other histone modifications, suggesting a cross-talk between modifications (Lee et al. 2010). Monoubiquitination of histone H2B Lys120 was shown to be required for H3 Lys79 methylation in vitro and in vivo (Briggs et al. 2002; Ng et al. 2002; McGinty et al. 2008). These findings suggest that there is cross-talk between H2B ubiquitination and histone H3 Lys79 methylation. However, it is not clear how DOT1L recognizes H2B ubiquitinated nucleosomes, thus leading to stimulation of DOT1L activity.

In addition to their catalytic activities, histone methyltransferases have been shown to have noncatalytic activities (Kim et al. 2015). A recent study showed that yeast Dot1 has nucleosome chaperone activity and enhances the ATP-dependent chromatin remodeling by CHD1, which is independent of Dot1 methyltransferase activity (Lee et al. 2018). This work suggested that Dot1/DOT1L might destabilize the nucleosome structure and help the remodeler to alter the nucleosome structure. However, the molecular details of DOT1L-mediated nucleosome destabilization have not been investigated.

Here, we present cryo-EM structures of DOT1L bound to unmodified or H2B ubiquitinated nucleosomes. The structures revealed that the location of DOT1L relative to the nucleosome is reoriented in the ubiquitinated nucleosome (Nuc_H2B-Ub) compared with the unmodified nucleosome (Nuc) and that DOT1L recognizes ubiquitinated histone H2B (H2B-Ub) via two main modes: A hydrophobic helix located at the C-terminal of the DOT1L catalytic domain recognizes H2B-Ub, and an arginine anchor in DOT1L interacts with the nucleosome H2A/H2B acidic patch. In addition, the cryo-EM structures reveal that DNA is detached from the histones and that the first helix of H3 and the C terminus of H2A are concomitantly disordered, indicating that DOT1L binding destabilizes the nucleosome structure. Furthermore, single-molecule

[*Keywords*: cryo-EM; histone; nucleosome; methylation; ubiquitin]
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Article published online ahead of print. Article and publication date are online at http://www.genesdev.org/cgi/doi/10.1101/gad.323790.118.

FRET analysis shows that H2B ubiquitination greatly increases DOT1L-mediated nucleosome destabilization, suggesting that H2B ubiquitination affects not only histone H3 methylation but also nucleosome destabilization by DOT1L. Overall, this work provides the first molecular insight into a cross-talk between H2B-Ub and H3 Lys79 methylation as well as a structural view of DOT1L-mediated nucleosome destabilization.

Results and Discussion

Cryo-EM structures of the DOT1L–nucleosome with or without ubiquitin

DOT1L methylates histone H3 Lys79 located on the body of nucleosome, and its activity is stimulated by H2B ubiquitin. To understand how DOT1L recognizes ubiquitinated nucleosomes, we determined cryo-EM structures of the DOT1L catalytic domain (amino acids 1–416) bound to unmodified or H2B ubiquitinated nucleosomes. We prepared H2B-Ub by chemical cross-linking (Morgan et al. 2016) and assembled the H2B-Ub nucleosome by mixing ubiquitinated histone octamers with 601 DNA (Supplemental Fig. S1A,B; Lowary and Widom 1998). We then purified DOT1L_nucleosomes (DOT1L_Nucs) and DOT1L_H2B ubiquitinated nucleosomes (DOT1L_Nuc_H2B-Ubs) by GraFix (Supplemental Fig. S2; Stark 2010). Micrographs from plunge-frozen DOT1L–nucleosome complexes were collected using Titan Krios 300KeV with a Gatan K2 Summit direct detector (Supplemental Table 1). The micrographs were processed and refined with Relion2 (Kimanius et al. 2016). The gold standard FSCs of the cryo-EM maps at 0.143 criteria indicate 6.8 Å or 7.3 Å resolutions for DOT1L_Nuc-H2B_Ub and DOT1L_Nuc_H2B-Ub, respectively (Supplemental Figs. S3, S4). Despite the relatively low resolutions, the secondary structures of histones and the grooves of DNA were well resolved (Fig. 1A,B; Supplemental Fig. S5). The cryo-EM maps clearly indicated that DOT1L is bound to only one side of the nucleosome. The cryo-EM map of DOT1L is relatively weak, suggesting that the binding of DOT1L to the nucleosome is flexible and that DOT1L might have multiple binding modes to the nucleosome. Comparison between the cryo-EM maps of DOT1L_Nuc and DOT1L_ Nuc_H2B-Ub clearly located the H2B ubiquitin (Fig. 1A,B,E–G).

