The N-Terminus of Drosophila SU(VAR)3−9 Mediates Dimerization and Regulates Its Methyltransferase Activity†

Ragnhild Eskeland,[§,#] Birgit Czermin,[§,#] Jörn Boeke,[#] Tiziana Bonaldi,[#] Jörg T. Regula,[‡] and Axel Imhof*[,#]

Adolf-Butenandt Institute, Department of Molecular Biology, Histone Modifications Group and Protein Analysis Unit, Ludwig-Maximillians University of Munich, Schillerstrasse 44, 80336 Munich, Germany

Received November 4, 2003; Revised Manuscript Received January 17, 2004

ABSTRACT: In most eukaryotes, the histone methyltransferase SU(VAR)3−9 and its orthologues play a major role in the function of centromeric heterochromatin. Although the methyltransferase domain is required for the formation of a fully functional centromere, mutations within other regions of the gene such as the N-terminus also have a strong impact on its in vivo function. To analyze the contribution of the N-terminus on the methyltransferase activity, we have expressed the full-length *Drosophila* SU(VAR)3−9 (dSU(VAR)3−9) together with various N-terminal deletions in *Escherichia coli* and analyzed the structural and enzymatic properties of the purified recombinant enzymes. Full-length dSU(VAR)3−9 specifically methylates lysine 9 within histone H3 on peptides, on intact histones, and, to a lesser extent, on nucleosomes. A detailed analysis of the reaction products shows that dSU(VAR)3−9 adds two methyl groups to an unmethylated H3 tail peptide in a nonprocessive manner. The full-length enzyme elutes with an apparent molecular weight of 160 kDa from a gel filtration column, which indicates the formation of a dimer. This property is dependent on an intact N-terminus. In contrast to the full-length enzymes, proteins lacking the N-terminus fail to dimerize, and show a 10-fold lower specific activity and a linear dependence of methyltransferase activity on enzyme concentration. A N-terminal peptide containing amino acids 1−152 of dSU(VAR)3−9 is sufficient to mediate this interaction in vitro. The dimerization of dSU(VAR)3−9 and the subsequent increase of its methyltransferase activity provide a starting point to understand the molecular details of the formation of heterochromatic structures in vivo.

Centromeres are conserved structures of eukaryotic chromosomes, which ensure their proper segregation during mitotic divisions (*1*, *2*). Most centromers are formed by association of the centromeric DNA with specific proteins such as CENP-A, -B, and -C (*3*, *4*) and have been shown to form clusters within interphase chromatin (*5*, *6*). Although centromeres as well as pericentromeric regions are typically rich in repetitive DNA, the main determinant of centromers seems to be of an epigenetic nature as they can also form ectopically within euchromatic arms (*7*, *8*). Centromeric regions of chromosomes are generally transcriptionally quiescent, a property that can "spread" into neighboring regions (*2*, *9*). The variable transcriptional activity of normally active genes, after their translocation close to heterochromatin, has been termed position effect variegation (PEV)[1] (*10*, *11*) (*9*).

Histones within centromeric regions are usually hypoacteylated (*12*, *13*) and methylated at lysine 9 within the H3 tail (*14*). The enzyme that is critical for this modification is the histone methyltransferase SU(VAR)3−9 (*15*). *Drosophila* SU(VAR)3−9 (dSU(VAR)3−9) has been initially identified in a genetic screen for suppressors of PEV (for a review see ref *16*). SU(VAR)3−9 or its orthologue CLR4 are required for heterochromatin-mediated gene silencing in *Drosophila* and *Schizosaccharomyces pombe* (*17*). Mouse SUV39 enzyme is important to maintain genome stability (*18*). Furthermore, it acts as a transcriptional repressor in transient as well as in stable transfection experiments in tissue culture cells (*19*, *20*).

Drosophila SU(VAR)3−9 belongs to a large class of proteins containing a SET domain. The SET domain confers methyltransferase activity and is crucial for the in vivo function of most SET-containing proteins. Although the SET domain is relatively well conserved, SET-methyltransferases show a remarkable structural and functional variability. SET domain proteins can exist as monomers (*21*−*25*) or dimers (*26*). In addition, some need auxiliary factors (*27*−*30*) or require nucleic acids for full activity (*31*, *32*).

In addition to the well-characterized SET domain, dSU(VAR)3−9 contains a chromo domain (*17*, *19*, *33*), a

† Funding for this work has been provided by a grant (IM23/4-1) from the Deutsche Forschungsgemeinschaft (DFG).

* Correspondence should be sent to Axel Imhof, Adolf-Butenandt Institute, Ludwig-Maximillians University of Munich, Schillerstr. 44, 80336 Muenchen, Germany. Tel.: - -49 89 5996 435 Fax: - -49 89 5996 425. E-mail: axel.imhof@mol-bio.med.uni-muenchen.de.

§ Both authors contributed equally to this work.

Department of Molecular Biology.

‡ Histone Modifications Group and Protein Analysis Unit.

[1] PEV, position effect variegation; PCR, polymerase chain reaction; PMSF, phenylmethanesulfonyl fluoride; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid); BSA, bovine serum albumin; EGTA, ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; DTT, DL-dithiothreitol; MTase, methyltransferase; HMT, histone methyltransferase; ACN, acetonitrile; TFA, trifluoroacetic acid; MALDI-TOF, matrix assisted laser desorption ionization-time-of-flight mass spectrometry; SAM, *S*-adenosyl methionine; SAH, *S*-adenosyl homocystein.

10.1021/bi035964s CCC: $27.50
Published on Web 03/06/2004

GTPase domain that is derived from a common exon used by dSU(VAR)3−9 and the eukaryotic translation initiation factor 2 and a relatively ill-defined N-terminal domain (*15*). The N-terminus is moderately conserved in humans, mice, and flies and interacts with at least two additional suppressors of PEV, HP1, and SU(VAR) 3−7 (*14*, *34*). A Structure−function analysis of the human SUV39H1 indicated that a deletion of the N-terminus leads to a failure of SUV39 to bind chromatin in vivo (*33*). A fragment of SUV39H1 containing just the N-terminus and the chromo domain binds efficiently to heterochromatin when expressed in tissue culture cells. This binding is thought to be mediated by HP1 and has been suggested to be a main component in the maintenance mechanism of histone methylation as the chromo domain of HP1 binds strongly to a H3 tail, which is methylated at lysine 9. In the absence of HP1, SU(VAR)3−9 is found at multiple sites along the chromosome arms, suggesting a role for HP1 in restricting SU(VAR)3−9 to centromeric heterochromatin. However, the HP1 interacting region is not sufficient to confer chromatin binding to an overexpressed fusion protein (*33*), suggesting an additional structural component in addition to HP1 binding, which is important for heterochromatin association. This hypothetical structural component may well be the conformation of dSU(VAR)3−9 itself. SUV39H1 forms distinct nuclear domains when overexpressed in vivo (*19*), a phenomenon that has already been described for certain ring finger proteins which form higher order aggregates in vivo and in vitro (*35*, *36*). In flies, overexpression of dSU(VAR)3−9 or other suppressors of PEV leads to a strong dosage-dependent enhancement of PEV independent of the dosage of the other partners. This strong dosage dependence argues against a single limiting factor regulating PEV but rather favors a model in which several factors participate in the assembly of a specific heterochromatin scaffold (*16*, *37*).

In this report, we show that full-length dSU(VAR)3−9 is a very active methyltransferase when expressed in bacteria. It adds two methyl groups to lysine 9 within the H3 tail in a nonprocessive manner. Moreover, the N-terminus of dSU(VAR)3−9 mediates an interaction between two SU(VAR)3−9 molecules, thereby increasing its ability to methylate H3. The N-terminus of dSU(VAR)3−9 has a bipartite interaction domain, which allows the formation of multimers of SU(VAR)3−9 proteins. This interaction between dSU(VAR)3−9 molecules may contribute to the clustering of centromers within living cells and the formation of nuclear substructures in vivo after expressing the N-terminus of SU(VAR)3−9 alone and may therefore explain the strong dosage dependence of the dSU(VAR)3−9 mediated enhancement of PEV.

MATERIALS AND METHODS

Cloning of dSU(VAR)3−9. Full-length dSU(VAR)3−9, deletion mutants, and point mutants were cloned into pET15b (Novagen) via NdeI and XhoI. The pET15b plasmid adds a 6× his-tag on the N-terminus. All inserts were created by PCR from a plasmid carrying the dSU(VAR)3−9 cDNA (kind gift of G. Reuter, Halle, Germany) and verified by DNA sequencing. GST fusion proteins were cloned by inserting a PCR generated EcoRI-XhoI fragment into a pGEX 4T-2 vector (Amersham).

