The Inherent Processivity of the Human de novo Methyltransferase 3A (DNMT3A) is Enhanced by DNMT3L

Celeste Holz-Schietinger§ and Norbert O. Reich‡§

Interdepartmental Program in Biomolecular Science & Engineering§, and Department of Chemistry & Biochemistry‡ University of California, Santa Barbara, California 93106-9510

Address correspondence to: Norbert O. Reich, Email: reich@chem.ucsb.edu. Phone: (805) 893-8368. Fax (805) 893-4120

Running Title: DNMT3A processivity and its regulation

Human DNMT3A is responsible for de novo DNA cytosine methylation patterning during development. Here, we show that DNMT3A methylates 5-8 CpG sites on human promoters before 50% of the initially bound enzyme dissociates from the DNA. Processive methylation is enhanced 3 fold in the presence of DNMT3L an inactive homology of DNMT3Ahjk, therefore providing a mechanism for the previously described DNMT3L activation of DNMT3A. DNMT3A processivity on human promoters is also regulated by DNA topology, where a two fold decrease in processivity was observed on supercoiled DNA in comparison to linear DNA. These results are the first observation that DNMT3A utilizes this mechanism of increasing catalytic efficiency. Processive de novo DNA methylation provides a mechanism that ensures multiple CpG sites undergo methylation for transcriptional regulation and silencing of newly integrated viral DNA.

The large family of DNA methyltransferases including both bacterial and mammalian enzymes displays highly variable degrees of processivity. The mammalian DNMT1 (18, 19) and bacterial M.SssI (20) are extremely processive, whereas others bacterial enzymes including M.EcoRI (21), are entirely lacking in processivity. In this study we investigated the enzymatic properties of the human de novo methyltransferase DNMT3A and determined its processivity using pulse-chase assays, kinetic modeling and determining $k_{cat}$ values for substrates of embryogenesis (5, 6). DNMT3A and the closely related DNMT3B carry out de novo methylation, laying down new DNA methylation patterns throughout the genome (7-9). Methylation patterns are then stably maintained over cell divisions by the maintenance methyltransferase DNMT1 (10, 11). Although 70-80% of CpG sites are methylated in the genome, some regions (CpG islands) are largely resistant to methylation, while others (retrotransposons and highly repetitive sequences) undergo extensive methylation (8). Determining how DNMT3A methylates an entire region in the genome is necessary to understand the temporal and spatial regulation of de novo methylation. Many mechanisms that control de novo methyltransferase specificity have been proposed, including cis-acting signals in the DNA sequence (12), DNA topology (13) histone modifications (14), interactions between DNMTs (15), auxiliary proteins (16) or RNA molecules (17). However, the underlying mechanisms leading to the acquisition of new methylation patterns remain poorly understood. The methylation of multiple, proximal CpG sites during a single binding event (processive catalysis) by DNMT3A or DNMT3B may contribute to the localized acquisition of methylation marks.

Copyright 2010 by The American Society for Biochemistry and Molecular Biology, Inc.
with varying number of CpG sites. We found DNMT3A is processive, spreading methylation to distal CpG sites. DNMT3A processivity is modulated by DNA topology and protein-protein interactions. DNMT3L, a known activator of DNMT3A catalytic activity, is shown to be a processivity factor of DNMT3A. The data presented supports a model of DNA methylation spreading through the action of processive catalysis.

METHODS

Materials

The DNA used as substrates, poly-dIdC (~1000 bp) (Sigma-Aldrich), poly-dAdT (~1000 bp) (Sigma-Aldrich), GCbox2 (5'-GGGAATTCAAGGGGGCGGGGCAATGTAGG G-3') duplex and Gusch (TCAAAAAGGATCTTCACCTAG) duplex (non-CpG containing sequence) were purchased from Midland Certified Reagent Company (Midland, TX). The recognition site for DNMT3A is underlined. Duplexes were prepared by annealing the complementary strands in a buffer containing 10 mM Tris-Cl pH 8.0, 1 mM EDTA, and 50 mM NaCl, heating the DNA to 85°C for 5 minutes and slow cooling the DNA. Annealing was confirmed by 12% PAGE analysis.