To further investigate the interactions between DOT1L and the nucleosome, we performed hydrogen/deuterium exchange mass spectrometry (HDX-MS). We incubated DOT1L alone, DOT1L_Nuc, and DOT1L_Nuc_H2B-Ub in a D_2O buffer for 10, 100, 1000, and 10,000 sec. The degrees of hydrogen/deuterium exchanges were analyzed by mass spectrometry (Supplemental Fig. S5). Although we were not able to analyze the HDX-MS for histones due to the limited data quality and low peptide coverage, HDX-MS experiments revealed that the deuterium uptakes were significantly decreased in several peptides in DOT1L upon nucleosome binding. These peptides include the peptide $_{291}$RVVELSPLKG$_{301}$ near the hydrophobic patch of the C-terminal helix and the peptide $_{278}$RINSRNLSDIGT$_{289}$, including the arginine anchor, which is known to interact with the acidic patch of the nucleosome (Figs. 1C, 3E). The deuterium uptake of the arginine anchor peptide was significantly decreased in DOT1L_Nuc compared with DOT1L alone and further decreased in DOT1L_Nuc_H2B-Ub. In addition, the deuterium uptake of the hydrophobic peptide (amino acids 291–301) was further decreased upon H2B ubiquitination. These data suggest that the H2B ubiquitination reorients DOT1L to have a tighter interaction with the nucleosome via the C-terminal hydrophobic helix and the arginine anchor.

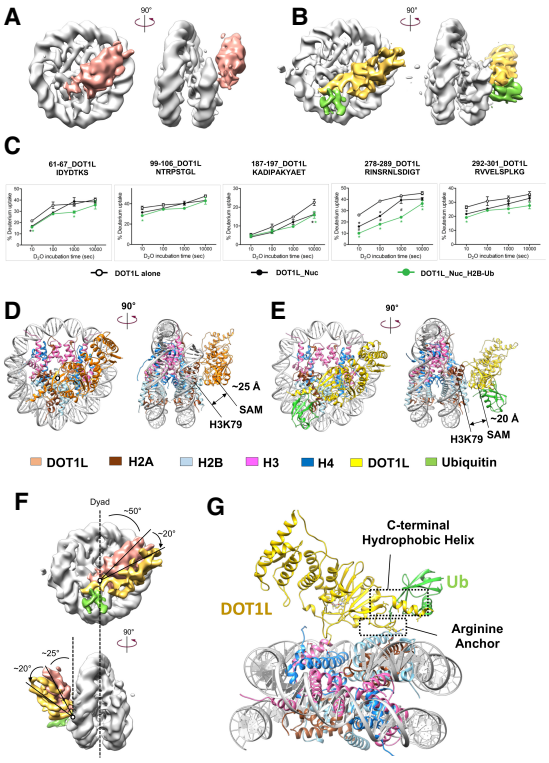

Figure 1. Cryo-EM structures of DOT1L_Nucs. (*A*) Cryo-EM map of DOT1L bound to unmodified nucleosome (DOT1L_Nuc). The nucleosome is shown in gray, and DOT1L is shown in pale red. The map of DOT1L was drawn in a lower contour than the nucleosome due to the weak map of DOT1L. (*B*) The deuterium uptake plots of selected DOT1L peptides of DOT1L showing the difference in the deuterium uptake rate between states. The significant difference ($P < 0.05$) is indicated for between DOT1L alone and DOT1L + nucleosome (*), between DOT1L alone and DOT1L + H2B ubiquitinated nucleosome (green asterisk), and between DOT1L + nucleosome and DOT1L + H2B ubiquitinated nucleosome (#). (*C*) Cryo-EM structure of DOT1L bound to H2B ubiquitinated nucleosome (Nuc_H2B-Ub). The nucleosome is shown in gray, DOT1L is shown in yellow, and ubiquitin is shown in green. (*D*) Atomic model of DOT1L_Nuc is shown in two orientations. (*E*) Atomic model of DOT1L_Nuc_H2B-Ub is shown in two orientations. (*F*) Superimpositions of cryo-EM maps of DOT1L_Nuc and DOT1L_Nuc_H2B-Ub. The axes for measuring the relative rotation angles from the twofold axis of the nucleosome are shown in dotted lines. (*G*) An enlarged view of the DOT1L_Nuc_H2B-Ub. The locations of the C-terminal hydrophobic helix and the arginine anchor are indicated with the dotted boxes.