Protein Purification. His-tagged dSU(VAR)3−9 and dSU(VAR)3−9 mutant polypeptides were expressed in *E. coli* BL21(DE3)pLys, and purified with Talon (Clontech) resin according to the manufacturer's instructions. For the molecular weight analysis, the Talon-purified proteins were loaded on a Superdex 200 column (HR 10/30, Amersham) or on a 5−20% sucrose gradient. The column was run isocratically in BC100 buffer (25 mM HEPES/KOH (pH 7.3), 100 mM NaCl, 1 mM $MgCl_2$, 0.5 mM EGTA, 0.1 mM EDTA, 10% glycerol (v/v), 1 mM DTT, and 0.2 mM PMSF) for 1.4 CV. 0.5 mL fractions were collected and 15 μL of each fraction were analyzed on a 10% SDS PAA gel. 5−20% (w/v) sucrose gradients were prepared in BC100 buffer with or without 3 M urea. The gradient was prepared using a Gradient Master 105/106 (BioComp) set at 2.40 min/81.5 deg/15 rpm. A 500 μL sample containing 10 μg of dSU(VAR)3−9 wild type or Δ213 or 20 μg of BSA was loaded on top of the gradient. Centrifugation was performed using a SW41 rotor (Beckman) at 41 000 rpm for 28 h at 4 °C. 0.5 mL fractions were collected and analyzed by 10% SDS−PAGE.

For activity assays, the enzyme was stabilized by addition of BSA to a final concentration of 100 ng/μL followed by dialysis against BC100 buffer. All recombinant dSU(VAR)3−9 were quantified by Coomassie staining with the ImageMaster 1D Elite v3.01 software package (Amersham) using BSA as a standard. Bacterially expressed HP1 was purified according to ref *38* and dialyzed against BC100.

Methyltransferase Activity. H3 peptides used contained amino acid 1−19 plus a C-terminal cysteine (ARTKQTARKSTGGKAPRKQC) and were either unmodified, dimethylated at K4, monomethylated at K9, acetylated at K9, or dimethylated at K9 (Peptide Speciality Laboratories, Heidelberg). Recombinant Drosophila histones were expressed and purified from *E. coli*, and reconstituted into octamers as described previously (*39*). Nucleosomes were reconstituted by salt dialysis. Recombinant histone H3 carrying the mutations K9A, K27A, or both, were expressed in bacteria from plasmids kindly provided by D. Reinberg. HMT assays were done in ddH_2O using H3-peptide, histone H3, octamer, or nucleosomes as substrates and *S*-adenosyl-[methyl-^{3}H]-L-methionine (25 μCi/mL) and/or *S*-adenosylmethionine (New England BioLabs) as methyl donor. Reactions were performed at 30 °C for 1−80 min. To stop the reaction, acetic acid was added to a final concentration of 5−10% (v/v). Kinetic assays were carried out in triplicates by varying the concentration of the H3 peptide (1−16 μM) or SAM (5−60 μM) at saturating amounts of SAM/peptide and analyzed by double reciprocal plots.

GST Pulldown. dSU(VAR)3−9 and mutants were translated in vitro from pET15b vectors, [^{35}S]methionine-labeled. GST-pulldowns were carried out as described earlier (*40*). In vitro translated proteins and HP1 (1 μg) were incubated in a total volume of 200 μL containing $NTEN_{100}$ (20 mM TrisHCl (pH 8), 100 mM NaCl, 1 mM EDTA, 0.5% (v/v) Nonidet P-40), 100 μg of BSA, and 5 μg of ethidium bromide. Washes were performed two times in $NTEN_{100}$ and four times in the $NTEN_{200}$. The bound proteins were eluted with SDS sample buffer and analyzed using a phosphoimager or a specific antibody in the case of HP1.

MALDI-TOF Analysis. To purify the methylated peptides from contaminating salts, the peptide solution was passed

over a pipet tip containing small amounts of C18 reversed phase material (ZipTip, Millipore). After three 10 μL wash steps with 0.1% TFA, the bound peptides were eluted with 1 μL of prepared matrix solution (saturated α-cyanohydroxy-cinnamic-acid (Sigma) dissolved in 50% ACN (v/v)/0.3% TFA (v/v)) directly onto the target plate. Samples were air-dried to allow cocrystallization of the peptides and the matrix, and the target plate was loaded in a Voyager DE STR spectrometer and analyzed.

RESULTS

Full-Length dSU(VAR)3−9 Is an Active Methyltransferase. To analyze the enzymatic properties of dSU(VAR)3−9, we expressed a recombinant full-length protein containing six histidine residues at the N-terminus in bacteria and purified it by affinity chromatography (Figure 1A). Recombinant dSU(VAR)3−9 methylates histone H3 in a mixture of histones as well as in reconstituted nucleosomes (Figure 1B). We defined the site of methylation using by using peptides premodified at lysine 4 or lysine 9 (Figure 1C). As lysine 27 is surrounded by almost identical amino acids and can be methylated by methyltransferases that also methylate lysine 9 (*30*, *41*, *42*), we also tested whether dSU(VAR)3−9 is able to methylate a histone H3 molecule carrying a mutation at lysine 9 or at lysine 27. The fact that dSU(VAR)3−9 only methylates the wildtype H3 molecule and the H3 mutated at lysine 27 but does not methylate mutants in which lysine 9 is mutated further confirmed our specificity analysis (Figure 1D). This is in good agreement with the finding that dSU(VAR)3−9 isolated from *Drosophila* embryos (*43*) as well as a N-terminally truncated version of dSU(VAR)3−9 (*14*) are able to methylate lysine 9 within the N-terminus of H3.

dSU(VAR)3−9 Is a Nonprocessive Enzyme with Similar Affinity for Unmethylated and Monomethylated H3K9. Lysines within histones exist in mono-, di-, and trimethylated forms and the different isoforms can have different molecular functions (*44*). Experiments using antibodies specific for either di- or trimethylated lysine 9 demonstrate that dSU(VAR)3−9 function is required for di- as well as trimethylation of histone H3 in *Drosophila* polytene chromosomes (*14*, *30*). We therefore analyzed the reaction products of an in vitro methylation reaction using full-length dSU(VAR)3−9 and a H3 peptide by MALDI-TOF (Figure 2A). A time course experiment of the methylation reaction using unmodified peptide shows that after 5 min about 20% of the peptides are found to be monomethylated and none of it is dimethylated. The dimethylated form only appears after 10 min of reaction time when almost 50% of all peptide has been converted into the monomethylated form. After 80 min, almost all of the unmethylated peptide is converted into either the mono- or the dimethylated form. We have not observed a significant amount of trimethylation by dSU(VAR)3−9 under those conditions (Figure 2A). We also used peptides that were either mono- or dimethylated at lysine 9 as substrate to exclude the possibility that dSU(VAR)3−9 could trimethylate a peptide that is already premodified. In both cases, we do not observe significant trimethylation of the peptide (Figure 2B,C). Only at a very high enzyme-to-substrate ratio or at extended reaction times we could observe a small fraction (less than 1%) of trimethylated peptide (data not shown). This is in good agreement with experiments using the human orthologue of dSU(VAR)3−9 SUV39H1, which also methylates a dimethylated peptide only with a very low efficiency (*15*).

