Heterologous expression reveals distinct enzymatic activities of two DOT1 histone methyltransferases of *Trypanosoma brucei*

Floor Frederiks[1,2], Tibor van Welsem[1,2], Gideon Oudgenoeg[2,3], Albert J. R. Heck[2,3], Christian J. Janzen[4,*] and Fred van Leeuwen[1,2,*]

[1]Division of Gene Regulation, The Netherlands Cancer Institute, 1066 CX, Amsterdam, The Netherlands
[2]Netherlands Proteomics Centre
[3]Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Research, and Utrecht University, 3584 CH, Utrecht, The Netherlands
[4]Department of Biology, Genetics, University of Munich (LMU), 82152 Martinsried, Germany
*Authors for correspondence (cjanzen@lmu.de; fred.v.leeuwen@nki.nl)

Accepted 31 August 2010
Journal of Cell Science 123, 4019-4023

doi:10.1242/jcs.073882

Summary

Dot1 is a highly conserved methyltransferase that modifies histone H3 on the nucleosome core surface. In contrast to yeast, flies, and humans where a single Dot1 enzyme is responsible for all methylation of H3 lysine 79 (H3K79), African trypanosomes express two DOT1 proteins that methylate histone H3K76 (corresponding to H3K79 in other organisms) in a cell-cycle-regulated manner. Whereas DOT1A is essential for normal cell cycle progression, DOT1B is involved in differentiation and control of antigenic variation of this protozoan parasite. Analysis of DOT1A and DOT1B in trypanosomes or in vitro, to understand how H3K76 methylation is controlled during the cell cycle, is complicated by the lack of genetic tools and biochemical assays. To eliminate these problems, we developed a heterologous expression system in yeast. Whereas *Trypanosoma brucei* DOT1A predominantly dimethylated H3K79, DOT1B trimethylated H3K79 even in the absence of dimethylation by DOT1A. Furthermore, DOT1A activity was selectively reduced by eliminating ubiquitylation of H2B. The tail of histone H4 was not required for activity of DOT1A or DOT1B. These findings in yeast provide new insights into possible mechanisms of regulation of H3K76 methylation in *Trypanosoma brucei*.

Key words: Dot1, Histone, Methylation, Chromatin, Yeast, Trypanosoma, Antigenic variation

Introduction

Post-translational modifications of histone proteins have crucial roles in DNA-related processes, such as gene regulation, DNA repair and replication (Campos and Reinberg, 2009; Dinant et al., 2008; Li et al., 2007). One highly conserved core modification is methylation of lysine 79 of histone H3 (H3K79) by the Dot1 family of histone methyltransferases. In contrast to yeast, flies, and humans, the protozoan parasite *Trypanosoma brucei* expresses two Dot1-like proteins that methylate histone H3K76, the *T. brucei* counterpart of H3K79. DOT1A is an essential enzyme, whereas DOT1B is dispensable for normal growth but is essential for the differentiation of bloodstream form to procyclic (insect-stage) trypanosomes (Janzen et al., 2006). Furthermore, DOT1B is involved in the tight repression and switching of telomeric variant surface glycoprotein (VSG) gene expression site promoters (Figueiredo et al., 2008; Stockdale et al., 2008), indicating a role for DOT1B in antigenic variation of this parasite (Taylor and Rudenko, 2006; Verstrepen and Fink, 2009).

DOT1A and DOT1B are most likely to exert their different functions by differentially affecting the methylation state of H3K76. Di- and trimethylation (H3K76me2 and K76me3, respectively) appear to be the predominant H3K76 methylation states in *T. brucei* (Janzen et al., 2006). Immunostaining experiments revealed that H3K76me3 is constitutively detectable throughout the cell cycle, whereas H3K76me2 is restricted to mitosis (Janzen et al., 2006). Genetic studies suggest that DOT1B is responsible for H3K76 trimethylation because inactivation of DOT1B results in loss of H3K76me3 and an increase in H3K76me2 (Janzen et al., 2006). RNAi-mediated depletion of the essential DOT1A protein results in a partial decrease in H3K76me2 (Janzen et al., 2006), indicating that DOT1A might be responsible for the H3K76me2 peak in M phase.