Based on the cryo-EM maps, we generated models of DOT1L_Nuc and DOT1L_Nuc_H2B-Ub by fitting the crystal structures of DOT1L (Protein Data Bank [PDB] ID 1NW3), the nucleosome (PDB ID 1KX3), and ubiquitin (PDB ID 1UBI), and the models were further refined with molecular dynamics flexible fitting (MDFF) (Fig. 1D,E; Supplemental Fig. S6; Ramage et al. 1994; Davey et al. 2002; Min et al. 2003; Trabuco et al. 2008). In the DOT1L_

Nuc complex, DOT1L bound to the nucleosome rotated clockwise by ~50 degrees from the twofold axis of the nucleosome (Fig. 1F). The C-terminal half of DOT1L containing the catalytic pocket is located near the target histone H3 Lys79. However, the N-terminal half of DOT1L does not interact with either DNA or histones. In this configuration, the region near the catalytic pocket is attached to the nucleosome, and the N-terminal half is detached from the nucleosome with ~25° from the symmetric axis of the nucleosome from the side view. In the DOT1L_Nuc_H2B-Ub complex, the overall binding mode of DOT1L is overall similar to the DOT1L_Nuc complex (Fig. 1B,E). Superimposition between cryo-EM maps of DOT1L_Nuc and DOT1L_Nuc_H2B-Ub revealed that the DOT1L with H2B-Ub nucleosome is further rotated ~20°, and the N-terminal half is also further detached from the nucleosome ~20° compared with DOT1L_Nuc (Fig. 1F,G).

The distances between S-adenosyl methionines (SAMs) and the target histone H3 Lys79 are ~25 Å for DOT1L_Nuc and ~20 Å for DOT1L_Nuc_H2B-Ub (Fig. 1D,E). The closer distance of DOT1L to the Nuc_H2B-Ub nucleosome than DOT1L to Nuc suggests that the H2B-Ub might reorient DOT1L toward the target histone H3 Lys79. However, the distances between SAM and H3 Lys79 in both structures are too far to methylate the target, suggesting that the current structures might be catalytically inactive states in which the substrate is not fully engaged. These results show that the C-terminal part of the DOT1L catalytic domain interacts with the nucleosome, while the N-terminal part is detached from the nucleosome, and DOT1L might have multiple binding orientations, suggesting that H2B ubiquitination might reorient DOT1L into a more active orientation.

The recognition of H2B ubiquitin by DOT1L via a hydrophobic C-terminal helix

Histone H3 Lys79 methylation by DOT1L is stimulated by H2B ubiquitination, indicating the direct interaction between DOT1L and the ubiquitin. Unfortunately, our cryo-EM map of DOT1L_Nuc_H2B-Ub alone did not allow us to place the exact rotational position of the ubiquitin due to the poor map of the ubiquitin. However, the following MDFF run that considered the cryo-EM density, the steric hinderance with DOT1L, and the interaction compatibility between residues of ubiquitin and of the DOT1L C-terminal domain allowed us to place ubiquitin. The resulting cryo-EM structures clearly show the close proximity of H2B ubiquitin and the C-terminal helix of the DOT1L catalytic domain (Fig. 2A; Supplemental Fig. S6B). A previous mutational analysis of the surface of ubiquitin revealed that the hydrophobic surface near L71 and L73 located at the C-terminal of ubiquitin is critical for DOT1L activity (Holt et al. 2015).