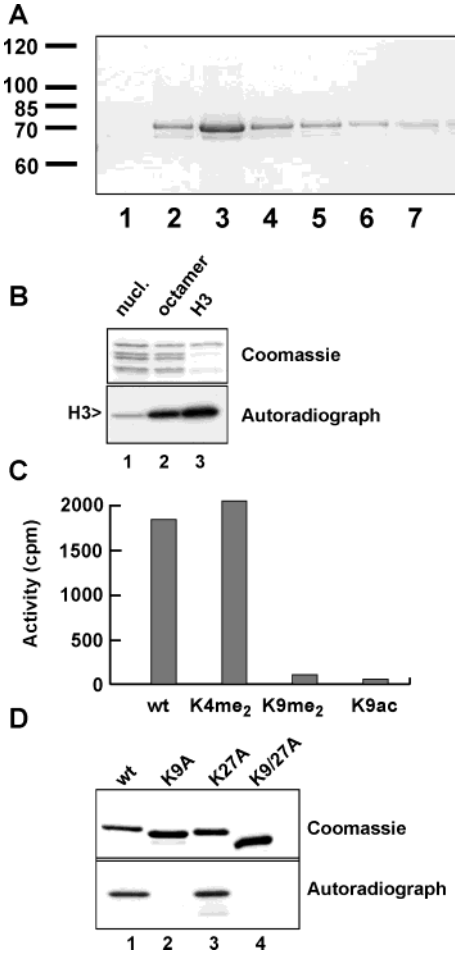

FIGURE 1: Characterization of recombinant dSU(VAR)3−9. (A) Elution of recombinant, His tagged dSU(VAR)3−9 from Talon beads. Ten microliters of the first eight fractions (lanes 1−8) was loaded onto a gel and subjected to 10% SDS−PAGE, and the gel was stained with Coomassie blue R250. (B) In vitro methylation reactions using different protein substrates. Nucleosomal arrays (lane 1) were reconstituted on pBS(KS) (Stratagene) by salt dialysis using recombinant octamers (lane 2), which were reconstituted from equimolar amounts of histones produced in *E. coli* and purified by gel-filtration chromatography. H3 (lane 3) was expressed and purified as described. The reaction products were then loaded and run on a 18% SDS PAA gel, and the gel was stained with Coomassie blue R250, dried, and exposed to a X-ray film (autoradiograph) for 24 h. (C) Peptides containing the first 19 amino acids of H3 were methylated by recombinant dSU(VAR)3−9 and radioactive SAM (Amersham). We used the unmodified peptide (wt), a peptide dimethylated at K4 ($K4me_2$) or at K9 ($K9me_2$) and a peptide acetylated at K9 (K9ac) as substrates. Incorporated radioactivity was measured by a filter-binding assay. (D) In vitro methylation reaction using either wt recombinant H3 (lane 1), H3 mutated at lysine 9 (lane 2), H3 mutated at lysine 27 (lane 3), or a H3 molecule mutated at both sites (lane 4). Histones were methylated by recombinant dSU(VAR)3−9 in the presence radioactive SAM, separated by SDS−PAGE, and analyzed by autoradiography.

A possible explanation for the slow appearance of the dimethylated peptide could be a dramatically reduced reaction velocity of the methylation reaction after the peptide has been monomethylated. To test this, we repeated the same experiments using the monomethylated peptide as a substrate and found very similar kinetics (Figure 2B,D). From these

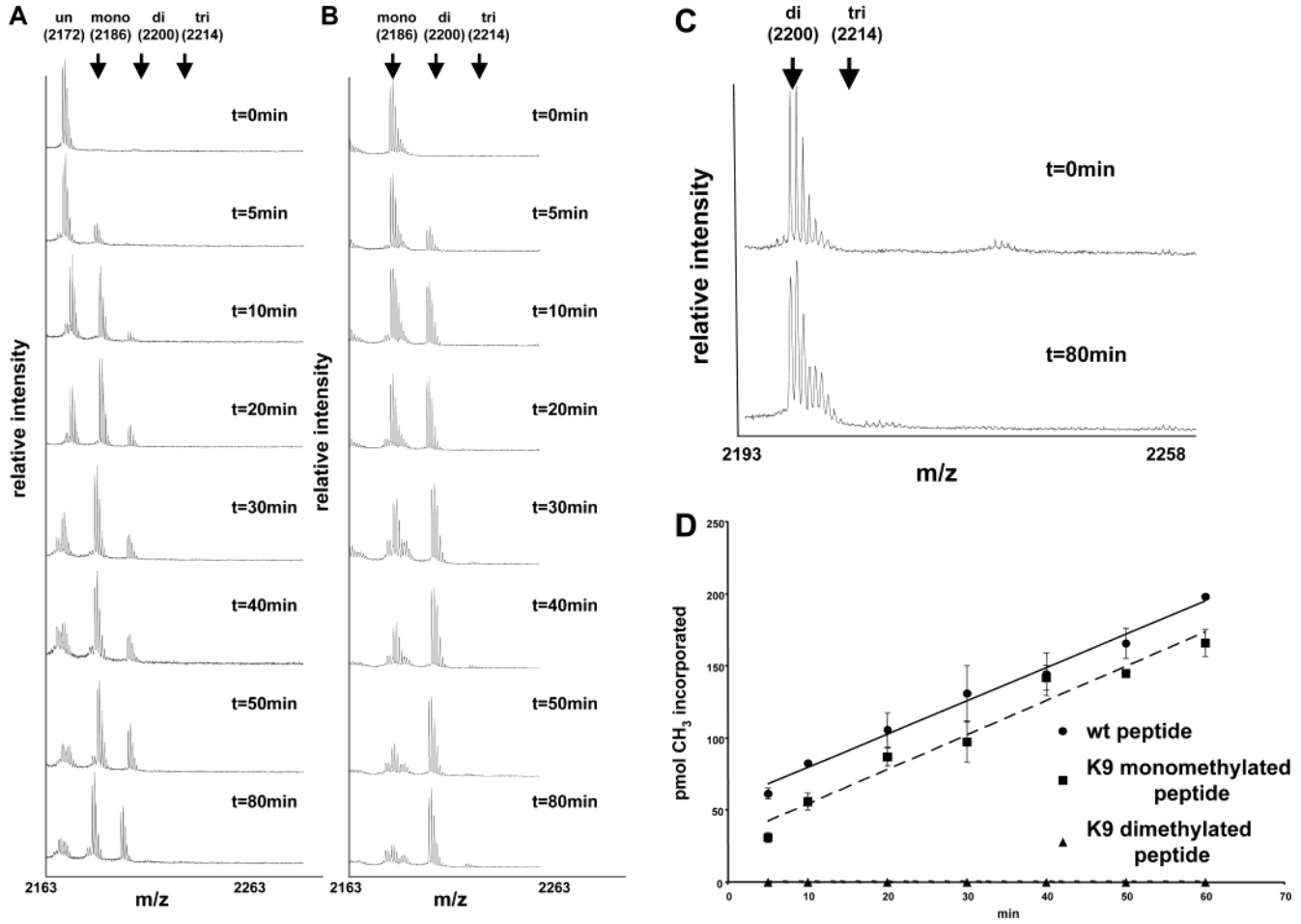

FIGURE 2: MALDI analysis of reaction products. MALDI-TOF analysis of the reaction products using 600 ng of dSU(VAR)3−9 and 460 pmol of a H3 peptide (see Figure 1), which was unmethylated (A), monomethylated (B), or dimethylated (C) at K9. The methylation reaction was stopped at different time points after starting the reaction and reaction products analyzed by MALDI-TOF. In case of the dimethylated peptide, only the 80-min time point is shown. (D) Time course of a methylation reaction as measured by a filter-binding assay.

experiments, we conclude that dSU(VAR)3−9 adds a single methyl group to the peptide, after which it dissociates from the SET domain and has to reassociate for a second methylation reaction. This behavior is in marked contrast to the trimethylase DIM5 of *Neurospora*, which processively methylates lysine 9 within a H3 peptide to the trimethylated form without releasing it into the solution (*45*) or to SET7/9, which has been shown to only monomethylate lysine 4 on the H3 tail (*25*).

The N-Terminus of dSU(VAR)3−9 Is Required for Full Catalytic Activity. To analyze the influence of the N-terminus of dSU(VAR)3−9 on its enzymatic activity, we expressed various mutant dSU(VAR)3−9 proteins in *E. coli* and studied their catalytic activity (Figure 3A,B). As expected, a mutation of dSU(VAR)3−9 in the SET domain (H561K) renders the enzyme inactive. Enzymes in which the N-terminal 152 or 213 amino acids were deleted are still able to methylate H3 peptides and histones albeit with an over all 10-fold lower methyltransferase activity (Figure 3C,D). Further deletion of the N-terminus including the chromo domain (Δ279) resulted in an additional decline of methylation activity, which we have not further analyzed.