To determine how the H3K76 methylation pattern is controlled in vivo, it is important to understand the substrate specificities of the two DOT1 enzymes and how they can be regulated. Dot1 enzymes recognize a part of H3 on the nucleosome surface that is in close proximity to histone H4 and DNA. They, therefore, are most likely to interact with a very complex binding surface. In-vitro studies on Dot1 proteins to determine their enzymatic properties are complicated by the lack of activity of Dot1 towards free histone H3 and short peptides, and its weak activity towards reconstituted nucleosome substrates (Feng et al., 2002; Janzen et al., 2006; McGinty et al., 2008; Sawada et al., 2004). Interestingly, DOT1B efficiently trimethylates H3K76-dimethylated native chromatin isolated from DOT1B knockout trypanosomes in vitro (Janzen et al., 2006). However, whether DOT1B requires prior dimethylation of H3K76 by DOT1A remained unknown. Since DOT1A is an essential enzyme it is currently not possible to purify native chromatin substrates that lack H3K76 methylation from *T. brucei* for in-vitro studies. To circumvent these problems, we used a heterologous expression system in *Saccharomyces cerevisiae* to investigate the activities and substrate specificities of DOT1A and DOT1B.

Results and Discussion

Characterization of the activity of DOT1A and DOT1B in yeast

To investigate the activity of DOT1A and DOT1B, they were expressed individually in *dot1Δ* yeast strains that lack endogenous H3K79 methylation. Quantitative mass spectrometry and immunoblot analysis using antibodies specific for the TAP tag and mono-, di-, and trimethylated H3K79 showed that DOT1A and DOT1B were expressed (supplementary material Fig. S1) and that they very efficiently methylated H3K79 in yeast (Fig. 1A-D). The highly conserved GxGxG motif (Fig. 1A-B) is essential for catalytic activity of Dot1 enzymes (Cheng et al., 2005; Cheng and Zhang, 2007; Frederiks et al., 2008). Catalytic mutants of DOT1A and DOT1B in which the central glycine of the GxGxG motif was mutated to arginine (DOT1A-G138R and DOT1B-G121R) showed no H3K79 methylation (Fig. 1D). We noticed that the expression of DOT1B-G121R was somewhat reduced (supplementary material Fig. S1). Interestingly, although DOT1A and DOT1B both methylated the vast majority of H3 in yeast, they showed a very distinct methylation pattern. DOT1A expression resulted in abundant H3K79me2 (Fig. 1C), which is in agreement with the observations in *T. brucei* (Janzen et al., 2006). Expression of DOT1B resulted in abundant H3K79me3 (Fig. 1C), confirming that DOT1B is responsible for trimethylation of H3K76 in *T. brucei* (Janzen et al., 2006). The almost complete absence of H3K79me1 and H3K79me2 suggests that DOT1B more efficiently converts the lower methylation states into higher methylation states than ScDot1. Importantly, our results show that DOT1B was able to trimethylate H3K79 without prior dimethylation of H3K79 by DOT1A. How this impinges on the regulation of H3K76 methylation in *T. brucei* is discussed below.

H2B ubiquitylation enhances methylation by DOT1A

Having established an expression system for DOT1A and DOT1B that recapitulates the properties of the enzymes in *T. brucei*, we took advantage of yeast genetics to investigate the possible modes of regulation of H3K76 methylation. In yeast and humans, ubiquitylation of a C-terminal lysine on H2B (H2Bub) by the ubiquitin ligase Bre1 stimulates the activity of Dot1 (Briggs et al., 2002; Frederiks et al., 2008; Krogan et al., 2003; McGinty et al., 2008; Nakanishi et al., 2009; Ng et al., 2002; Schulze et al., 2009; Shahbazian et al., 2005; Wood et al., 2003; Zhu et al., 2005). The molecular mechanism of this activation is unknown but the positive effect of H2Bub on the activity of human Dot1 does not require additional factors (McGinty et al., 2008). Therefore, we first asked whether methylation by TbDOT1 in yeast is affected by ubiquitylation of H2BK123 (H2BK123ub). For that purpose, DOT1A and DOT1B were expressed in *dot1Δbre1Δ* cells, which lack endogenous H3K79 methylation and H2BK123ub. In this *bre1Δ* background, ScDot1 showed less H3K79me3 and more H3K79me1 than in wild-type cells (Fig. 1E), confirming the slow accumulation of methylation in the absence of H2BK123ub (Frederiks et al., 2008). The DOT1A activity was similarly affected by H2BK123ub, albeit somewhat less than ScDot1. By contrast, DOT1B showed only a subtle increase in H3K79me2 and H3K79me1 in *bre1Δ* cells, indicating a minor effect of H2BK123ub on this enzyme (Fig. 1E). Thus, chromatin ubiquitylation can differentially affect the activity of *T. brucei* DOT1 enzymes in yeast.

