Methylation of H3-Lysine 79 Is Mediated by a New Family of HMTases without a SET Domain

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Summary

The N-terminal tails of core histones are subjected to multiple covalent modifications, including acetylation, methylation, and phosphorylation [1]. Similar to acetylation, histone methylation has emerged as an important player in regulating chromatin dynamics and gene activity [2–4]. Histone methylation occurs on arginine and lysine residues and is catalyzed by two families of proteins, the protein arginine methyltransferase family and the SET-domain-containing methyltransferase family [3]. Here, we report that lysine 79 (K79) of H3, located in the globular domain, can be methylated. K79 methylation occurs in a variety of organisms ranging from yeast to human. In budding yeast, K79 methylation is mediated by the silencing protein DOT1. Consistent with conservation of K79 methylation, DOT1 homologs can be found in a variety of eukaryotic organisms. We identified a human DOT1-like (DOT1L) protein and demonstrated that this protein possesses intrinsic H3-K79-specific histone methyltransferase (HMTase) activity in vitro and in vivo. Furthermore, we found that K79 methylation level is regulated throughout the cell cycle. Thus, our studies reveal a new methylation site and define a novel family of histone lysine methyltransferase.

Results and Discussion

Identification of H3-K79 as a Novel Methylation Site

Histone methylation has emerged as an important player in regulating gene expression and chromatin function [2–4]. Histone methylation occurs on arginine and lysine residues at the N-terminal tails of histones H3 and H4 and is catalyzed by two distinct family of proteins, the PRMT1 and the SET-domain-containing family of proteins [3]. Since the discovery of the first histone lysine methyltransferase [5], other lysine methyltransferases that methylate histone H3 at lysines 4, 9, 27, and 36

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have been reported [6–14]. One common feature of these histone lysine methyltransferases is that they all contain a SET domain that is required for their enzymatic activity. Thus, SET domain is believed to be a signature motif for histone lysine methyltransferase [3].

So far, all the known methylation sites are located at the N terminus of histones H3 and H4 [2, 3]. Due to the limitations of detection methods, other potential modifications either at low abundance or located in the histone globular domain might be missed. We therefore employed mass spectrometry to identify potential novel modification sites. Histone H3 purified from HeLa cells was subjected to tryptic digestion. Analysis of the resulting peptides revealed that most of the major ions matched the calculated monoisotopic masses of predicted peptides (Figure 1Aa). However, one ion (m/z = 1349.72) is 14.03 Da heavier than the predicted mass of an H3 tryptic peptide "EIAQDFKTDLR" corresponding to amino acids 73–83. Such a mass difference could be a result of methylation (DeltaMass database: http://www.abrf.org/index.cfm/dm.home). To explore this possibility, the two peptides with masses of 1335.69 and 1349.72 were subjected to tandem (MS/MS) mass spectrometric sequencing. Results shown in Figures 1Ab and 1Ac unequivocally demonstrate that both peptides correspond to EIAQDFKTDLR and that the heavier peptide is monomethylated on K79 (Figures 1Ab and 1Ac).

Based on the published nucleosome structure [15], K79 is located in a loop connecting the first and the second α helixes (Figure 1Ad). This region is exposed and is adjacent to the interface between H3/H4 tetramer and the H2A/H2B dimer. Although K79 is not directly involved in the formation of the interface, it is in a position capable of influencing the access to the interface, raising the possibility that methylation on K79 may play an important role in regulating the access of protein factors to chromatin.