Kinetic Parameters for Full-Length Recombinant dSU(VAR)3−9. To further quantify the effect of the N-terminus on the HMT activity of dSU(VAR)3−9, we used a standard filter-binding assay to analyze the kinetics of dSU(VAR)3−9 methyltransferase reaction. Under standard reaction conditions, the reaction is linear for at least 60 min (Figure 2D). To prevent possible product inhibition effects, we measured the incorporated radioactivity after 1 min, when less than 5% of product was formed (see Figure 2). In test experiments, we verified that this amount of *S*-adenosyl homocystein (SAH) or of the methylated H3 peptide did not significantly inhibit the reaction in our assays when added externally (data not shown). In the reminder of the paper, the reaction velocity is therefore expressed as pmol of CH_3 incorporated min^{-1}. Determination of the kinetic parameters of the full-length dSU(VAR)3−9 revealed a V_{max} for the methylation reaction of 95.2 nmol min^{-1} mg^{-1} and a K_m-[SAM] of 25.9 μM (Figure 4A), assuming that all molecules are active. This translates into a k_{cat} of 0.11 s^{-1}, which is 2-fold higher than the reported k_{cat} of the full-length Rubisco methyltransferase LSMT (0.05 s^{-1}) (*23*). More importantly, full-length dSU(VAR)3−9 is approximately 20 times faster than another SET containing histone methyltransferase enzyme SET9 (k_{cat} = 0.005 s^{-1}) (*23*). Deletion of the N-terminal domain does not change the K_m for SAM or the H3 peptide, indicating that there is no major change in substrate affinity. Strikingly, the V_{max} and the k_{cat} of the N-terminally truncated version Δ213 are about 20 times

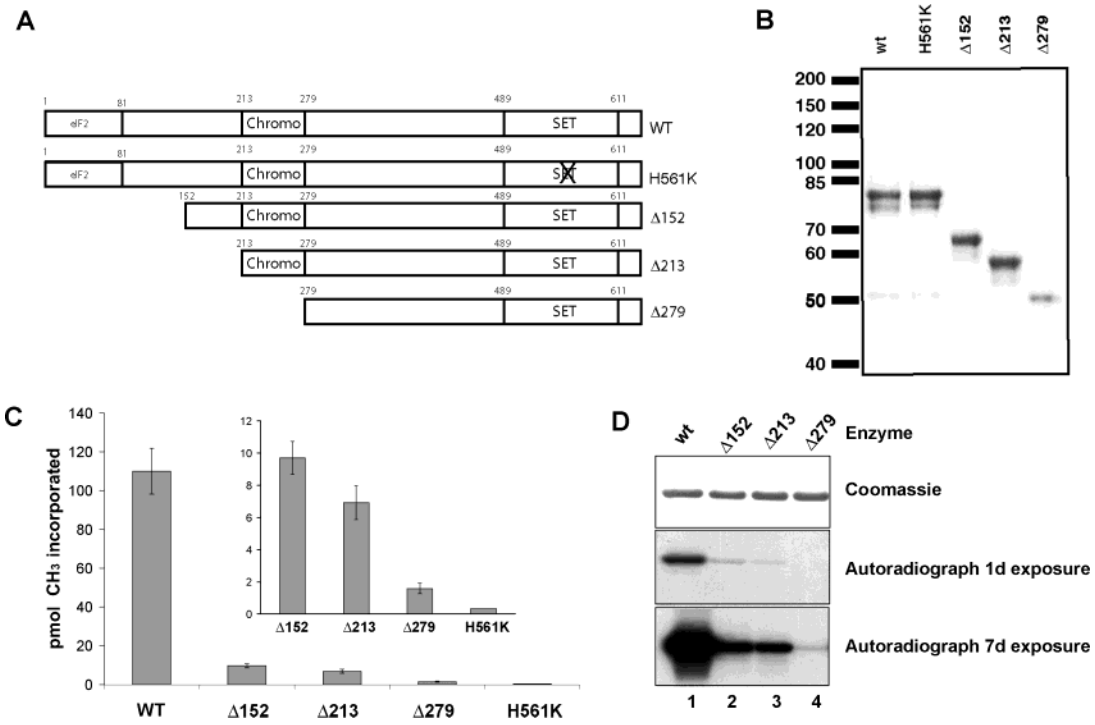

FIGURE 3: Expression and purification of dSU(VAR)3−9 mutants. (A) Scheme of dSU(VAR)3−9 mutants generated and expressed. In *Drosophila* dSU(VAR)3−9 mRNA is generated by alternative splicing of the translation initiation factor (eIF2) RNA leading to an additional 81 amino acids at the N-terminus of the protein, which are common between eIF2 and dSU(VAR)3−9. For the point mutation within the SET domain (H561K) histidine 561 was mutated into a lysine. (B) Coomassie-stained gel of Talon purified dSU(VAR)3−9 mutant proteins. (C) Comparison of HMT activity of various dSU(VAR)3−9 mutants on a H3 peptide and on recombinant H3 molecules (D). For a better comparison, the inset of panel C shows the activities of the N-terminal deletions and a point mutation within the SET domain only. To compare different activities on intact H3 molecules, an autoradiograph of a 1-day (top panel) and a 7-day (bottom panel) exposure is shown.

lower than the ones observed for the full-length enzyme and more similar to the kinetic parameters of SET9 (6.8 nmol min^{-1} mg^{-1} and 0.005 s^{-1}, respectively) (Figure 4A). This substantial increase in the catalytic efficiency of the full-length enzyme may be due to a conformational change or an association of monomeric enzymes into multimers. As multimeric enzymes often show a concentration-dependent increase of specific activity as expressed in substrate molecules converted per molecule of enzyme (*46*), we determined the relationship between the velocity of the reaction and the enzyme concentration. When the rate of methyl peptide formation was plotted against the corresponding enzyme concentration, a nonlinear plot was obtained (Figure 4B). This behavior is not observed when using the N-terminally truncated proteins, which only show a linear dependence of methyltransferase activity on enzyme concentration. Replotting the number of CH_3 groups incorporated per enzyme molecule revealed a steady increase of activity until the specific activity reached a plateau at an enzyme concentration of approximately 0.5 μM for the wild type. In contrast, virtually no change was observed for the N-terminally truncated enzyme (Figure 4C). On the basis of these observations, we reasoned that under our assay conditions the full-length enzyme is indeed able to form multimers, which have a higher specific activity than the monomers and that at a concentration of 0.5 μM and above virtually all molecules exist as multimers.

The N-Terminus Is Important for Dimerization and Activity of dSU(VAR)3−9 in Vitro. To test this hypothesis, we analyzed the molecular weight of the recombinant protein by gel filtration chromatography and density gradient centrifugation. The full-length dSU(VAR)3−9 elutes with an apparent molecular weight of 160 kDa, which is indicative of a dimer from a Superdex 200 gel filtration column (Figure 5A). The deletion of the N-terminus of dSU(VAR)3−9 results in a complete loss of this dimerization as the N-terminally truncated protein elutes with an apparent molecular weight of 60 kDa corresponding to a monomeric species (Figure 5A bottom panel). To further confirm the dimerization of dSU(VAR)3−9, we loaded the purified, recombinant enzyme on a 5−20% sucrose gradient under denaturing and nondenaturing conditions. As expected, under native conditions the full-length dSU(VAR)3−9 is found in fractions corresponding to higher molecular weight when compared to the Δ213, whereas it behaves virtually identical under denaturing conditions (Figure 5B). The N-terminally deleted protein as well as the BSA standard display a minor shift to an apparently smaller molecular weight in the presence of urea, which is probably due to a change in density of the denaturing solution (arrows in Figure 5B).