DOT1A and DOT1B do not require the tail of histone H4

The negatively charged C-terminus of ScDot1 directly binds to a positively charged patch on the N-terminal tail of histone H4 in vitro, and this interaction is required for full activity of ScDot1 in vivo (Altaf et al., 2007; Fingerman et al., 2007). Many of the positively charged residues on the H4 tail are conserved between yeast and trypanosomes, although the overall sequence of the tail is less conserved than that of the core (Fig. 2A). ScDot1 also binds to nucleosomes via its N-terminal domain in vitro (Altaf et al., 2007; Sawada et al., 2004). Interestingly, DOT1A and DOT1B lack the known chromatin-binding domains (Fig. 1A), indicating that they have different targeting mechanisms or, perhaps, lack specific targeting mechanisms, the latter of which would be in line with their global activity in *T. brucei* (Janzen et al., 2006). Each canonical histone protein in yeast is encoded by only two genes, which offers the possibility to delete one copy and make mutations

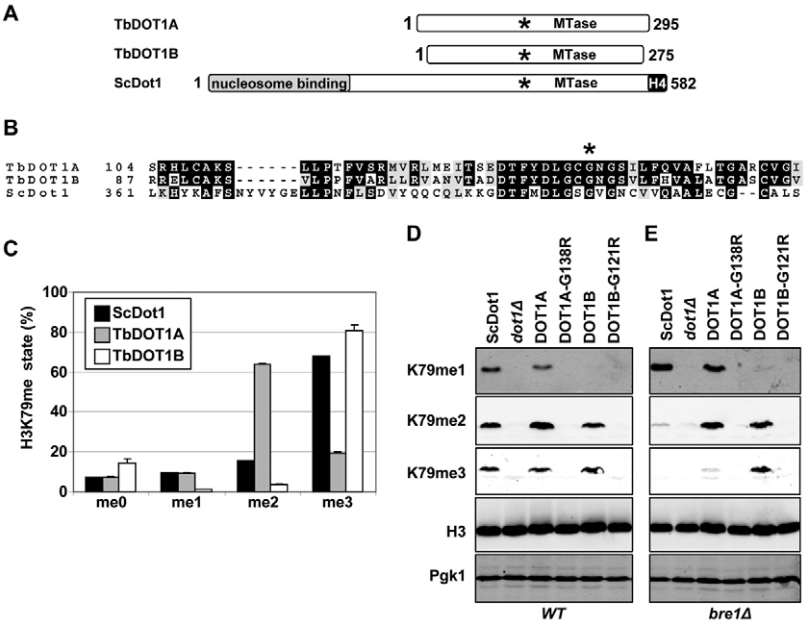

Fig. 1. Characterization of the activity of DOT1A and DOT1B in yeast. (**A**) Schematic representation of TbDOT1A, TbDOT1B and ScDot1. Asterisks indicate the highly conserved GxGxG motif of the catalytic domain. (**B**) Active sites of Dot1 enzymes aligned with ClustalW and BoxShade (EMBnet). (**C**) Quantitative mass spectrometry analysis of H3K79 methylation in wild-type yeast strain UCC7164 or *dot1Δ* strain UCC7183 expressing DOT1A or DOT1B. Error bars indicate the spread of the data of two biological duplicates. No H3K79 methylation was detected in a *dot1Δ* strain (see also panel D). (**D**) Immunoblot analysis of H3K79 methylation in *dot1Δ* strain UCC7183 expressing TbDOT1 proteins. ScDot1 corresponds to endogenous Dot1 in wild-type strain UCC7164 carrying an empty vector. Antibodies against the C-terminus of histone H3 and Pgk1 were used as loading controls. (**E**) Methylation of H3K79 by DOT1A and DOT1B or inactive mutants of these enzymes in a *dot1Δ bre1Δ* strain that lacks H2BK123 ubiquitylation and endogenous Dot1. The blots in D and E were analyzed on the same gel and scanned at the same time, and can therefore be directly compared.