H3-K79 Methylation Is Conserved from Yeast to Human

To confirm the above finding and to explore the possible conservation of K79 methylation in other organisms, we generated a polyclonal antibody against a dimethyl-K79 H3 peptide "IAQDFmKTDLRF." To examine the specificity of the antibody, recombinant histone H3 that is either not methylated or methylated on K4 or K9 by SET7 [8] or SUV39H1 [5], respectively, were subjected to Coomassie staining and Western blot analysis. As a positive control, equivalent amounts of core histones purified from HeLa cells were also analyzed. Results shown in Figure 1B demonstrate that while the antibody recognized histone H3 purified from HeLa cells, it did not recognize unmethylated or K4- or K9-methylated H3 (Figure 1B, bottom panel). In addition, competition with two peptides of identical amino acid sequences with or without dimethylation on K79 demonstrates that only the methylated peptide is capable of abolishing the reac-
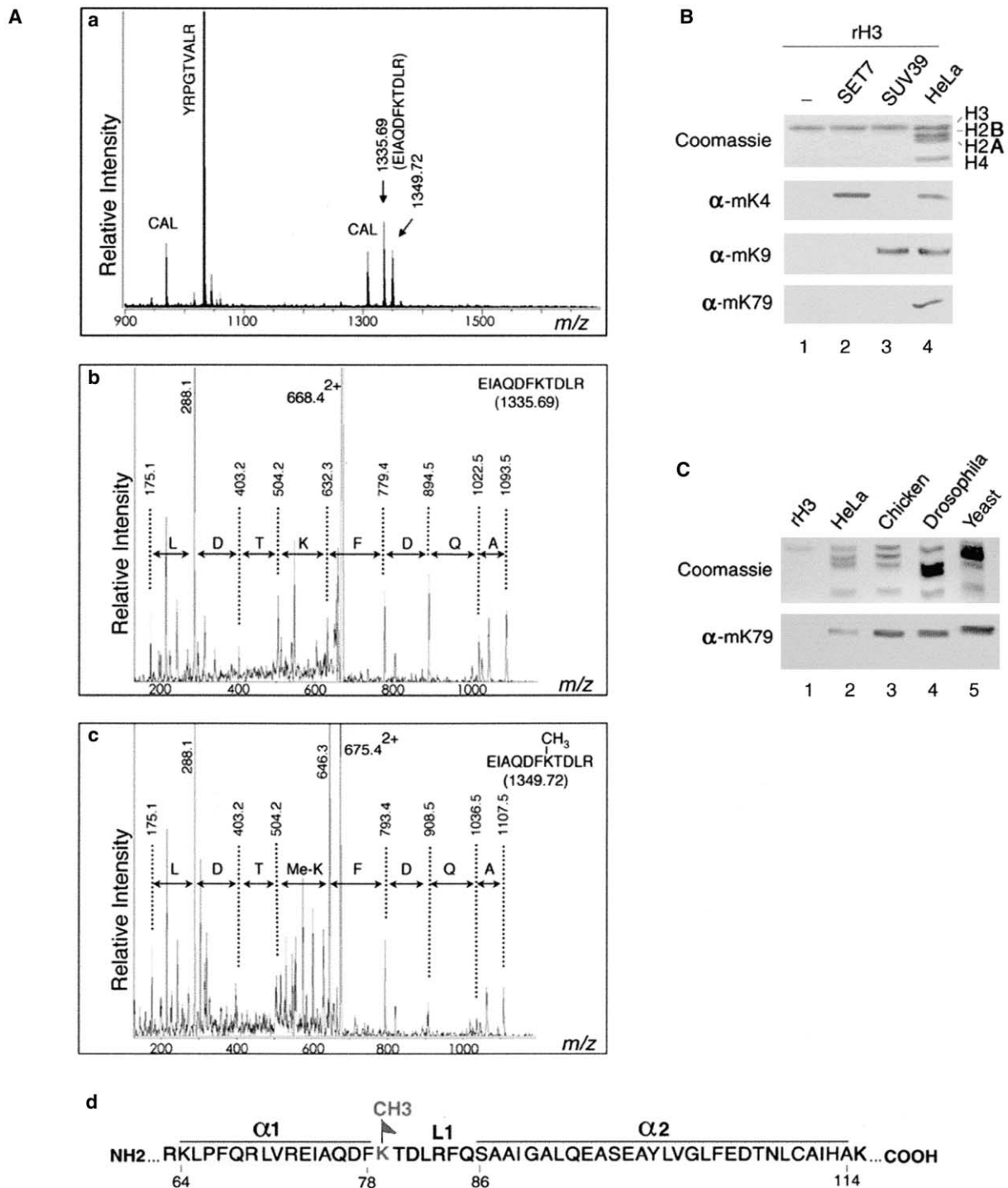
Figure 1. H3-K79 Methylation Is Conserved from Yeast to Human