The N-Terminus Mediates Interactions within dSU(VAR)3−9. As the deletion of the first 152 amino acids leads to a loss of dimer formation, we expressed the N-terminal 152 amino acids of dSU(VAR)3−9 as a GST fusion protein in bacteria and used it as an affinity resin in GST pull-down experiments. The bound fusion protein was incubated either with recombinant HP1 or with in vitro translated dSU(VAR)3−9 proteins. As has been shown previously (*14*, *34*), the N-terminus is able to interact with HP1 in vitro (Figure 6A, right panel). This domain also inter-

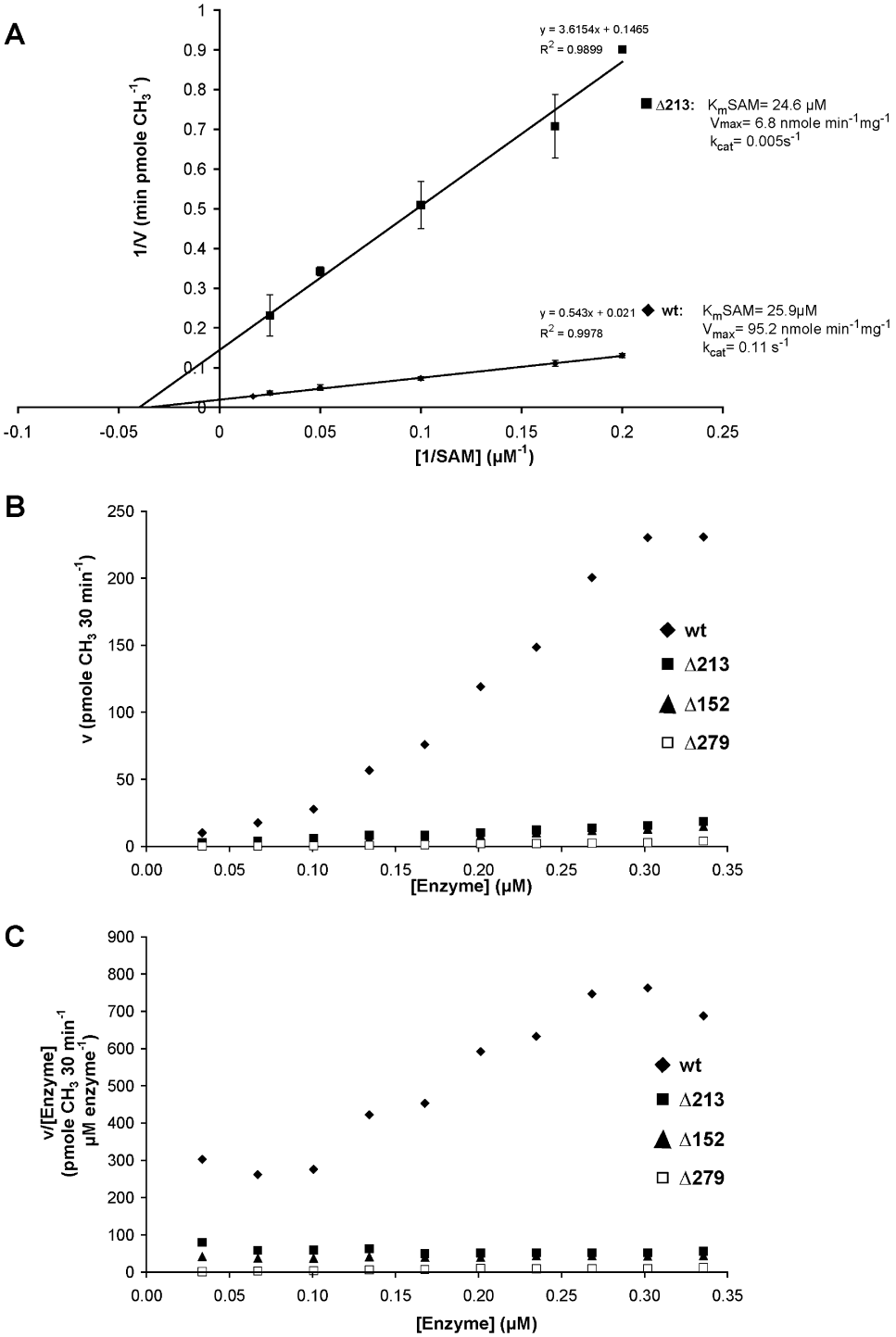

FIGURE 4: Kinetic properties of recombinant dSU(VAR)3−9. (A) Double reciprocal plots of in vitro methylation reactions using a H3 tail peptide and either wt (500 ng) or a N-terminally truncated protein (Δ213; 1 μg) using various concentrations of SAM under saturating peptide concentrations were used to determine the K_m for SAM. (B) Concentration-dependent increase of enzyme activity. Different concentrations of wt and N-terminally truncated dSU(VA)3−9 were incubated with H3 peptide and SAM at saturating concentrations for 30 min. After stopping the reaction, the amount of incorporated radioactivity was measured and plotted against the concentration of enzyme. (C) The ratio of the incorporated radioactivity to the concentration of enzyme present was replotted against the enzyme concentration to determine the increase in specific activity.

acts very efficiently with in vitro translated dSU(VAR)3−9, suggesting a role of this domain in inter- or intramolecular interactions. Surprisingly, this domain is also able to interact with a polypeptide lacking the first 152 amino acids. To map the region within dSU(VAR)3−9 that interacts with the first 152 amino acids, we have generated a series of mutations within dSU(VAR)3−9 and analyzed their capability to bind to the N-terminus (Figure 6A,B). Using these mutant proteins, we could narrow down the interaction domain to amino acids 152−213 (Figure 6C). This suggests the existence of a bipartite interaction motif that can potentially mediate a intra- as well as an intermolecular interaction. To further confirm this result, we expressed the amino acids 152−213 as a GST fusion protein and did another series of GST pull down experiments. As expected, the GST(152−213) protein is able to interact with the full-length protein

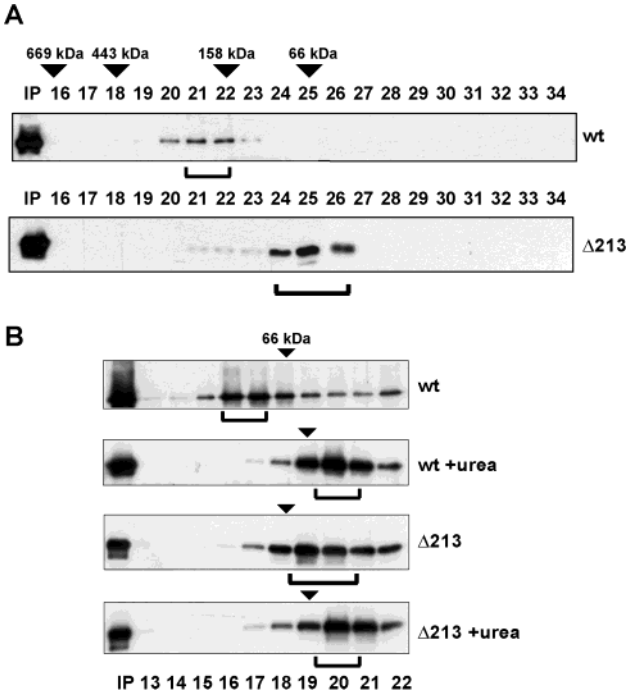

FIGURE 5: Molecular weight determination of dSU(VAR)3−9. (A) Elution profiles of wt dSU(VAR)3−9 (top) and the N-terminal deletion construct Δ213 (bottom). Retention of the molecular weight standards is indicated at the top. (B) Profile of a 5−20% sucrose gradient in the presence (panels 2 and 4) or absence of 3 M urea (panels 1 and 3). The position of the 66-kDa marker protein is indicated as a black triangle at the top of each gel. Note the slight shift (1 fraction) of the 66-kDa marker and the Δ213 protein, which is due to the presence of urea in the buffer compared to the significant shift in case of the wt protein (>3 fractions).

but only weakly binds to a protein lacking the first 152 amino acids (Figure 7A,B). On the basis of this result, we strongly favor a model in which dSU(VAR)3−9 interacts with itself via a domain swapping mechanism similar to what has been described for other dimeric proteins (*47*).

DISCUSSION

Recombinant dSU(VAR)3−9 is a catalytically active methyltransferase and is able to methylate lysine 9 within histone H3. The main conclusions from our experiments are that the full-length enzyme transfers two methyl groups to lysine 9 within an H3 tail peptide in a nonprocessive manner and that the enzyme requires the N-terminus for homodimerization and for full activity in vitro. The N-terminus of dSU(VAR)3−9 can be subdivided into two parts, which can interact with each other.

The initial rate kinetics of the methylation reaction show that dSU(VAR)3−9 has a very similar K_m for the protein substrate and a similar turnover rate as another SET containing methyltransferase, Rubisco LSMT (*23*), but a significantly higher K_m for SAM. This suggests that to have full catalytic activity the concentration of SAM has to be kept at a relatively high concentration within the cell. The intracellular SAM concentration varies between 20 and 40 μM (*48*) depending on the cell type used, which is in the range of the K_m of dSU(VAR)3−9. Under these conditions, the rate of methylation by dSU(VAR)3−9 is directly proportional to the SAM concentration and can therefore directly respond to variations of cofactor concentration. This high SAM concentration is maintained by the main SAM synthase enzyme, SU(Z)5, which, like dSU(VAR)3−9, is a suppressor for position effect variegation in *Drosophila* (*49*). It will be interesting to see whether the Su(z)5 gene product is randomly distributed throughout the cell or if it shows a specific localization maybe directing it to the sites of dSU(VAR)3−9 action. The concentration of SAM could therefore be an important regulator of heterochromatin spreading.