Bacterial plasmid pBR322 (NEB) was produced by transforming the plasmid in XL blue cells (E. coli, with no topoisomerase mutations), which were then grown in LB media overnight at 37°C. The plasmid was extracted by a Hispeed plasmid midi kit (Qiagen). The human promoters used as substrates were amplified from genomic DNA extracted from HeLa cells. PCR conditions include Taq DNA polymerase (10 unit/100 μl rxn NEB), dNTPs (0.2 mM), primers (0.5 μM), thermo buffer II (1x), glycerol (1.3 %), genomic DNA (200 ng/100 μl). Reactions were run for 5 mins at 95°C, then Taq DNA polymerase was added, followed by 30 cycles at 30 sec at 94°C, 30 sec at (EFS1 (62°C), p21 (53°C), p15 (55°C), ODC (63°C), β-actin (55°C), Smarca (55°C), CDC (55°C), Survivin (55°C), H-cad (55°C), Xist (53°C)), 1 min at 72°C then 10 mins at 72°C. The primers for the promoters are listed in supplementary TABLE 1. The PCR products were then used as the substrate for further amplification of each promoter. The sequences of the PCR products were confirmed by sequencing.

Plasmid pCpGL was kindly provided by Michael Rehli (22). The p21-pCpGL substrate was created by amplification of the p21 promoter, with PCR conditions include phusion high-fidelity DNA polymerase,(2 unit/100 μl rxn NEB), dNTPs (0.2 mM), primers (0.5 μM), HF buffer (1x), genomic DNA (200 ng/100 μl). Reactions were run for 4 mins at 98°C, followed by 35 cycles for 10 sec at 98°C, 25 sec at 68°C, 1 min at 72°C then 10 mins at 72°C. Promoters digested with HindIII and BamHI, and then ligated into digested and gel purified pCpGL. Inserted sequence was confirmed by sequencing. The pCpGL and p21-pCpGL substrates were produced by transforming into GT115 competent cells (InvivoGen) then grown overnight in LB media at 37°C. The plasmid was extracted using a Hispeed plasmid midi kit (Qiagen). The p21-pCpGL was digested with BamHI endonuclease overnight at 37°C and purified using a Qiagen PCR clean up kit. The form of the substrates was confirmed by analyzes on 0.8% agarose gel stained with ethidium bromide. Bacterial plasmids prepared in this way have an average superhelical density of -0.06 (23).

DNA concentrations are given in base pairs unless noted otherwise. Concentrations were determined by the absorbance at 260 nm, using the molar absorptive coefficients, poly-dIdC 6.9 mM -1 cm-1, poly-dAdT 6.6 mM-1 cm-1 and promoters and plasmids 6.8 mM -1 cm -1 for the number of base pairs and Gusch 0.4123 M -1 cm-1, GCbox2 0.3038 M -1 cm-1 for the number of molecules. Unlabeled S-adenosyl-L-methionine (AdoMet) was purchased from Sigma-Aldrich; t-[methyl-3H] adenosyl-L-methionine was purchased from GE Lifesciences.

Expression constructs

The plasmids used for E. coli recombinant protein expression include pET28a-hDNMT3A Copt, and pET28a-hDNMT3A catalytic_domain (Δ1-611) as described in Purdy et al. 2010 (24). pTYB1-3L used to express hDNMT3L was kindly provided by Frederic Chedin (University of California Davis).