(A) Identification of H3-K79 methylation. (Aa) MALDI-TOF MS of histone H3. HeLa histone H3 was digested with trypsin, and resulting peptides were concentrated on a Poros 50 R2 (Perseptive Biosystems; Framingham, MA) reversed-phase micro-tip and analyzed by MALDI-reTOF MS using a Reflex III instrument (Bruker Daltonics; Bremen, Germany), as described [8]. Only the relevant portion of the spectrum is shown. Internal calibrants (6.25 fmoles each; "CAL") and three prominent ions with predicted H3-peptide sequences are indicated. The ion with a m/z value of 1349.72 is 14.03 Da heavier than the mass of "EIAQDFKTDLR." (Ab and Ac) Nano-ES MS/MS. Tryptic digest mixtures were analyzed by electrospray ionization (ESI) MS using an API 300 triple quadrupole instrument (Applied Biosystems/MDS SCIEX; Thornhill, Canada), modified with a continuous-flow NanoES source, as described [25]. Precursor ions (m/z = 668.4 in panel [Ab]; m/z = 675.4 in [Ac]), corresponding to doubly charged (z = 2) versions of peptide ions marked "1335.69" and "1349.72" in panel (Aa), were selected for collision-induced dissociation (CID)-based MS/MS analysis. Both fragment ion spectra were inspected for y″ ion series; deduced sequences are indicated (note that y″ ion series originate at the C terminus and therefore read backward). (Ad) Location of K79 of histone H3 relative to the two α helices (α1 and α2) and loop 1 (L1) based on the published nucleosome structure [15].

(B) Characterization of the H3-mK79 antibody. HeLa core histones, recombinant histone H3 that are not methylated, methylated by SET7, or SUV39 were analyzed by Coomassie staining and Western blots using the mK4-, mK9-, and mK79-specific antibodies. The mK79-specific antibody was raised against a H3 peptide containing dimethylated K79 (IAQDFmKTDLRF).

(C) H3-K79 methylation occurs in a wide range of organisms. Equivalent amounts of histones, as evidenced by Coomassie staining, from different organisms indicated on top of the panel were analyzed by Western blot using the H3-mK79-specific antibody.

A

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yDOT1  290  ETILPDLNASFDNSDTKGFVNAINLYNKMIREIPRQR--IIDHLETIDKIPRSFIHDFLHIVYTRSIHPQANKLKHYKAFSN
cDOT1L  88  VPLPSDAVVGNPDKFSQ-YIDALNLKLAHPDVLSRVP--AKHHEITSTRLCSLTVAIKLAELAYRFSVPDANVLRHYAVGTS
hDOT1L  53  LAMENYVLIDYDTKSFESMQRLCDKYNRAIDSIHQLWKGTTQPM-KLNTRPSTGLLRHILQQVYNHSVTDPEKLNNYEPFSP
dDOT1L  58  SAFEETNLHQIDTACYKTMTGLVDRFNKAVDSIVALEKGTSLPAERLNKFAHPSLLRHILQLVYNAAVLDPDKLNQYEPFSP

                                                                    post I
                                                   I
yDOT1       YVYGELLPNFLSDVYQQCQLKKGDTFMDLGSGVGNCVVQAALECGCALSFGCEIMDDASDLTILQYEELKKRCKLYGMRLNN
cDOT1L      TVYGELHCSQMASFVDQLNMGPSDYFMDLGSGIGHLVNFVAAYARTQMSVGVELMDNLAEIAEKNKEFNERLLNHFGKKVYA
hDOT1L      EVYGETSFDLVAQMIDEIKMTDDDLFVDLGSGQVVLQVAAATNCKHHYGVEKADIPAKYAETMDREFRKWMKWYGKK--H
dDOT1L      EVYGETSYELVQQMLKHVTVSKEDTFIDLGSGVGQVVLQMAGSFPLKTCIGIEKADTPARYAERMDVIFRQYMGWFGKR--F

              II                              III
yDOT1       VEFSLKKSFVDNNRVAELIPQCDVILVNNFLFDEDLNKKVEKILQTAKVGCKIISLKSL-RSLTYQINFYNVENIFNRLKVQ
cDOT1L      TRF-IHGSFTSPAVIREIQTKATVILANNVRFDPELKLQLKEILMGCKDRTRIISSEPLVPSRARQTNSRRADDFVKISDES
hDOT1L      AEYTLERGDFLSEEWRERIANTSVIFVNNFAFGPEVDHQLKERFANMKEGGRIVSSKPFAP-LNFRINSRNLSDIGTIMRVV
dDOT1L      CEYKLIKGDFLVDEHRENITSSTLVFVNNFAFGPTVDHQLKERFADLRDGARVVSSKSFCP-LNFRITDRNLSDIGTIMHVS

yDOT1       RYDLKEDSVSWTHSGGEYYISTVMEDVDESLFSPAARGRRNRGTPVKYTR 582
cDOT1L      LLNLVDNNVSWTSRKVPFYLTTINRNKVSPFYCVRF                365
hDOT1L      ELSPLKGSVSWTGKPVSYYLHTIDRTILENYFSSLKNPKLREEQEAARRR 344
dDOT1L      EIPPLKGSVSWTCKPVSYYLHVIDRTILERYFQRLKTKGGNDHESVGTVR 350
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B