Recently several labs have characterized the processivity of various SET containing enzymes revealing remarkable differences in their ability to add one, two, or three methyl groups to their corresponding substrate (*25*, *45*). Furthermore, mono-, di-, and trimethylated histones have different functions (*44*). By analyzing the reaction products of recombinant dSU(VAR)3−9 in vitro, we find that it very efficiently adds two methyl groups to its substrate but is very poor in adding a third one. Moreover, we only see a trimethylated peptide (less than 1% of total product) at a high enzyme-to-substrate ratio (Figure 2D and data not shown). However, deletion of dSU(VAR)3−9 in vivo leads to a disappearance of trimethylated H3 molecules at the chromocenter of polytene chromosomes (*30*). The fact that we can observe a small trimethylating activity of recombinant dSU(VAR)3−9 argues against a sterical inhibition of trimethylation within the active center by the enzyme as it is the case for SET9 (*50*).

SET domain containing enzymes have been classified in several different classes based on their primary amino acid sequence (*51*). The only enzyme of the SU(VAR)3−9 class that has been kinetically characterized in detail is the *Neurospora* enzyme DIM5. Our experiments show that dSU(VAR)3−9, in contrast to DIM5, is not processive and mainly dimethylates a peptide containing lysine 9. Further analysis of additional point mutations within the SET domain will reveal the structural basis of this intriguing difference. An obvious candidate for an amino acid important for this difference is the R238 of DIM5, which is replaced by a histidine in dSU(VAR)3−9 and its orthologues in mouse and humans (*15*). In fact, it has been shown that a mutation within SUV39H1 converting H320 into an arginine residue strongly increases activity in vitro (*15*).

It has been speculated that a targeted recruitment of SET1 to the promoter region of an active gene can lead to a limited trimethylation due to a very high local concentration of enzyme (*44*, *52*), whereas the reminder of the chromatin is only dimethylated. As we observe a nonlinear increase of HMT activity dependent on the enzyme concentration in vitro, it is possible that comparable mechanisms of the regulation of the methylation state also affect the methylation capability of dSU(VAR)3−9 in vivo. Alternatively, the ability of dSU(VAR)3−9 to trimethylate may be regulated by phosphorylation of the protein (*19*, *53*), or through interaction with additional cofactors (*19*) as has been shown recently in the case of ESET (*54*). Furthermore, the trimethylation of K9 could be mediated by a different HMT enzyme, which is able to add a third methyl group to already dimethylated H3. One of the potential enzymes with such a trimethylating activity is the polycomb group protein enhancer of zeste (*30*).

Besides this kinetic observation, the most interesting observation we have made in analyzing the recombinant dSU(VAR)3−9 protein is the fact that it requires the

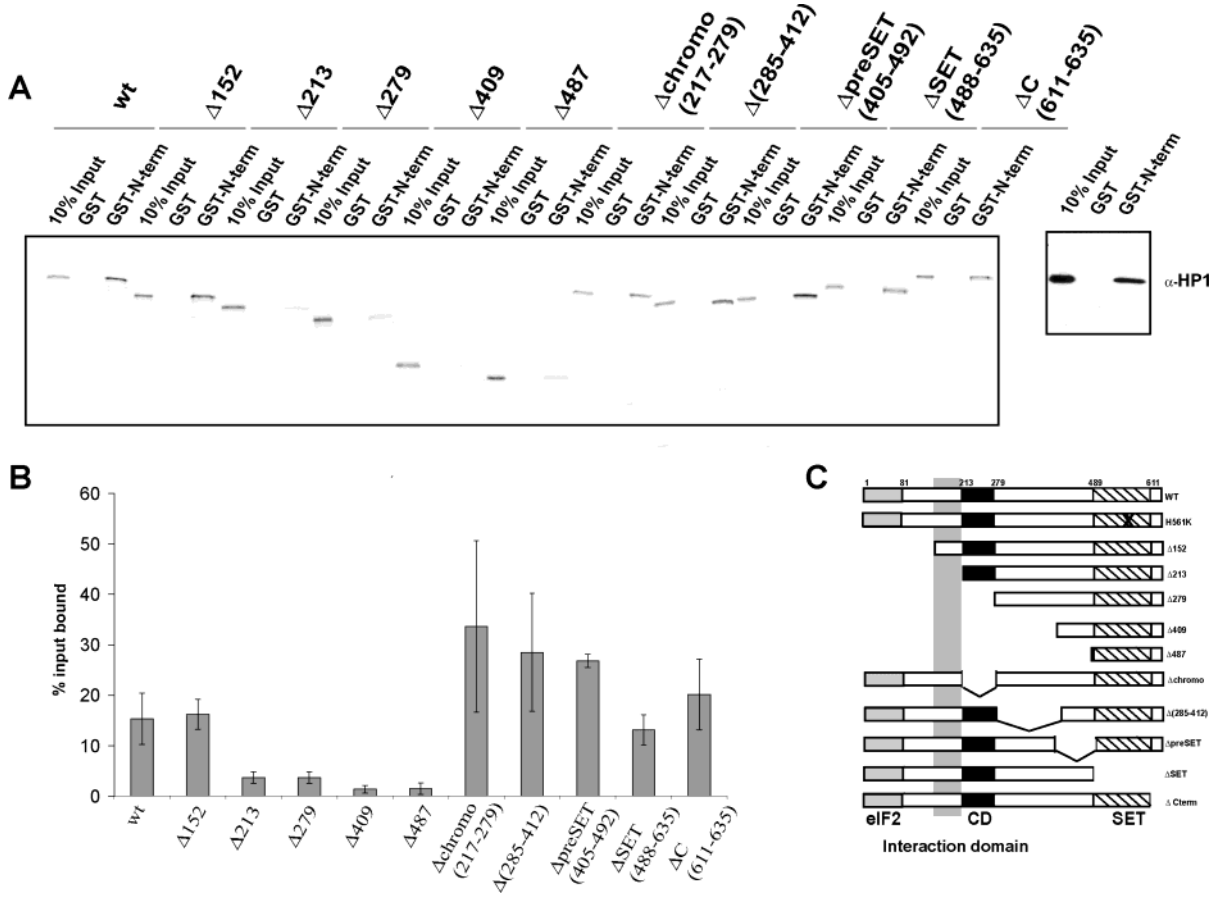

FIGURE 6: In vitro interaction assays to map the dimerization domain. The first 152 amino acids of dSU(VAR)3−9 are expressed as a GST fusion proteins and used to affinity purify either recombinant HP1 (A, right panel) or in vitro translated dSU(VAR)3−9 (A, left panel). HP1 was detected by Western blotting using a monoclonal antibody against HP1 (C1A9, kind gift of S. Elgin) and dSU(VAR)3−9 was detected by autoradiography. (B) Quantification of the binding affinities of the various dSU(VAR)3−9 proteins. Error bars represent the variations of at least three different experiments. (C) Schematic representations of the dSU(VAR)3−9 proteins used and the position of the dimerization domain.

N-terminus for its full in vitro activity. Our experiments strongly suggest that this N-terminus allows the recombinant protein to form dimers in solution, which greatly enhances its activity. However, we cannot fully exclude the possibility that intramolecular interactions within the N-terminus induce a major conformational shift that causes the full-length protein to migrate with an apparent molecular weight of a dimer on a gel filtration column as well as a density gradient. The observed concentration-dependent increase in specific enzyme activity, as expressed in molecules SAM converted per molecule of enzyme, argues against this possibility as the different conformation should not change as a function of enzyme concentration. Interestingly, the arginine methyltransferase PRMT1 and the SET domain containing lysine methyltransferase vSET also form oligomers in vitro (*26*, *55*, *56*). Both enzymes are nonprocessive and the multimerization of the protein has been suggested to facilitate the methylation reaction by increasing the probability of the monomethylated product to bind to a second enzyme molecule. This is in contrast to other SET domain proteins such as DIM-5 and SET7/9, which are either processive or only transfer one methyl group respectively and do not dimerize in vitro. Interestingly, the catalytic capacity of full-length dSU(VAR)3−9 is about 20 times higher than the one of SET9 or a N-terminally truncated version of dSU(VAR)3−9, which both fail to dimerize in solution suggesting that the dimerization can indeed be used to significantly enhance HMT activity.