In our model, the C-terminal helix in DOT1L is facing the previously identified hydrophobic surface, including L71 and L73 in the ubiquitin in H2B. The C-terminal helix (321–332 amino acids) of the DOT1L catalytic domain exhibits an amphipathic character in which one side of the helix is hydrophobic and the other side is hydrophilic. Several hydrophobic residues (L322, F326, and L329) in the helix together with hydrophobic residues (L284 and I290) in the loop near the helix form a hydrophobic patch (Fig. 2A,B). These data suggest that DOT1L might recognize

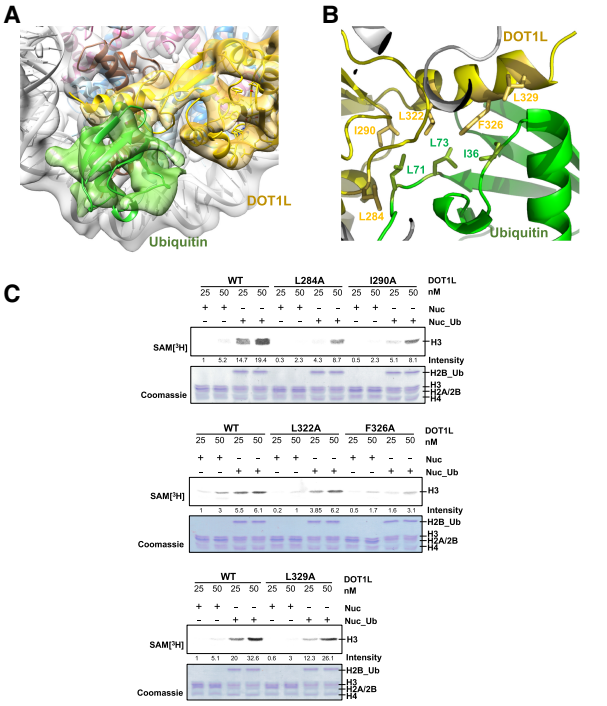

Figure 2. DOT1L recognizes the H2B Lys120 ubiquitin via the C-terminal hydrophobic helix. (*A*) Cryo-EM map with the model near the C-terminal hydrophobic helix. (*B*) Detailed interaction between the C-terminal hydrophobic helix and H2B ubiquitin. (*C*) Histone methyltransferase assay with wild type (WT) and the mutants, in which the hydrophobic residues (L284, I290, L322, F326, and L329) were mutated to alanines. (*Top* panel) Autoradiographs for methylated histone H3 Lys79 with ^{3}H-SAM. (*Bottom* panel) Half of each reaction was run in a separate gel and stained with Coomassie blue for a loading control. Intensities of the methylated histones were measured and are written as numbers *below* the gel.

H2B ubiquitin via the hydrophobic C-terminal helix. Specifically, L322, F326, and L329 in the C-terminal hydrophobic helix in DOT1L seem to interact with the previously identified hydrophobic residues (L71 and L73) together with I36 in H2B ubiquitin, and L284 is located near the C terminus of the ubiquitin (Fig. 2B). To determine whether these hydrophobic residues in DOT1L are critical for DOT1L activity, we mutated these hydrophobic residues to alanines (L284A, I290A, L322A, F326A, and L329A) and examined the DOT1L HMTase activity (Fig. 2C; Supplemental Fig. S1C). Except for L322A, all alanine mutants significantly decreased DOT1 activity. These results reveal that DOT1L recognizes H2B-Ub via the C-terminal hydrophobic helix of DOT1L and that these interactions are important for DOT1L HMTase activity.