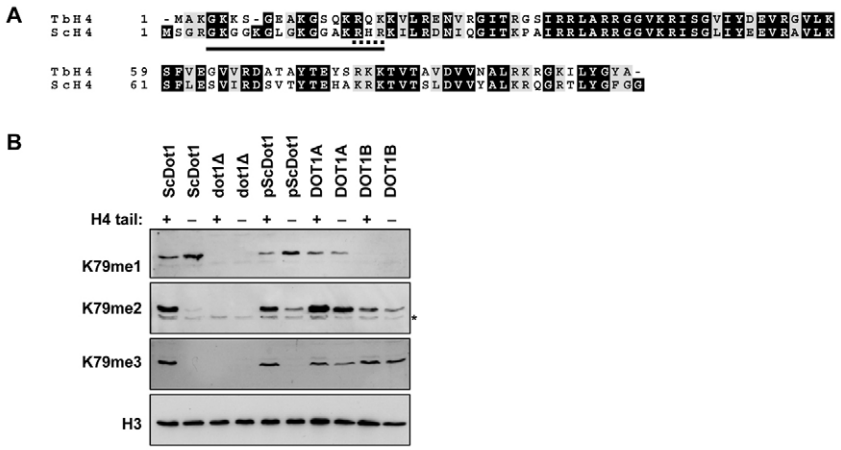

Fig. 2. DOT1A and DOT1B do not require the tail of histone H4. (**A**) Alignment of TbH4 and ScH4. The tail region deleted in H4Δ4-19 is underlined. Dotted line indicates positively charged positions crucial for ScDot1 activity. (**B**) Immunoblot analysis of the role of the N-terminal tail of H4 (H4Δ4-19; + or – H4-tail) in H3K79 methylation by DOT1A and DOT1B in a *dot1Δ* strain. ScDot1 indicates endogenous Dot1 in a *DOT1+* strain, pScDot1 indicates Dot1 expressed from a 2μ-plasmid. Asterisk indicates a non-specific band.

in the remaining copy. Using this possibility, we investigated the activity of DOT1A and DOT1B in yeast cells that lack the tail of histone H4 (H4Δ4-19; Fig. 2A). In agreement with previous findings (Altaf et al., 2007; Fingerman et al., 2007), in these cells the activity of ScDot1 was severely reduced compared with wild-type cells, as indicated by the loss of H3K79me3 and H3K79me2 (Fig. 2B). However, H3K79 methylation by DOT1A and DOT1B was not or only slightly reduced in cells expressing H4Δ4-19 as compared with wild type cells (Fig. 2B). Therefore, DOT1A and DOT1B did not require interactions with the H4-tail for efficient methylation in yeast.

The context of H3K79 influences the activity of DOT1B

There are several amino acid differences between trypanosome and yeast histone H3, in particular in the N-terminal tail and the sequence surrounding H3K76 and H3K79 (Fig. 3A). Our results show that DOT1A and DOT1B methylate H3K79 despite the divergent flanking sequence (Fig. 1). To more closely mimic the *T. brucei* H3 protein substrate, we replaced the yeast H3K79 flanking sequence (see the box in Fig. 3A) with the trypanosome H3K76 flanking sequence (Fig. 3A,B). The activity of DOT1A, DOT1B and ScDot1 towards the chimeric histone H3 in vivo was determined by immunoblot analysis using antibodies specific for TbH3K76me2 and TbH3K76me3, which were raised against methylated TbH3K76 peptides that are identical to the chimeric protein (Janzen et al., 2006), although the lysine residue is still at position 79 in yeast. Antibodies against H3K76me1 are currently not available. DOT1A generated H3K76me2 and low levels of H3K76me3 (Fig. 3C). This is similar to the activity of DOT1A towards wild-type yeast H3K79 (Fig. 1), even though yeast H3K79me and *T. brucei* H3K76me blots cannot be directly compared in a quantitative manner. By contrast, DOT1B expression