The mapping of the dimerization domain via GST pull down experiments revealed a bipartite dimeriztaion motif. This organization points to a dimerization via a domain swapping mechanism in which identical domains are exchanged between two proteins to form a homodimer (*57*). On the basis of our experiments, we would suggest that the amino acids 1−152 can interact with the residues 152−213 either intra- or intermolecularly and that the domain swapped dimer has a higher catalytic capacity than the monomer. The different conformation of a loop between two swapped domains in the monomeric and the dimeric form of an enzyme can have a significant effect on the function of a remote active site (*47*). Such an induced conformational change may provide an alternative explanation for the higher activity of the dimeric form of dSU(VAR)3−9. Domain swapping has also been implicated in the formation of oligomeric assemblies of proteins (*58*). Although we have strong evidence for dimer formation and could not identify higher order oligomers in gel filtration chromatography or density gradient experiments, at present we cannot fully exclude the existence of oligomeric species that may assembly from dimers and are disrupted in the course of our analysis.

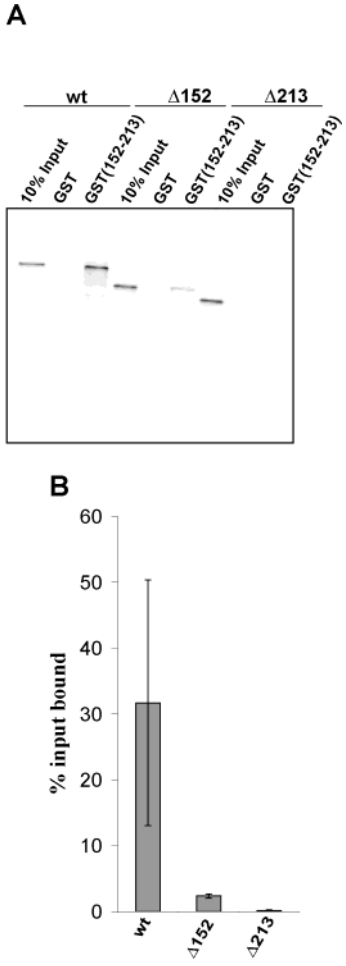

FIGURE 7: In vitro interaction assay using amino acids 152−213 as a GST fusion protein to affinity purify in vitro translated dSU(VAR)3−9, and two N-terminally truncated versions of dSU(VA)3−9 (A). (B) Quantification of the binding affinities of the various dSU(VAR)3−9 proteins. Error bars represent the variations of at least three different experiments.

The concentration-dependent self-interaction of dSU(VAR)3−9 may also be a way to regulate its function within the cell. One well-known features of dSU(VAR)3−9 is its strict dosage-dependent effect on PEV. Our observation that dSU(VAR)3−9's specific HMT activity increases in a nonlinear way provides a possible explanation for this effect. The human orthologue of dSU(VAR)3−9, SUV39H1, forms distinct nuclear bodies when overexpressed in tissue culture cells (*19*), which can be dispersed by modulating the phosphorylation state of the enzyme. From our in vitro experiments, we would suggest that the N-terminus plays an important role in the formation of those nuclear bodies.

ACKNOWLEDGMENT

We would like to thank Sally Elgin, Gunter Reuter, Danny Reinberg, and Joel Eissenberg for valuable reagents. We also would like to thank Peter Becker, Alex Brehm, and Josephine Sutcliffe for valuable comments on the manuscript and Felix Hartlepp for recombinant GST protein.

REFERENCES

1. Choo, K. H. (1997) Centromere DNA dynamics: latent centromeres and neocentromere formation. *Am. J. Hum. Genet. 61*, 1225−33.
2. Karpen, G. H., and Allshire, R. C. (1997) The case for epigenetic effects on centromere identity and function. *Trends Genet. 13*, 489−96.
3. Earnshaw, W. C., and Cooke, C. A. (1989) Proteins of the inner and outer centromere of mitotic chromosomes. *Genome 31*, 541−52.
4. Sugata, N., Li, S., Earnshaw, W. C., Yen, T. J., Yoda, K., Masumoto, H., Munekata, E., Warburton, P. E., and Todokoro, K. (2000) Human CENP-H multimers colocalize with CENP-A and CENP-C at active centromere−kinetochore complexes. *Hum. Mol. Genet. 9*, 2919−26.
5. Weimer, R., Haaf, T., Kruger, J., Poot, M., and Schmid, M. (1992) Characterization of centromere arrangements and test for random distribution in G0, G1, S, G2, G1, and early S′ phase in human lymphocytes. *Hum. Genet. 88*, 673−82.
6. Zalensky, A. O., Allen, M. J., Kobayashi, A., Zalenskaya, I. A., Balhorn, R., and Bradbury, E. M. (1995) Well-defined genome architecture in the human sperm nucleus. *Chromosoma 103*, 577−90.
7. Warburton, P. E., Dolled, M., Mahmood, R., Alonso, A., Li, S., Naritomi, K., Tohma, T., Nagai, T., Hasegawa, T., Ohashi, H., Govaerts, L. C., Eussen, B. H., Van Hemel, J. O., Lozzio, C., Schwartz, S., Dowhanick-Morrissette, J. J., Spinner, N. B., Rivera, H., Crolla, J. A., Yu, C., and Warburton, D. (2000) Molecular cytogenetic analysis of eight inversion duplications of human chromosome 13q that each contain a neocentromere. *Am. J. Hum. Genet. 66*, 1794−806.
8. Depinet, T. W., Zackowski, J. L., Earnshaw, W. C., Kaffe, S., Sekhon, G. S., Stallard, R., Sullivan, B. A., Vance, G. H., Van Dyke, D. L., Willard, H. F., Zinn, A. B., and Schwartz, S. (1997) Characterization of neo-centromeres in marker chromosomes lacking detectable alpha-satellite DNA. *Hum. Mol. Genet. 6*, 1195−204.
9. Richards, E. J., and Elgin, S. C. (2002) Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell 108*, 489−500.
10. Reuter, G., and Spierer, P. (1992) Position effect variegation and chromatin proteins. *Bioessays 14*, 605−12.
11. Henikoff, S. (1992) Position effect and related phenomena. *Curr. Opin. Genet. Dev. 2*, 907−12.
12. Choo, K. H. (2000) Centromerization. *Trends Cell Biol. 10*, 182−8.
13. Pidoux, A. L., and Allshire, R. C. (2000) Centromeres: getting a grip of chromosomes. *Curr. Opin. Cell Biol. 12*, 308−19.
14. Schotta, G., Ebert, A., Krauss, V., Fischer, A., Hoffmann, J., Rea, S., Jenuwein, T., Dorn, R., and Reuter, G. (2002) Central role of Drosophila SU(VAR)3−9 in histone H3−K9 methylation and heterochromatic gene silencing. *EMBO. J. 21*, 1121−31.
15. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B. D., Sun, Z. W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C. P., Allis, C. D., and Jenuwein, T. (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature 406*, 593−9.
16. Schotta, G., Ebert, A., Dorn, R., and Reuter, G. (2003) Position-effect variegation and the genetic dissection of chromatin regulation in Drosophila. *Semin. Cell Dev. Biol. 14*, 67−75.
17. Ivanova, A. V., Bonaduce, M. J., Ivanov, S. V., and Klar, A. J. (1998) The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. *Nat. Genet. 19*, 192−5.
18. Peters, A. H., O'Carroll, D., Scherthan, H., Mechtler, K., Sauer, S., Schofer, C., Weipoltshammer, K., Pagani, M., Lachner, M., Kohlmaier, A., Opravil, S., Doyle, M., Sibilia, M., and Jenuwein, T. (2001) Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell 107*, 323−37.
19. Firestein, R., Cui, X., Huie, P., and Cleary, M. L. (2000) Set domain-dependent regulation of transcriptional silencing and growth control by SUV39H1, a mammalian ortholog of Drosophila Su(var)3−9. *Mol. Cell Biol. 20*, 4900−9.
20. Snowden, A. W., Gregory, P. D., Case, C. C., and Pabo, C. O. (2002) Gene-Specific Targeting of H3K9 Methylation Is Sufficient for Initiating Repression In Vivo. *Curr. Biol. 12*, 2159−66.
21. Min, J., Zhang, X., Cheng, X., Grewal, S. I., and Xu, R. M. (2002) Structure of the SET domain histone lysine methyltransferase Clr4. *Nat. Struct. Biol. 9*, 828−32.
22. Zhang, X., Tamaru, H., Khan, S. I., Horton, J. R., Keefe, L. J., Selker, E. U., and Cheng, X. (2002) Structure of the Neurospora SET domain protein DIM-5, a histone H3 lysine methyltransferase. *Cell 111*, 117−27.