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            10         20         30         40         50         60         70
MGEKLELRLK  SPVGAEPAVY  PWPLPVYDKH  HDAAHEIIET  IRWVCEEIPD  LKLAMENYVL  IDYDTKSFES  MQRLCDKYNR    80
AIDSIHQLWK  GTTQPMKLNT  RPSTGLLRHI  LQQVYNHSVT  DPEKLNNYEP  FSPEVYGETS  FDLVAQMIDE  IKMTDDDLFV   160
DLGSGVGQVV  LQVAAATNCK  HHYGVEKADI  PAKYAETMDR  EFRKWMKWYG  KKHAEYTLER  GDFLSEEWRE  RIANTSVIFV   240
NNFAFGPEVD  HQLKERFANM  KEGGRIVSSK  PFAPLNFRIN  SRNLSDIGTI  MRVVELSPLK  GSVSWTGKPV  SYYLHTIDRT   320
ILENYFSSLK  NPKLREEQEA  ARRQQRESK   SNAATPTKGP  EGKVAGPADA  PMDSGAEEEK  AGAATVKKPS  PSKARKKKLN   400
KKGRKMAGRK  RGRPKKMNTA  NPERKPKKNQ  TALDALHAQT  VSQTAASSPQ  DAYRSPHSPF  YQLPPSVQRH  SPNPLLVAPT   480
PPALQKLLES  FKIQYLQFLA  YTKTPQYKAS  LQELLGQEKE  KNAQLLGAAQ  QLLSHCQAQK  EEIRRLFQQK  LDELGVKALT   560
YNDLIQAQKE  ISAHNQQLRE  QSEQLEQDNR  ALRGQSLQLL  KARCEELQLD  WATLSLEKLL  KEKQALKSQI  SEKQRHCLEL   640
QISIVELEKS  QRQQELLQLK  SCVPPDDALS  LHLRGKGALG  RELEPDASRL  HLELDCTKFS  LPHLSSMSPE  LSMNGQAAGY   720
ELCGVLSRPS  SKQNTPQYLA  SPLDQEVVPC  TPSHVGRPRL  EKLSGLAAPD  YTRLSPAKIV  LRRHLSQDHT  VPGRPAASEL   800
HSRAEHTKEN  GLPYQSPSVP  GSMKLSPQDP  RPLSPGALQL  AGEKSSEKGL  RERAYGSSGE  LITSLPISIP  LSTVQPNKLP   880
VSIPLASVVL  PSRAERARST  PSPVLQPRDP  SSTLEKQIGA  NAHGAGSRSL  ALAPAGFSYA  GSVAISGALA  GSPASLTPGA   960
EPATLDESSS  SGSLFATVGS  RSSTPQHPLL  LAQPRNSLPA  SPAHQLSSSP  RLGGAAQGPL  PEASKGDLPS  DSGFSDPESE  1040
AKRRIVFTIT  TGAGSAKQSP  SSKHSPLTAS  ARGDCVPSHG  QDSRRRGRRK  RASAGTPSLS  AGVSPKRRAL  PSVAGLFTQP  1120
SGSPLNLNSM  VSNINQPLEI  TAISSPETSL  KSSPVPYQDH  DQPPVLKKER  PLSQTNGAHY  SPLTSDEEPG  SEDEPSSARI  1200
ERKIATISLE  SKSPPKTLEN  GGGLAGRKPA  PAGEPVNSSK  WKSTFSPISD  IGLAKSADSP  LQASSALSQN  SLFTFRPALE  1280
EPSADAKLAA  HPRKGFPGSL  SGADGLSPGT  NPANGCTFGG  GLAADLSLHS  FSDGASLPHK  GPEAAGLSSP  LSFPSQRGKE  1360
GSDANPFLSK  RQLDGLAGLK  GEGSRGKEAG  EGGLPLCGPT  DKTPLLSGKA  AKARDREVDL  KNGHNLFISA  AAVPPGSLLS  1440
GPGLAPAASS  AGGAASSAQT  HRSFLGPFPP  GPQFALGPMS  LQANLGSVAG  SSVLQSLFSS  VPAAAGLVHV  SSAATRLTNS  1520
HAMGSFSGVA  GGTVGGN                                                                           1537
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Figure 2. Identification of a Human DOT1-like Protein

(A) Alignment of the amino acid sequences of the DOT1 family proteins. Only the most conserved regions are shown. Sequences used in the alignment include yeast DOT1 (NP_010728) and its homologs from human (AF509504), *Drosophila* (AAF54122), and *C. elegans* (NP_510056). We note that four additional putative *C. elegans* proteins in GenBank (NP_509981, NP_490970, NP_509997, NP_508351) also show significant homology to DOT1. Sequences predicted to be involved in SAM binding [17] are indicated. Numbers represent the amino acid number of respective proteins. Gaps are indicated by "-". Amino acids that are identical or have similar properties are shaded.