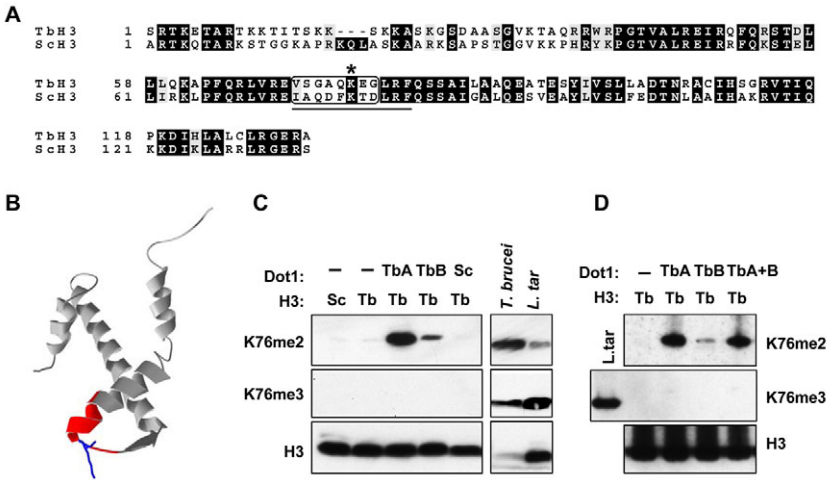

Fig. 3. The sequence flanking H3K79 affects methylation by TbDOT1 and ScDot1. (**A**) Alignment of histone H3 from *T. brucei* and *S. cerevisiae*. Box indicates the ScH3K79 region that was replaced by the TbK76 region. Asterisk indicates ScH3K79/TbH3K76. The underline indicates the peptide used to generate the antibodies specific for methylated TbH3K76 and ScH3K79. (**B**) Ribbon representation of yeast histone H3 (residues 38–134; PDB 1ID3) generated by Swiss-PDB viewer 3.7. The region that was replaced by the H3K76 region is indicated in red. Lysine 79 is shown with side chain in blue. (**C**) Immunoblot analysis of H3K76 methylation by DOT1A, DOT1B or ScDot1 on the chimeric H3 protein carrying the TbH3K76 region (Tb). Whole-cell lysates from *T. brucei* and *L. tarentolae* (L. tar) were used as positive controls for the H3K76me2 and H3K76me3 antibodies. The antibody against the C-terminus of yeast H3 is not affected by the swap of the core region and recognizes the H3 protein from trypanosomes, albeit with lower affinity (data not shown). (**D**) H3K76 methylation in strains co-expressing DOT1A and DOT1B.

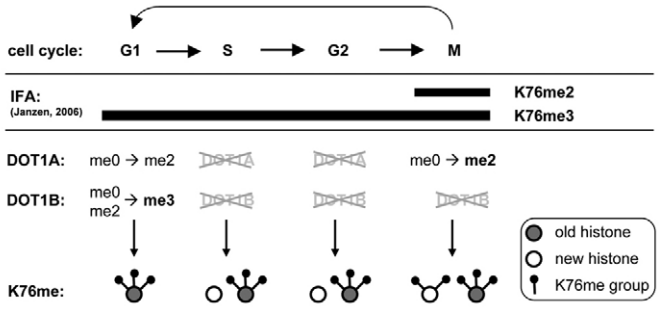

Fig. 4. A model for cell cycle regulation of H3K76 methylation in *T. brucei*. Using immunostaining (IFA) techniques, H3K76me3 is detectable throughout the *T. brucei* cell cycle, whereas H3K76me2 is not detectable from G1 until the end of G2 and is restricted to M phase (Janzen et al., 2006). Here, we show that DOT1B can methylate H3 in the absence of prior methylation by DOT1A (see Fig. 1). Details of the model are described in the text.

resulted in undetectable H3K76me3 and low H3K76me2 levels (Fig. 3C). ScDot1 was unable to di-, and tri-methylate the chimeric substrate (Fig. 3C). To determine whether DOT1B requires prior methylation of the chimeric H3 protein, DOT1A and DOT1B were co-expressed. This, however, did not result in detectable H3K76me3. Therefore, DOT1B was unable to efficiently methylate the chimeric H3 protein, even when H3K76me2 was present (Fig. 3D). We expect that in the context of the full yeast nucleosome, the *T. brucei* K76 region may adopt an unusual structure, even though the flanks of the loop are unchanged, and that DOT1B is more sensitive to the structural change.

Implications for regulation of histone methylation by DOT1A and DOT1B in *T. brucei*

To understand the cell-cycle related functions of DOT1A and the role of DOT1B in control of VSG expression sites, it is important to determine the enzymatic properties and modes of regulation of these enzymes. Unfortunately, methods that have been used for other Dot1 proteins can not be applied to the TbDOT1 enzymes. Human Dot1L has been well characterized by analysis of the activity of recombinant Dot1L towards defined reconstituted recombinant chromatin substrates or purified yeast chromatin templates (Chatterjee et al., 2010; McGinty et al., 2008; McGinty et al., 2009; Mohan et al., 2010). Attempts to reconstitute trypanosome chromatin have failed so far, and one of the two DOT1 proteins is essential, precluding the possibility to purify nucleosomes with unmodified H3K76 from DOT1-deficient parasites (Janzen et al., 2006). The enzymatic properties of yeast Dot1 have been best characterized by in vivo manipulation of the single yeast Dot1 enzyme using genetic tools (Frederiks et al., 2008; van Leeuwen et al., 2002). Since DOT1A is essential, a similar genetic approach is not possible for trypanosomes (Janzen et al., 2006). To circumvent these restrictions, we developed a heterologous expression system. Heterologous expression has previously been successfully applied for characterization of many other proteins (e.g. Jeong et al., 2007; Kunz et al., 2004; Luo et al., 2002; Marini et al., 2008). One possible limitation of heterologous expression is that the *T. brucei* DOT1 proteins expressed in yeast might lack their putative natural binding partners or might interact with unknown yeast proteins. Other approaches have similar intrinsic limitations, however. The activity of DOT1 proteins in their native environment in trypanosomes might also be influenced by putative interactions with additional factors and in in-vitro studies (if developed in the future) it is possible that crucial components or conditions are missing. However, the TbDOT1 proteins are mainly composed of a conserved methyltransferase core domain and lack the domains that are found in yeast or human Dot1 and that are known to be involved in protein- and DNA-interactions (Fig. 1). Furthermore, the observed activities of DOT1A and DOT1B in yeast are in agreement with our previous observations in *T. brucei* (Janzen et al., 2006).