DOT1L recognizes the H2A/H2B acidic patch in the nucleosome via an arginine anchor

Several nucleosome-binding proteins use arginine anchors to interact with the H2A/H2B acidic patch (Makde et al. 2010; Armache et al. 2011; Kato et al. 2013; Morgan et al. 2016). Interestingly, two conserved arginines (R278 and R282) exist in the loop between amino acids 268 and 286 in DOT1L. The cryo-EM structures together

with the HDX-MS data show that these arginines recognize the H2A/H2B acidic patch, suggesting that DOT1L might also use the arginine anchor to recognize the acidic patch in the nucleosome (Fig. 3A–C). The structure shows that R282 seems to directly interact with E61 and E63 from H2A, and E110 from H2B and R278 is located close to these glutamates (Fig. 3B). To examine the role of the arginine anchor for DOT1L activity, we mutagenized the arginines to alanines (R278A and R282A) and measured DOT1L HMTase activities (Fig. 3D; Supplemental Fig. S1C). The arginine mutant $DOT1L_{R282A}$, but not $DOT1L_{R278A}$, dramatically decreased DOT1L activity. These mutagenesis studies show that the arginine anchor is critical for H2B-Ub-mediated DOT1L activation via interacting with the acidic patch in the nucleosome as other nucleosome-binding proteins.

DOT1L binding destabilizes the nucleosome structure

Unexpectedly, the cryo-EM structures show that DNA is detached from the histone octamer and that the degree of

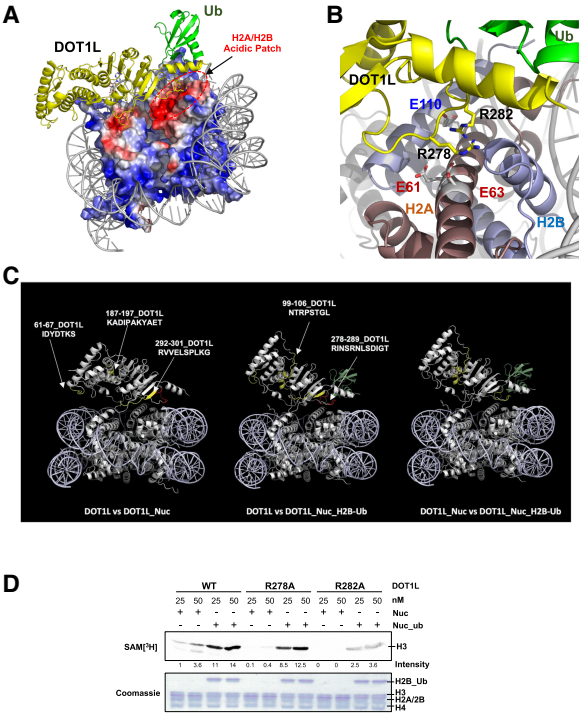

Figure 3. DOT1L recognizes the H2A/H2B acidic patch with the arginine anchor. (*A*) The arginine anchor is located near the H2A/H2B acidic patch. (*B*) Detailed view of the interaction between the arginines (R278 and R282) in the arginine anchor and the glutamates ($E61_{H2A}$, $E63_{H2A}$, and $E110_{H2B}$) in the H2A/H2B acidic patch. (*C*) The peptides analyzed in the HDX-MS analysis in Figure 1C are shown in the structures. The peptides colored in red indicate a higher difference in the deuterium uptakes than the peptides in yellow. The arginine anchor is located in the peptide colored in red (amino acids 278–289). H2B ubiquitin is shown in pale green, and DNA is shown in pale blue. (*D*) DOT1L HMTase assay with WT and the arginine anchor mutants (R278A and R282A). (*Top* panel) Autoradiographs for methylated histone H3 Lys79 with ^{3}H-SAM. (*Bottom* panel) Half of each reaction was run in a separate gel and stained with Coomassie blue for a loading control. Intensities of the methylated histones were measured and are written as numbers *below* the gel.

the detachment is greater in DOT1L_Nuc_H2B-Ub compared with DOT1L_Nuc (Fig. 4A). Furthermore, the first helix of histone H3 and the C terminus of histone H2B were concomitantly disordered, with DNA being detached (Fig. 4B). These results suggest that the DOT1L binding to the nucleosome might destabilize the nucleosome structure. Consistent with these observations, a recent study showed that DOT1L-bound nucleosome is more readily remodeled by the CHD1 ATP-dependent chromatin remodeler than the nucleosome alone (Lee et al. 2018).