(B) Amino acid sequence of human DOT1L. Predicted SAM binding motifs are underlined. Mutation of the shaded amino acids abolished the HMTase activity of hDOT1L.

tivity of the antibody toward the HeLa histone H3 (data not shown). Thus, we conclude that the antibody is H3-mK79 specific.

The fact that K79 and adjacent sequences of H3 are conserved in different organisms prompted us to test the possibility of H3-K79 methylation occuring in other organisms. Thus, core histones isolated from chicken, *Drosophila*, and budding yeast were analyzed by Western blotting using the H3-mK79-specific antibody. As negative and positive controls, recombinant H3 and HeLa core histones were included in the assay. Results shown in Figure 1C demonstrate that H3-K79 methylation occurs in all the organisms analyzed. Thus, we con-

clude that H3-K79 methylation is conserved from yeast to human.

Human DOT1-like Protein Is an H3-K79-Specific Methyltransferase

Having demonstrated that H3-K79 methylation occurs in yeast, we attempted to identify the responsible enzyme in this organism. With the H3-mK79-specific antibody in hand, we decided to take a candidate approach. We reasoned that deletion of the K79 HMTase should lead to loss or reduction of K79 methylation that could be detected by Western blotting using the mK79-specific antibody. The candidate genes that we screened include
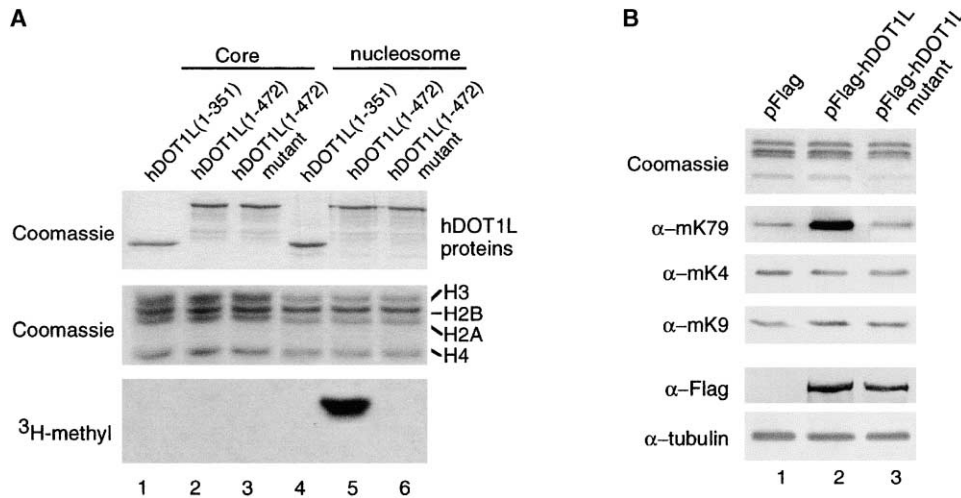
Figure 3. hDOT1L Is a Nucleosomal H3-K79-Specific HMTase

(A) Recombinant hDOT1L is a nucleosomal H3-specific HMTase in vitro. About 0.2 μg of different version of recombinant hDOT1L proteins were incubated with 10 μg of core histones or equivalent amounts of nucleosomes as described [8]. The reaction mixtures were resolved in SDS-PAGE followed by Coomassie staining and fluorogram. Recombinant hDOT1L proteins shown in the top panel are 15-fold of what were used in HMTase assay.

(B) hDOT1L methylates H3-K79 in vivo. Empty vector, as well as vectors that encode Flag-tagged wild-type or mutant hDOT1L were transfected into 293T cells using the Qiagen Effectene Transfection Reagent. Two days after transfection, cells were collected for the preparation of total cell lysates and histones. Expression of wild-type and mutant hDOT1L was verified by Western blots using anti-Flag antibody. Equal loading of lysates was confirmed by probing for tubulin. Equal loading of histones was verified by Coomassie staining.

the six yeast SET domain-containing proteins (Set1 to Set 6) as well as those that predicted to encode SAM binding domain-containing proteins [16, 17]. This screen resulted in the identification of DOT1 as the HMTase responsible for K79 methylation in yeast [18].