Our characterization of the *T. brucei* Dot1 enzymes in yeast showed that DOT1A and DOT1B have distinct catalytic properties. Therefore, regulation of the degree of histone methylation can be achieved by differential regulation of the two enzymes, something that is not possible in yeast or humans, where a single Dot1 enzyme is present. That DOT1B can di- and trimethylate H3K79 in the absence of prior H3K76 methylation by DOT1A (Fig. 1) has implications for the regulation of DOT1B to establish the appearance of H3K76me2 during M phase. Using immunostaining techniques, H3K76me3 is detectable throughout the *T. brucei* cell cycle, whereas H3K76me2 is not detectable from G1 until the end of G2, and restricted to M phase and cytokinesis (Janzen et al., 2006). Our new findings suggest that DOT1B is most likely to be inactive during S and M phases towards the newly deposited unmodified histones to allow dimethylation of unmethylated H3K76 by DOT1A in M phase (Fig. 4). Briefly, our findings, together with our previous observations (Janzen et al., 2006) suggest (1) no or low activity of DOTA and DOT1B in S phase and G2, when new unmodified histones are present in the chromatin, (2) subsequent activation of DOT1A to dimethylate H3K76 on the new histones in M phase and, (3) activation of DOT1B after exit from M phase to convert lower H3K76 methylation states including H3K76me2 to H3K76me3 (Fig. 4). Alternatively, the H3K76 methylation state might be regulated by cell cycle-specific demethylases. However, no H3K76 or H3K79 demethylases have been described to date, and this model does not explain why DOT1A is present and why it is required for normal cell cycle progression.

Although the TbDOT1 proteins were not expressed in their native environment, our results offer some suggestions for possible mechanisms of regulation of H3K76 methylation in trypanosomes. For example, DOT1A activity was higher in yeast cells that contain ubiquitylated H2B (Fig. 1E). However, the specific histone crosstalk between H2B and H3K79 or H3K76 does probably not exist in *T. brucei* because the canonical H2B of *T. brucei* does not contain a lysine at the C-terminal tail, and K129 of the histone variant H2BV is not required for H3K76 methylation and most likely not ubiquitylated (Mandava et al., 2008). Therefore, the mechanism by which H2Bub affects Dot1 enzymes might be rather non-specific; for example, by changing the chromatin structure and, thereby, altering access to the substrate lysine on the nucleosome core. Another possibility is that ubiquitylation of another chromatin protein complements the role of H2Bub in *T. brucei*. This is not unlikely because it has recently been show that the exact structure of the ubiquitin moiety and its position on the nucleosome core are not crucial for the activation of human Dot1 (Chatterjee et al., 2010; McGinty et al., 2009). Finally, our finding that the ScH4-tail is not required for activity of DOT1A or DOT1B suggests that H3K76 methylation by DOT1A and DOT1B is not directly modulated by interactions of other chromatin proteins with the histone H4 tail, a mechanism that has been suggested for the yeast

heterochromatin protein Sir3 (Altaf et al., 2007; Fingerman et al., 2007; Norris and Boeke, 2010). For a full understanding of the possible mechanisms of regulation of DOT1A and DOT1B it will be important to develop new genetic and biochemical tools to determine the behavior of the two proteins in their native environment and in defined in-vitro assays.

Materials and Methods

Yeast strains and plasmids

Yeast cells were grown as described previously (Frederiks et al., 2008). Strains and plasmids are described in the supplementary material Tables 1 and 2.