To examine whether the binding of DOT1L destabilizes the nucleosome structure, we analyzed the detachment of DNA from histones using single-molecule FRET experiments. We labeled Cy3 at $H2A_{T120C}$ and Cy5 at the 5′ end of DNA proximal to Cy3 as a FRET pair (Supplemental Fig. S7). The unmodified Nuc or Nuc_H2B-Ub containing the FRET pair was immobilized with biotins conjugated at the other end of DNA on a streptavidin-coated surface (Fig. 5A). DOT1L without SAM was then injected, and the FRET changes were monitored (Fig. 5B). Upon DOT1L injection, the Cy5 signal slightly increased, indicating DOT1L binding to nucleosomes. After initial DOT1L binding, FRET down-spikes were clearly observed, which indicates the detachment of DNA from histones.

We next measured and compared the dwell times (t_d) of the low FRETs, which indicate the duration periods that the DNA stays detached from histones (Fig. 5C). In Nuc_H2B-Ub (Nuc-Ub), DOT1L binding increased the dwell time (t_d) of the low-FRET state approximately ninefold compared with nucleosome alone, while in unmodified Nuc (Nuc), DOT1L binding slightly increased the dwell time (t_d) of the low-FRET state. These data clearly show that the DNA detachment lasts substantially longer in Nuc_H2B-Ub than unmodified Nuc upon DOT1L binding. Furthermore, we examined whether the C-terminal ubiquitin-binding helix (DOT1L F326A) or the arginine anchor (DOT1L R282A) mutations affect the unwrapping of DNA. First, we measured whether these mutants affect the binding ability of DOT1L toward the nucleosome by gel shift assay and single-molecule FRET (Supplemental Figs. 8, 9).

Interestingly, these mutants, which have defects in HMTase activities, showed nucleosome binding comparable with the wild type (WT). However, Figure 5C clearly shows that both mutants exhibited substantial decreases in the dwell time (t_d) of the low FRET, indicating that these mutants are defective in DNA unwrapping. These results from the single-molecule FRET analysis are consistent with our cryo-EM structures showing that the DNA is further detached from histones in DOT1L_ Nuc_H2B-Ub than DOT1L_Nuc. In addition, these data suggest that the interactions between DOT1L and the nucleosome via the C-terminal Ub-binding helix and the arginine anchor might also play roles in the DNA unwrapping as well as HMTase activity and that the DNA unwrapping is likely an attribute of DOT1L function.

To rule out the possibility that DOT1L prefers DNA-unwrapped nucleosome as substrates, we prepared nucleosomes in which a few residues in histone H3 contacting the exit and entry of nucleosomal DNA were mutated (H39A, Y41A, and R49D), leading to DNA unwrapping, and the DNA unwrapping was confirmed by MNase analysis (Supplemental Fig. S10). We then used these nucleosomes for DOT1L HMTase assay. Supplemental Fig. S10 shows that DOT1L methylates the WT nucleosome and the DNA-unwrapped nucleosomes comparably.