Protein Expression

Full length DNMT3A and DNMT3L were expressed in E. coli strain Rosetta2 (DE3) pLysS (from Novagen), and the DNMT3A catalytic
domain was expressed in *E. coli* strain ER2566 (New England Biolabs) that had previously been transformed with the pLYsS plasmid (Novagen). Cell cultures were grown in 2xYT media at 37 °C to an OD<sub>600</sub> of 0.9 (DNMT3A), 0.4 (DNMT3L), 0.7 (DNMT3A catalytic domain). The temperature was lowered for DNMT3A full length and DNMT3A catalytic domain to 28 °C and expression was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (Fisher Scientific). Induction was two hours for DNMT3A and DNMT3L and four hours for the DNMT3A catalytic domain. Cells were harvested by centrifugation and frozen at -80 °C.

**Full Length DNMT3A Purification**

DNMT3A was purified from cells lysed in 50 mM Tris-HCl, 500 mM NaCl, pH 7.5 with 1 mM EDTA, 0.5 mM DTT, 0.2% (v/v) triton X-100, 0.4 mM PMSF, and 1% (v/v) protease inhibitor cocktail (Sigma-Aldrich) with a French press in the presence of DNase I 25 ug/ml, then clarified by centrifugation. The supernatant was diluted to 300 mM NaCl and loaded onto a Biorex-70 column (BioRad). The column was washed with 50 mM Tris-Cl, 300 mM NaCl, pH 7.0 with 1 mM EDTA, 0.5 mM DTT, 0.1% triton X-100, 0.2 mM PMSF, and 1 μM pepstatin A (BR wash buffer), followed by the same buffer but with 400 mM NaCl. The protein was eluted in BR wash buffer containing 800 mM NaCl (and no EDTA). The eluant was brought to 40 mM imidazole (Ni wash buffer), loaded on a 2 ml His-bind resin (Novagen) followed by a short wash of the same buffer with no Triton X-100. Protein was eluted with Ni wash buffer (400 mM NaCl, 400 mM imidazole and no Triton X-100). The protein was then dialyzed into storage buffer (50 mM Tris-Cl, 200 mM NaCl, 1 mM EDTA, 20% (v/v) glycerol, pH 7.2 with 0.5 mM DTT) and stored at -80 °C.

**Purification of DNMT3A catalytic domain**

The catalytic domain (Δ1-611) was purified as described in Purdy et al. 2010 (24). Briefly the catalytic domain was purified by a DEAE column, a Nickel-Sepharose column, followed by a BioRex-70 column and dialyzed into storage buffer.

**Purification of DNMT3L**

DNMT3L was purified from cells lysed by French press in nickel buffer (20 mM Tris-HCl pH 7.5, 500 mM EDTA, 40 mM imidazole with 0.2% Triton X-100, 10% glycerol) with 25 μg/ml DNase I and 1 mM PMSF, then clarified by centrifugation. Supernatant loaded onto a Ni-NTA Agarose (Qiagen). The column was washed with nickel buffer and eluted with nickel buffer with 500 mM imidazole. The eluted protein was dialyzed against chitin binding buffer (20 mM Tris-HCl, pH 8.5, 200 mM NaCl, 0.2% Triton X-100, and 10% glycerol) and bound to the chitin resin. DNMT3L was cleaved from the bound chitin binding protein using chitin cleavage buffer (20 mM Tris-HCl, pH 8.5, 500 mM NaCl, 100 mM dithiothreitol, 0.2% Triton X-100, and 10% glycerol). The eluted protein was dialyzed into storage buffer and frozen at -80 °C (25).

Protein purities of 85, 90 and 95% for full length DNMT3A, DNMT3A catalytic domain and DNMT3L respectively, were estimated by densitometry of Coomassie stained SDS-PAGE gels. Protein concentrations were determined by using calculated (26) 280 nm extinction coefficients which were within 20% of Bradford assays results using bovine serum albumin as a standard. The total concentration was calculated by multiplying the total protein concentration by the fraction of purity.