Dot1 was originally identified in a genetic screen for genes whose overexpression disrupts telomeric silencing [19]. Disruption or overexpression of Dot1 not only impaired telomeric silencing but also reduced silencing at mating type and rDNA loci [19]. In addition to participating in silencing, DOT1 also plays an important role in meiotic checkpoint control [20]. Given that K79 methylation is conserved from yeast to human (Figure 1C), we expect that DOT1-like (DOT1L) proteins exist in other organisms. A Blast search revealed several putative proteins with significant sequence homology to DOT1. Sequence alignment of these proteins revealed several conserved blocks (Figure 2A) that were predicted to be involved in SAM binding [17]. The Blast search also revealed that a hypothetical human protein (GenBank accession number AAC08316) encoded by a gene located on 19p13.3 is likely the human DOT1L. However, this hypothetical protein is incomplete at both 5′ and 3′ ends. To clone the full-length cDNA encoding hDOT1L, several EST clones were obtained and sequenced. Two overlapping EST clones (BF507396 and BF982417) contain a single open reading frame (ORF) which is predicted to encode 1537 amino acids with a calculated mass of 165 kDa (Figure 2B). The fact that the nucleotide sequence around the first methionine conforms to the Kozak initiator sequence in combination with the fact that the cDNA contains an upstream stop codon indicate that the 1537 amino acids encoded by the cDNA represent the full-length protein. Analysis of the hDOT1L pro-

tein sequences revealed no known functional motif other than a putative SAM binding domain (Figure 2B).

To determine whether hDOT1L possesses intrinsic HMTase activity, two recombinant proteins corresponding to the N-terminal 351 and 472 amino acids of hDOT1L, respectively, were produced in *E. coli*. The N terminus of hDOT1L was chosen because this region contains the putative SAM binding motif and is most conserved among the different DOT1L proteins (Figure 2A). HMTase assays revealed neither protein possesses significant enzymatic activity when free core histones were used as substrates (Figure 3A, lanes 1–3). However, when nucleosomes were used as substrates, hDOT1L(1-472) exhibits significant HMTase activity, although hDOT1L(1-351) is inactive (Figure 3A, lanes 4 and 5). To demonstrate that the HMTase activity depends on its ability to bind to SAM, we generated a mutated version of hDOT1L(1-472) by changing the highly conserved $GSG_{163-165}$ sequence within motif I (Figure 2A) to RCR. This mutation completely abolished the HMTase activity (Figure 3A, compare lanes 5 and 6). Taken together, these results demonstrate that hDOT1L is a nucleosomal H3-specific HMTase and that both the SAM binding motif and the sequences between amino acids 351 and 472 are critical for its enzymatic activity.