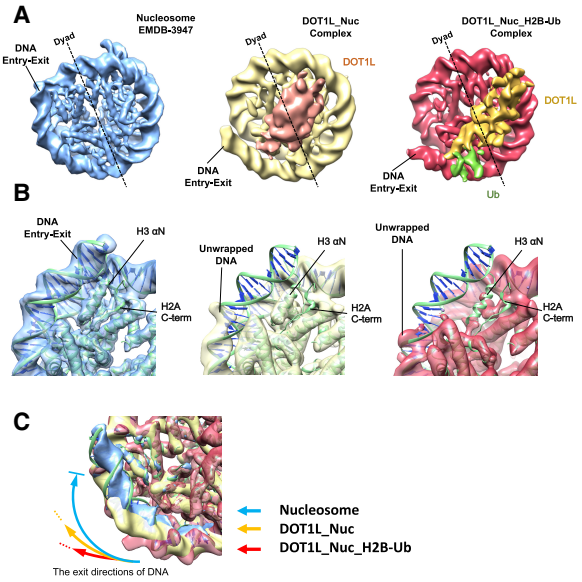

Figure 4. DOT1L-mediated nucleosome destabilization. (*A*) DOT1L binding to nucleosome leads to detachment of DNA and disorder of the first helix of histone H3 and the C terminus of histone H2B. Cryo-EM maps comparing the positions of the DNA exit and entry sites in the canonical nucleosome (EMDB-3947), DOT1L_Nuc, and DOT1L_Nuc_H2B-Ub. The DNA at the exit/entry are progressively detached from DOT1L_Nuc to DOT1L_Nuc_H2B-Ub. (*B*) Detailed view near the DNA exit/entry sites and the histone H3 and histone H2A, which are disordered in DOT1L complexes. (*C*) Comparison of the exit directions of DNA in the canonical nucleosome, DOT1L_Nuc, and DOT1L_Nuc_H2B-Ub.

These results suggest that DNA unwrapping does not affect DOT1L HMTase activity.

Overall, our single-molecule FRET combined with the cryo-EM structures suggest that DOT1L binding to nucleosomes together with the ubiquitination of H2B greatly destabilizes the nucleosome structure. These data demonstrate the molecular basis of the noncatalytic function of DOT1L, which destabilizes the nucleosome structure (Figs. 4C, 5D). Furthermore, these results add another layer of complexity to the cross-talk, where H2B ubiquitination regulates the DOT1L-mediated nucleosome destabilization in addition to histone H3 Lys79 methylation. As previous works showed that H2B ubiquitination plays important roles in transcription elongation and nucleosome dynamics, DOT1L-mediated DNA unwrapping might facilitate this process (Wu et al. 2014; Krajewski et al. 2018; Lee et al. 2018). At this moment, it is not clear how DOT1L binding induces the detachment of DNA with the concomitant disorder of the parts of histones. There are no substantial conformational changes in histones between Nuc alone and Nuc bound to DOT1L, although we cannot rule out that the conformational change is too small to be observed at the current resolution. A recent cryo-EM work on the nucleosome itself showed that the nucleosome is a highly dynamic structure, as the captured cryo-EM structures of nucleosomes in which DNA is unwrapped as well as DNA are bulged out (Bilokapic et al. 2018). Therefore, it can be imagined that DOT1L binding to nucleosome might stabilize one of these dynamic intermediate structures of the nucleosome. However, further biophysical and biochemical studies would be required to reveal the exact mechanism of the DNA unwrapping mediated by DOT1L.

Nonetheless, our single-molecule studies combined with the structures imply that DOT1L has nucleosome-destabilizing activity as well as histone methyltransferase activity and that both activities are affected by H2B ubiquitination.

The results presented here provide the first structural insights into the recognition of the H2B ubiquitinated nucleosome by DOT1L. DOT1L recognizes the H2B ubiquitinated nucleosome via the hydrophobic C-terminal helix for the ubiquitin and the basic anchors for the acidic patch. Furthermore, this work provides the structural evidence of DOT1L-mediated nucleosome destabilization that is enhanced by H2B ubiquitination. These data