**Methylation Assays**

Reactions were carried out at 37 °C in a buffer of 50 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA, 20 mM NaCl, 2 μM AdoMet (a 6 Ci/mmol mix of unlabeled and 3H methyl labeled) at pH 7.8. Aliquots of 20 μl were removed from a larger reaction. Reactions were quenched by addition of 500 μM unlabeled AdoMet and digestion with 50 μg/mL proteinase K at 37 °C for 5 mins (27). Samples were spotted onto Whatman DE81 filters then washed, dried, and counted as described previously (28).

**k<sub>cat</sub> values**

k<sub>cat</sub> values were determined as described previously except with DNMT3A at 150 nM and 50 mM Tris-HCl, pH 7.8 in place of phosphate. For each substrate the k<sub>cat</sub> value was obtained by varying the substrate concentration (10 concentrations per substrate between 0-50 μM bp).
Each reaction was initiated with DNA and run for 1 hr at 37°C and quenched as stated above. The data was fit to Michaelis-Menten, or Substrate Inhibition equations using Prism Version 4.0 (GraphPad Software). $k_{cat}$ values were calculated by dividing the $V_{\text{max}}$ values by the amount of active enzyme in the reaction. The $k_{cat}$ values for each substrate were compared to the number of CpG sites and the size of the promoters, and data fit to a linear regression using the Prism Version 4.0 program.

**Processivity assays**

Processivity assays were carried out with DNMT3A or the DNMT3A-catalytic domain at 50 nM. The reaction was initiated following a 3 minute preincubation at 37°C by the addition of substrate DNA: poly-dIdC (5 μM), pBR322 (10 μM), ESF1 (10 μM) or p21-pCpGL (10 μM). The addition of 200 μM of plasmid pCpGL (lacks CpG sites (22) after 20 minutes (unless otherwise noted)) forms the “chase” condition. The addition of this same “chase” DNA at the start of the reaction concurrent with DNA substrate addition provides a control.

**DNMT3L assays**

DNMT3L was tested for its activation of DNMT3A by varying DNMT3L from 12.5 nM to 200 nM with 50 nM DNMT3A and poly-dIdC at 25 μM. DNMT3A and DNMT3L were preincubated in reaction buffer with AdoMet for 1 hr at 37°C before starting the reaction with DNA. Reactions were run for 1 hr, stopped as stated above and methylation counted. A 1:1 ratio (50 nM) with the 1 hr preincubation (with AdoMet) gave maximum amount of activity, which was used for all DNMT3L assays. The fold stimulation was calculated by product formed by DNMT3A with DNMT3L divided by product formed by DNMT3A without DNMT3L. Activation by DNMT3L on DNMT3A on poly-dIdC and GCbox2 were carried out with DNA at 20 μM and the reaction run for 1 hr. Four replicates were used in each reaction to obtain the error bars (mean standard deviation). Processivity assays which include DNMT3L had the same conditions (buffer, enzyme and DNA concentrations) as the DNMT3A processivity assays which included the preincubation of DNMT3L with DNMT3A as stated above.

**Processivity data analyses**

The data was fit to a nonlinear regression (one phase decay). Data fit separately before the experimental reaction had chaser DNA added (time 0-20 mins) and after chaser DNA was added (20-260 mins), using Prism Version 4.0 (GraphPad Software). Error bars represent the mean standard deviation of at least three replicates. The number of turnovers is calculated by product formed (nM) divided by catalytic active sites (nM) (total protein concentration times percent active sites (18%)). The percent active sites (18%) was determined as previously described in Purdy et al. (24). Inactive DNMT3A that can bind DNA should not affect the assays or the measurement of processivity since the substrate DNA is in great excess to the total amount of enzyme (active and non-active enzyme).

The change in product formation between the non-chase and chase experiment is related as shown in Equation 1.

$$r = \frac{P'_{\text{chase}}}{P'_{\text{non-chase}}}$$

(Eq. 1)

Where $P'_{\text{chase}}$ is the product formed after chaser DNA is added and $P'_{\text{non-chase}}$ is the product formed without chaser for the same give time. $r$ is a ratio of the product formed in the chase compared to non-chase experiment, providing a measure of the enzyme dissociation from the DNA.