Having demonstrated the HMTase activity of human DOT1L in vitro, we attempted to demonstrate its HMTase activity in vivo. To this end, mammalian expression vectors encoding a Flag-tagged hDOT1L and a motif I mutant were transfected into 293T cells. Core histones purified from the transfected cells were analyzed by Coomassie and Western blotting using antibodies specific for methylated K4, K9, or K79. Results shown in Figure 3B demonstrate that overexpression of
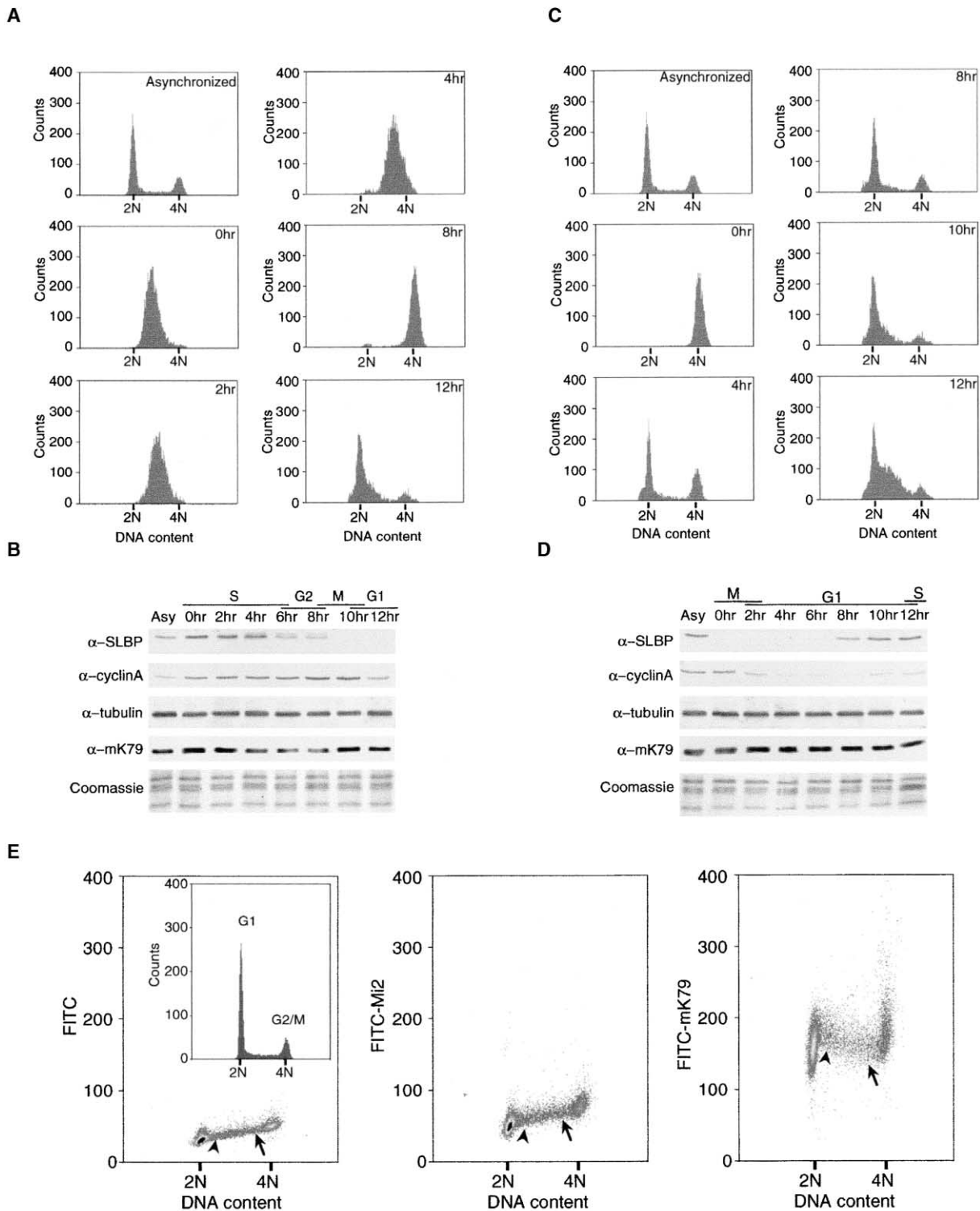
Figure 4. H3-K79 Methylation Level Is Cell Cycle Regulated

(A) Cells released from double thymidine block were analyzed by flow cytometry. The numbers of cells (arbitrary units) were plotted against DNA content.

(B) Cell extracts and histones derived from samples analyzed in (A) were analyzed by Western blots. SLBP and cyclin A were used as cell cycle markers; tubulin was used as loading control. Equal loading of histones was revealed by Coomassie staining. The H3-K79 methylation level was analyzed by probing with the mK79-specific antibodies. The cell cycle stage of each sample is indicated on top of the panel. "asy" represents asynchronized cell extracts.

(C and D) Identical to (A and B) except that the cells were arrested at M phase before release.

(E) mK79 level in relation to cell DNA content. Cells were labeled with FITC-conjugated goat anti-rabbit secondary antibody in the absence (left panel) or presence of primary antibody (middle and right panels) as indicated. DNA in each cell was labeled with PI. Each spot represents an individual cell. The numbers represent arbitrary FITC unit. Unlabeled cells were analyzed by flow cytometry (left panel insert) to serve as standard for DNA content. The intensity of FITC signal (measurement of Mi-2 or mK79) is plotted against PI signal (measurement of DNA content that correlates with cell cycle stages). Arrowheads and arrows represent early S phase and late S phase, respectively.

hDOT1L significantly increased H3-K79 methylation while having no effect on K4 and K9 methylation (compare lanes 1 and 2). The increased K79 methylation is dependent on an intact motif I as transfection of a motif I mutant did not affect K79 methylation (compare lanes 1 and 3). This differential effect is not caused by differential expression, since both constructs expressed hDOT1L at a similar level (Figure 3B, bottom two panels). Based on these results, we conclude that hDOT1L is an H3-K79-specific HMTase in vivo.