Immunoblots and mass spectrometry

Histone methylation was analyzed by mass spectrometry and immunoblots as described previously (Frederiks et al., 2008). Primary antibodies used for immunodetections: H3, H3K79me1, H3K79me2 and H3K79me3 (Frederiks et al., 2008), Pgk1 (Invitrogen, A-6457), TAP (Open Biosystems, CAB1001), HA (12CA5), H3K76me2 and H3K76me3 (Janzen et al., 2006).

We thank Saara Vainio and Pankaj Tripathi for whole-cell lysates of *T. brucei* and *Leishmania tarentolae*. We thank members of the van Leeuwen and Janzen laboratories, and Piet Borst and Michael Boshart for critical reading of the manuscript. This work was supported by the EU 6th framework program (NOE 'The Epigenome' LSHG-CT-2004-503433), The Netherlands Organization for Scientific Research (NWO), The Netherlands Genomics Initiative and by the SFB TR5 of the LMU Munich. The authors declare that they have no competing financial interest.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/123/23/4019/DC1

References

Altaf, M., Utley, R. T., Lacoste, N., Tan, S., Briggs, S. D. and 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.

Bartel, P., Chien, C. T., Sternglanz, R. and Fields, S. (1993). Elimination of false positives that arise in using the two-hybrid system. *Biotechniques* **14**, 920-924.

Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P. and Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**, 115-132.

Briggs, S. D., Xiao, T., Sun, Z. W., Caldwell, J. A., Shabanowitz, J., Hunt, D. F., Allis, C. D. and Strahl, B. D. (2002). Gene silencing: trans-histone regulatory pathway in chromatin. *Nature* **418**, 498.

Campos, E. I. and Reinberg, D. (2009). Histones: annotating chromatin. *Annu. Rev. Genet.* **43**, 559-599.

Chatterjee, C., McGinty, R. K., Fierz, B. and Muir, T. W. (2010). Disulfide-directed histone ubiquitylation reveals plasticity in hDot1L activation. *Nat. Chem. Biol.* **6**, 267-269.

Cheng, X. and Zhang, X. (2007). Structural dynamics of protein lysine methylation and demethylation. *Mutat. Res.* **618**, 102-115.

Cheng, X., Collins, R. E. and Zhang, X. (2005). Structural and sequence motifs of protein (histone) methylation enzymes. *Annu. Rev. Biophys. Biomol. Struct.* **34**, 267-294.

Dinant, C., Houtsmuller, A. B. and Vermeulen, W. (2008). Chromatin structure and DNA damage repair. *Epigenetics Chromatin* **1**, 9.

Feng, Q., Wang, H., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Struhl, K. and 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.

Figueiredo, L., Janzen, C. and Cross, G. (2008). A histone methyltransferase modulates antigenic variation in African trypanosomes. *PLoS Biol.* **6**, e161.

Fingerman, I. M., Li, H. C. and Briggs, S. D. (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. and van Leeuwen, F. (2008). Nonprocessive methylation by Dot1 leads to functional redundancy of histone H3K79 methylation states. *Nat. Struct. Mol. Biol.* **15**, 550-557.

Frederiks, F., Heynen, G. J., van Deventer, S. J., Janssen, H. and van Leeuwen, F. (2009). Two Dot1 isoforms in Saccharomyces cerevisiae as a result of leaky scanning by the ribosome. *Nucleic Acids Res.* **21**, 7047-7058.

Goldstein, A. L. and McCusker, J. H. (1999). Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. *Yeast* **15**, 1541-1553.

Janzen, C. J., Hake, S. B., Lowell, J. E. and Cross, G. A. (2006). Selective di- or trimethylation of histone H3 lysine 76 by two DOT1 homologs is important for cell cycle regulation in Trypanosoma brucei. *Mol. Cell* **23**, 497-507.

Jeong, H., Herskowitz, I., Kroetz, D. L. and Rine, J. (2007). Function-altering SNPs in the human multidrug transporter gene ABCB1 identified using a Saccharomyces-based assay. *PLoS Genet.* **3**, e39.

Krogan, N. J., Dover, J., Wood, A., Schneider, J., Heidt, J., Boateng, M. A., Dean, K., Ryan, O. W., Golshani, A., Johnston, M. et al. (2003). The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol. Cell* **11**, 721-729.

Kunz, S., Kloeckner, T., Essen, L. O., Seebeck, T. and Boshart, M. (2004). TbPDE1, a novel class I phosphodiesterase of Trypanosoma brucei. *Eur. J. Biochem.* **271**, 637-647.

Li, B., Carey, M. and Workman, J. L. (2007). The role of chromatin during transcription. *Cell.* **128**, 707-719.