**Modeling of Processivity Data.**

The processivity profiles were analyzed using the nonlinear regression package in Mathematica (Wolfram Inc.). The results were reported as the best fit values ± standard error. The processivity can be schematically described as

$$ES_0 \xrightarrow{k} EP_1 \xrightarrow{k} EP_2 \xrightarrow{k} \cdots \xrightarrow{k} \cdots EP_i \xrightarrow{k} \cdots$$

(SCHHEMIE 1)

Every $k$ is a turnover rate constant for methylaton and every $k_{off}$ is the dissociation rate constant for the enzyme-DNA complex. $EP_i$ represents the enzyme that has processed $i$ steps, and $ES_0$ is the initial enzyme-DNA complex. $ES_0$ is equal to the total active enzyme concentration in the assay since the enzyme and the DNA concentration are well above the dissociation constant for the
enzyme-DNA complex. ES₀ is calculated by the amount of total enzyme multiplied by the percent active enzyme.

The following equations, modified from those described in Svedruzic and Reich (2005) (19) were used:

\[
[EP_i(t)] = \frac{k_i t^{i-1}}{t!} [ES_0] e^{-(k + k_{off}) t}
\]  
\[\text{Eq. 2}\]

\[
[P_i] = \frac{k_i}{(k + k_{off})} \left(1 - e^{-(k + k_{off}) t} - e^{-(k + k_{off}) t} \sum_{i=1}^{n} \frac{(k + k_{off})^i t^i}{i!}\right)
\]  
\[\text{Eq. 3}\]

The equation for product formed in a processive reaction is a sum of all processive steps (\(i = 1\) to \(n\)) and is equal to

\[
[P] = \sum_{i=1}^{n} P_i
\]  
\[\text{Eq. 4}\]

\(k\) and \(k_{off}\) where modeled using equation 3. The processivity probability \((p)\) was determined using

\[
p = \frac{k}{k + k_{off}}
\]  
\[\text{Eq. 5}\]

The probability for the processive step \(n\) is equal to

\[
p_n = (p)^n.
\]  
\[\text{Eq. 6}\]

Where \(P_n\) of 0.5 is equal to \(n^{1/2}\) which is the average number of catalytic turnovers before half of the enzymes are dissociated from the original substrate.

**RESULTS**

Full length DNMT3A, DNMT3A catalytic domain, and DNMT3L were purified to 85%, 90% and 95% purity respectively (supplemental FIGURE 1). The enzyme shows maximum activity on poly-dIdC DNA. With this substrate, DNMT3A has a turnover rate constant of \(1.2 \pm 0.3\) hr\(^{-1}\), where the turnover rate constant is defined as the maximal turnover rate divided by the active protein concentration. This value falls into the range reported previously for recombinantly expressed DNMT3A of \(1.8\) hr\(^{-1}\) to \(1.07\) hr\(^{-1}\) (5, 24, 25, 27, 29). We recently showed that DNMT3A preparations are only partially active (18%) (24). Taking this into consideration, the turnover rate constant with poly-dIdC becomes \(6.7\) hr\(^{-1}\) (TABLE 1). During steady-state turnover, the enzyme retains greater than 75 percent activity over the four hour methylation reaction (supplementary FIGURE 2).

**Design of the processivity assay**

The processivity assay determines the length of time the enzyme stays associated with its DNA substrate and the number of turnovers using a pulse-chase experiment. Product formation is measured by the amount of tritiated methyl groups transferred by the enzyme to the DNA in a given time. Our conditions involve a 100-200-fold excess of DNA over enzyme, unless otherwise noted. Thus, each DNA molecule will be occupied by a single DNMT3A enzyme and the enzyme is allowed to carry out catalysis on multiple sites along a single piece of DNA. After given time periods, the reaction is challenged with a 40-fold excess of chaser DNA (a plasmid, pCpG\(^L\), lacking the recognition site for DNMT3A) compared to substrate DNA. A processive enzyme will continue to methylate the original substrate and the chaser DNA will have no, or a delayed impact, on DNA methylation. A non-processive enzyme will dissociate after one methyl transfer; thus, after
the addition of chaser DNA, the enzyme will bind
the excess chaser DNA leading to an immediate
decrease in methylation activity.