23. Trievel, R. C., Beach, B. M., Dirk, L. M., Houtz, R. L., and Hurley, J. H. (2002) Structure and catalytic mechanism of a SET domain protein methyltransferase. *Cell 111*, 91−103.
24. Wilson, J. R., Jing, C., Walker, P. A., Martin, S. R., Howell, S. A., Blackburn, G. M., Gamblin, S. J., and Xiao, B. (2002) Crystal structure and functional analysis of the histone methyltransferase SET7/9. *Cell 111*, 105−15.
25. Xiao, B., Jing, C., Wilson, J. R., Walker, P. A., Vasisht, N., Kelly, G., Howell, S., Taylor, I. A., Blackburn, G. M., and Gamblin, S. J. (2003) Structure and catalytic mechanism of the human histone methyltransferase SET7/9. *Nature 421*, 652−6.
26. Manzur, K. L., Farooq, A., Zeng, L., Plotnikova, O., Koch, A. W., Sachchidanand, and Zhou, M. M. (2003) A dimeric viral SET domain methyltransferase specific to Lys27 of histone H3. *Nat. Struct. Biol. 10*, 187−96.
27. Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R. S., and Zhang, Y. (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science 298*, 1039−43.
28. Muller, J., Hart, C. M., Francis, N. J., Vargas, M. L., Sengupta, A., Wild, B., Miller, E. L., O'Connor, M. B., Kingston, R. E., and Simon, J. A. (2002) Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell 111*, 197−208.
29. Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (2002) Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev. 16*, 2893−905.
30. Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A., and Pirrotta, V. (2002) Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell 111*, 185−96.
31. Fang, J., Feng, Q., Ketel, C. S., Wang, H., Cao, R., Xia, L., Erdjument-Bromage, H., Tempst, P., Simon, J. A., and Zhang, Y. (2002) Purification and Functional Characterization of SET8, a Nucleosomal Histone H4-Lysine 20-Specific Methyltransferase. *Curr. Biol. 12*, 1086−99.
32. Nishioka, K., Rice, J. C., Sarma, K., Erdjument-Bromage, H., Werner, J., Wang, Y., Chuikov, S., Valenzuela, P., Tempst, P., Steward, R., Lis, J. T., Allis, C. D., and Reinberg, D. (2002) PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Mol. Cell 9*, 1201−13.
33. Melcher, M., Schmid, M., Aagaard, L., Selenko, P., Laible, G., and Jenuwein, T. (2000) Structure−function analysis of SUV39H1 reveals a dominant role in heterochromatin organization, chromosome segregation, and mitotic progression. *Mol. Cell Biol. 20*, 3728−41.
34. Yamamoto, K., and Sonoda, M. (2003) Self-interaction of heterochromatin protein 1 is required for direct binding to histone methyltransferase, SUV39H1. *Biochem. Biophys. Res. Commun. 301*, 287−92.
35. Melnick, A., and Licht, J. D. (1999) Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood 93*, 3167−215.
36. Kentsis, A., Gordon, R. E., and Borden, K. L. (2002) Control of biochemical reactions through supramolecular RING domain self-assembly. *Proc. Natl. Acad. Sci. U.S.A. 99*, 15404−9.
37. Henikoff, S. (1996) Dosage-dependent modification of position-effect variegation in Drosophila. *Bioessays 18*, 401−9.
38. Zhao, T., and Eissenberg, J. C. (1999) Phosphorylation of heterochromatin protein 1 by casein kinase II is required for efficient heterochromatin binding in Drosophila. *J. Biol. Chem. 274*, 15095−100.
39. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature 389*, 251−60.
40. Brackertz, M., Boeke, J., Zhang, R., and Renkawitz, R. (2002) Two highly related p66 proteins comprise a new family of potent transcriptional repressors interacting with MBD2 and MBD3. *J. Biol. Chem. 277*, 40958−66.
41. Ogawa, H., Ishiguro, K., Gaubatz, S., Livingston, D. M., and Nakatani, Y. (2002) A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science 296*, 1132−6.
42. Tachibana, M., Sugimoto, K., Fukushima, T., and Shinkai, Y. (2001) Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. *J. Biol. Chem. 276*, 25309−17.
43. Czermin, B., Schotta, G., Hulsmann, B. B., Brehm, A., Becker, P. B., Reuter, G., and Imhof, A. (2001) Physical and functional association of SU(VAR)3−9 and HDAC1 in Drosophila. *EMBO Rep. 2*, 915−9.
44. Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, J., Bernstein, B. E., Emre, N. C., Schreiber, S. L., Mellor, J., and Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. *Nature 419*, 407−11.
45. Tamaru, H., Zhang, X., McMillen, D., Singh, P. B., Nakayama, J. I., Grewal, S. I., Allis, C. D., Cheng, X., and Selker, E. U. (2003) Trimethylated lysine 9 of histone H3 is a mark for DNA methylation in Neurospora crassa. *Nat. Genet. 34*, 75−9.
46. Bheemanaik, S., Chandrashekaran, S., Nagaraja, V., and Rao, D. N. (2003) Kinetic and catalytic properties of dimeric KpnI DNA methyltransferase. *J. Biol. Chem. 278*, 7863−74.
47. Schymkowitz, J. W., Rousseau, F., Wilkinson, H. R., Friedler, A., and Itzhaki, L. S. (2001) Observation of signal transduction in three-dimensional domain swapping. *Nat. Struct. Biol. 8*, 888−92.
48. Hoffman, D. R., Marion, D. W., Cornatzer, W. E., and Duerre, J. A. (1980) S-Adenosylmethionine and S-adenosylhomocystein metabolism in isolated rat liver. Effects of L-methionine, L-homocystein, and adenosine. *J. Biol. Chem. 255*, 10822−7.
49. Larsson, J., and Rasmuson-Lestander, A. (1994) Molecular cloning of the S-adenosylmethionine synthetase gene in Drosophila melanogaster. *FEBS Lett. 342*, 329−33.
50. Zhang, X., Yang, Z., Khan, S. I., Horton, J. R., Tamaru, H., Selker, E. U., and Cheng, X. (2003) Structural basis for the product specificity of histone lysine methyltransferases. *Mol. Cell 12*, 177−85.
51. Baumbusch, L. O., Thorstensen, T., Krauss, V., Fischer, A., Naumann, K., Assalkhou, R., Schulz, I., Reuter, G., and Aalen, R. B. (2001) The *Arabidopsis thaliana* genome contains at least 29 active genes encoding SET domain proteins that can be assigned to four evolutionarily conserved classes. *Nucleic Acids Res. 29*, 4319−33.
52. Ng, H. H., Robert, F., Young, R. A., and Struhl, K. (2003) Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol. Cell 11*, 709−19.
53. Aagaard, L., Schmid, M., Warburton, P., and Jenuwein, T. (2000) Mitotic phosphorylation of SUV39H1, a novel component of active centromeres, coincides with transient accumulation at mammalian centromeres. *J. Cell Sci. 113*, 817−29.
54. Wang, H., An, W., Cao, R., Xia, L., Erdjument-Bromage, H., Chatton, B., Tempst, P., Roeder, R. G., and Zhang, Y. (2003) mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. *Mol. Cell 12*, 475−87.
55. Tang, J., Frankel, A., Cook, R. J., Kim, S., Paik, W. K., Williams, K. R., Clarke, S., and Herschman, H. R. (2000) PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. *J. Biol. Chem. 275*, 7723−30.
56. Tang, J., Gary, J. D., Clarke, S., and Herschman, H. R. (1998) PRMT 3, a type I protein arginine *N*-methyltransferase that differs from PRMT1 in its oligomerization, subcellular localization, substrate specificity, and regulation. *J. Biol. Chem. 273*, 16935−45.
57. Newcomer, M. E. (2002) Protein folding and three-dimensional domain swapping: a strained relationship? *Curr. Opin. Struct. Biol. 12*, 48−53.
58. McGee, A. W., Dakoji, S. R., Olsen, O., Bredt, D. S., Lim, W. A., and Prehoda, K. E. (2001) Structure of the SH3-guanylate kinase module from PSD-95 suggests a mechanism for regulated assembly of MAGUK scaffolding proteins. *Mol. Cell 8*, 1291−301.

BI035964S