Luo, S., Scott, D. A. and Docampo, R. (2002). Trypanosoma cruzi H+-ATPase 1 (TcHA1) and 2 (TcHA2) genes complement yeast mutants defective in H+ pumps and encode plasma membrane P-type H+-ATPases with different enzymatic properties. *J. Biol. Chem.* **277**, 44497-44506.

Mandava, V., Janzen, C. J. and Cross, G. A. (2008). Trypanosome H2Bv replaces H2B in nucleosomes enriched for H3 K4 and K76 trimethylation. *Biochem. Biophys. Res. Commun.* **368**, 846-851.

Marini, N. J., Gin, J., Ziegle, J., Keho, K. H., Ginzinger, D., Gilbert, D. A. and Rine, J. (2008). The prevalence of folate-remedial MTHFR enzyme variants in humans. *Proc. Natl. Acad. Sci. USA* **105**, 8055-8060.

McGinty, R. K., Kim, J., Chatterjee, C., Roeder, R. G. and Muir, T. W. (2008). Chemically ubiquitylated histone H2B stimulates hDot1L-mediated intranucleosomal methylation. *Nature* **453**, 812-816.

McGinty, R. K., Kohn, M., Chatterjee, C., Chiang, K. P., Pratt, M. R. and Muir, T. W. (2009). Structure-activity analysis of semisynthetic nucleosomes: mechanistic insights into the stimulation of Dot1L by ubiquitylated histone H2B. *ACS Chem. Biol.* **4**, 958-968.

Mohan, M., Herz, H. M., Takahashi, Y. H., Lin, C., Lai, K. C., Zhang, Y., Washburn, M. P., Florens, L. and Shilatifard, A. (2010). Linking H3K79 trimethylation to Wnt signaling through a novel Dot1-containing complex (DotCom). *Genes Dev.* **24**, 574-589.

Nakanishi, S., Lee, J. S., Gardner, K. E., Gardner, J. M., Takahashi, Y. H., Chandrasekharan, M. B., Sun, Z. W., Osley, M. A., Strahl, B. D., Jaspersen, S. L. et al. (2009). Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1. *J. Cell Biol.* **186**, 371-377.

Ng, H. H., Xu, R. M., Zhang, Y. and Struhl, K. (2002). Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J. Biol Chem.* **277**, 34655-34657.

Norris, A. and Boeke, J. D. (2010). Silent information regulator 3: the Goldilocks of the silencing complex. *Genes Dev.* **24**, 115-122.

Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M. and Seraphin, B. (1999). A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol* **17**, 1030-1032.

Sawada, K., Yang, Z., Horton, J. R., Collins, R. E., Zhang, X. and 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.

Schulze, J. M., Jackson, J., Nakanishi, S., Gardner, J. M., Hentrich, T., Haug, J., Johnston, M., Jaspersen, S. L., Kobor, M. S. and 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.

Shahbazian, M. D., Zhang, K. and Grunstein, M. (2005). Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. *Mol. Cell* **19**, 271-277.

Singer, M. S., Kahana, A., Wolf, A. J., Meisinger, L. L., Peterson, S. E., Goggin, C., Mahowald, M. and Gottschling, D. E. (1998). Identification of high-copy disruptors of telomeric silencing in *Saccharomyces cerevisiae*. *Genetics* **150**, 613-632.

Stockdale, C., Swiderski, M., Barry, J. D. and McCulloch, R. (2008). Antigenic variation in *Trypanosoma brucei*: joining the DOTs. *PLoS Biol.* **6**, e185.

Taylor, J. E. and Rudenko, G. (2006). Switching trypanosome coats: what's in the wardrobe? *Trends Genet.* **22**, 614-620.

van Leeuwen, F., Gafken, P. R. and Gottschling, D. E. (2002). Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* **109**, 745-756.

Verstrepen, K. J. and Fink, G. R. (2009). Genetic and epigenetic mechanisms underlying cell-surface variability in protozoa and fungi. *Annu. Rev. Genet.* **43**, 1-24.

Wach, A., Brachat, A., Alberti-Segui, C., Rebischung, C. and Philippsen, P. (1997). Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. *Yeast* **13**, 1065-1075.

Wood, A., Schneider, J., Dover, J., Johnston, M. and Shilatifard, A. (2003). The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. *J. Biol. Chem.* **278**, 34739-34742.

Zhu, B., Zheng, Y., Pham, A. D., Mandal, S. S., Erdjument-Bromage, H., Tempst, P. and Reinberg, D. (2005). Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. *Mol. Cell* **20**, 601-611.