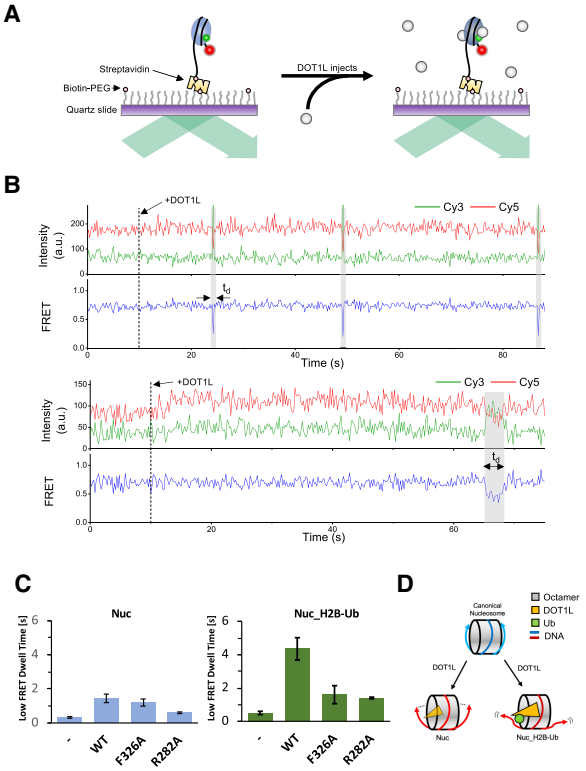

Figure 5. Single-molecule FRET showing the destabilization of nucleosome upon DOT1L binding. (*A*) Experimental scheme. Nucleosomes labeled with a FRET pair (green circle for Cy3 and red circle for Cy5) were immobilized on a microscope slide. DOT1L was added, while single-molecule fluorescence signals were observed. (*B*) Representative fluorescence intensity (green for Cy3 and red for Cy5) and corresponding FRET (blue) time traces of the unmodified nucleosome (*top*) and the ubiquitinated nucleosome (*bottom*). DOT1L was injected at 10 sec (dashed lines and black arrows). The increase of Cy5 signal after DOT1L addition indicates the binding of DOT1L to a nucleosome. DNA detachment (FRET down-spike) with duration t_d (gray region) was more clearly observed after DOT1L binding. (*C*) Low FRET dwell times of unmodified and ubiquitinated nucleosomes in the absence and presence of DOT1L WT, the DOT1L F326A mutant, and the DOT1L R282A mutant. The data are shown as means ± SEM of five independent experiments. The number of molecules used for the analysis were 181 for Nuc alone, 181 for Nuc + DOT1L WT, 153 for Nuc + DOT1L F326A, 147 for Nuc + DOT1L R281A, 170 for Nuc-Ub, 170 for Nuc-Ub + DOT1L WT, 134 for Nuc-Ub + DOT1L F326A, and 169 for Nuc + DOT1L R281A. (*D*) A schematic model for DOT1L-mediated nucleosome destabilization.

implicate the complexity of a cross-talk between H2B ubiquitinylation and DOT1L activity.

Materials and methods

Protein purification

The DOT1L catalytic domain was expressed as a GST fusion in *Escherichia coli* and purified with glutahione S-transferase (GST) affinity chromatography followed by cation exchange chromatography and size exclusion chromatography. The ubiquitinated H2B was chemically cross-linked with Ub_{G76C} and $H2B_{K120C}$ mutants and further purified using N-terminal His tag at ubiquitin.

Cryo-EM

DOT1L_Nuc complexes were prepared with ultracentrifugation in the presence of glutaraldehyde as a cross-linker. Micrograph images were collected using a Titan Krios 300KeV with a K2 Summit direct detector.

Single-molecule FRET

DNA in the nucleosomes was labeled with Cy5 at one end and biotin at the other end, and Cy3 was labeled at the H2A T120C residue. The nucleosome was immobilized, and florescence images were acquired using a total internal reflection fluorescence microscope.

Detailed Materials and Methods are described in the Supplemental Material.

Acknowledgments

We thank the Song laboratory members for helpful discussion. We thank Dr. Carol Soomin Cho and Dr. Soung-Hun Roh for critical comments on the manuscript. We also thank the staff at the SciLifeLab, Stockholm, for the data collection. This work is supported by grants from the National Research Foundation (NRF) of Korea to J.-J.S. (NRF-2016R1A2B3006293 and NRF-2016K1A1A2912057) and K.Y.C. (NRF-2012R1A5A2A28671860), and a grant from the Swedish Research Council to H.H. (2016-03810). The computing resource was supported by the Global Science Experimental Data Hub Center (GSDC), Korea Institute of Science and Technology Information (KISTI; NRF-2018R1A6A7052113). S.J. is a recipient of the Global Fellowship (NRF-2016H1A2A1908806) from Korea National Research Foundation.

Author contributions: S.J. and J.-J.S. conceived the idea, and S.J., T.J., H.H., and J.-J.S. determined the cryo-EM structures. C.K. and S.H. performed the single-molecule FRET experiments, H.-S.Y. and K.Y.C. performed the HDX-MS experiments, and S.J.K. performed the molecular dynamics flexible fitting. S.J. and J.-J.S. wrote the manuscript, and all authors reviewed the data and the manuscript.

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