Methylation on K79 Is Regulated during the Cell Cycle

Previous studies have demonstrated that the total histone methylation level is regulated during the cell cycle [21]. Thus, we asked whether K79 methylation level changes during the cell cycle. To obtain a population of synchronous cells, we arrested HeLa cells at the G1/S border using a double thymidine block. After releasing the arrested cells from the thymidine block, cells were collected every 2 hr for flow cytometry as well as for the preparation of protein extracts and histones. Flow cytometry analysis (Figure 4A) indicated that more than 95% of the cells progress through S phase and enter G2 synchronously. The cells were successfully arrested at the G1/S border before release, as evidenced by the accumulation of the histone mRNA stem-loop-binding-protein (SLBP) (Figure 4B). As demonstrated previously [22], SLBP levels stayed high throughout S phase and dropped rapidly as cells exited S phase (Figure 4B). The cells completed mitosis about 12 hr after release, as evidenced by the degradation of cyclin A when cells enter anaphase (Figure 4B). To determine whether K79 methylation levels change during the cell cycle, histones isolated from corresponding cells were subjected to Western blotting using the mK79-specific antibody. This analysis demonstrates that K79 methylation level decreases during S phase and reaches the lowest level in G2 and increases during M phase (Figure 4B).

To study the K79 methylation status in G1 phase, we arrested the cells with thymidine, released them, and then arrested them in mitosis with nocodazole. After releasing from the nocodazole block, the cells progress through G1 to S phase synchronously (Figure 4C). The cells completed mitosis 2 hr after release from the nocodazole block, as evidenced by the degradation of cyclin A (Figure 4D). The cells then started to enter S phase about 10 hr after release, as evidenced by the accumulation of SLBP (Figure 4D). Western blot analysis of the histones from cells collected at different time points after the nocodazole release indicates that the K79 methylation remains at a high level throughout the G1 phase (Figure 4D).

To further confirm the cell cycle-dependent changes in K79 methylation, we performed bivariate analysis of DNA content and mK79 level using a method similar to that used in analyzing cell cycle-dependent changes of cyclins using asynchronous cells [23]. For each individual cell, DNA was labeled with propidium iodide (red), while K79-methylated-H3 was labeled with an FITC-conjugated secondary antibody (green). The intensities of green color and red color of a particular cell reflect K79 methylation level and DNA content, respectively. As expected, negative controls without a primary antibody or with antibody against Mi-2, a component of a nucleosome remodeling and deacetylase complex [24], resulted in a slight increase in FITC signals when cells go from G1 phase to G2/M phase (Figure 4E, left and middle panels). This result is expected as cell size becomes bigger during this transition. However, labeling of the cells with the mK79-specific antibody resulted in a decrease in FITC-labeling when cells go through S phase (Figure 4E, right panel, compare early and late S phases). It is unlikely that the decrease in mK79 level during S phase is caused by "dilution" by nascent histones during chromatin assembly, as similar decrease is not observed in other methylation sites (data not shown). By using two different methods, we demonstrate that the K79 methylation level decreases during S phase, reaches its lowest level in G2, increases during M phase, and maintains at a high level during G1 phase.

In this study, we present evidence that K79 within the globular domain of histone H3 is methylated and this modification is conserved from yeast to human (Figure 1). Based on the studies in yeast, we isolated a human DOT1 homolog and show that the human protein possesses intrinsic HMTase activity toward K79 (Figure 3). Thus, our studies establish that both the K79 methylation and the responsible enzymes are evolutionarily conserved. Since DOT1 and its homologs do not contain a SET domain, a signature motif present in all the known histone lysine methyltransferases [3], the DOT1 family of proteins represents a novel class of histone lysine methyltransferase. What might be the function of K79 methylation? Previous studies indicate that DOT1 is involved in telomere silencing [19] and meiotic checkpoint control [20]. Consistent with its role in telomeric silencing, deletion of Dot1 gene resulted in mislocalization of the silencing protein Sir2 and Sir3 [20]. Mutations on H3-K79 or SAM binding motif of DOT1 resulted in impaired telomeric silencing [18], suggesting that Dot1 regulates telomeric silencing predominantly through methylation of H3-K79. Whether the function of DOT1 in meiotic checkpoint control is also directly linked to the methylation of K79 remains to be determined. In addition, the observation that K79 methylation level changes during the mitotic cell cycle (Figure 4) raises the possibility that this modification might also play an important role in mitotic cell cycle. It is worth noting that K79 is reasonably close to both the N-terminal tail of H4 and the C-terminal helix of H2B in the nucleosome structure [15]. It is known that H4 N-terminal tail is extensively modified. In addition, K120 in the H2B C-terminal helix is ubiquitinated. Whether K79 methylation plays a role in regulating these modifications remains to be determined.

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Accession Numbers