The effects on turnover with the addition
of chaser DNA is compared to the activity of the
enzyme without chaser. An additional control has
both the substrate and chaser DNA added to the
enzyme at the start of the reaction to confirm the
chase DNA suppresses product formation. In the
experimental reaction, chaser DNA is added 20
minutes into the methylation assay. The change in
product formation between the non-chase and
chase experiment is given as r (Equation 1) for an
indication of how fast the enzyme disassociates
from its substrate. An r of 1 indicates that the
chase and nonchase has the same amount of
product formed, showing no dissociation. An r
value of zero, shows no product is formed after
chaser DNA is added, indication of no processive
catalysis. Processivity was also calculated for
DNMT3A using kinetic modeling originally
developed for helicase processivity studies (30,
31) and previously used for DNMT1 (19). The
modeling is based on the shape of the curve for
non-chase experiment, which determines the
methylation rate (k) and dissociation rate (koff)
(Equation 4). A processivity value (p, Equation 5)
was determined from the k and koff rates. The
processivity values were used to calculate n½
which indicates the average numbers of catalytic
turnovers before half of the enzymes are
dissociated from the original substrate.

We tested three different chaser DNA
sequences, pCpGL, poly-dAdT, and Gusch (a
small 20 oligonucleotide with no CpG sites).
pCpGL is a 3872 bp plasmid lacking the
recognition site for DNMT3A (CG). We found
poly-dAdT at 40-fold excess of the substrate DNA
(poly-dIdC) did not decrease product formation
when added at the start of the reaction
(supplemental FIGURE 4), Gusch also had little
effect on product formation, with less than 10 %
decrease in product formation. DNMT3A had
limited methylation on pCpGL (kcat 0.11 ± 0.03 h⁻¹)
and when mixed at 40-fold excess poly-dIdC,
eliminated > 90 % of product formation. Similar
results were previously seen with the highly
processive M.SssI (20). The significant decrease in
DNMT3A activity in the presence of CpG-lacking
DNA suggests DNMT3A can interact with DNA
devoid of CpG residues but may not interact with
substrates lacking cytosines (poly-dAdT or
Gusch).

**M. SssI processivity**

The processivity assay was first tested on
a known processive DNA methyltransferase M.
SssI (20) with the same recognition sequence as
DNMT3A. The pulse-chase assay showed that
adding in excess chaser DNA did not decrease
product formation, and modeling showed an n½
of 346, demonstrating as expected M. SssI is highly
processive (supplemental FIGURE 3).

**DNMT3A is processive on a variety of substrates
and is inhibited by supercoiling**

Using poly-dIdC, DNMT3A carries out
multiple rounds of catalysis before dissociating.
78% of the product formed in the absence of any
chase DNA is formed with the chase reaction at 90
minutes (FIGURE 1A) (r = 0.78 ± 0.01) (TABLE
2). Modeling the non-chase data revealed that
DNMT3A on poly-dIdC has a processivity value
(p) of 0.98 and an n½ of 27 indicating that 27
turnovers occur before 50% of the enzyme is
dissociated from the poly-dIdC substrate (TABLE
3).

We then used biologically relevant DNA
substrates for additional tests of DNMT3A
processivity. These include the EFS1 human
promoter, amplified from HeLa genomic DNA
which contains a CpG island with 85 CpG sites in
a 936 bp linear fragment (FIGURE 1B) and
supercoiled pBR322 (FIGURE 1C), a bacterial
plasmid which has 329 CpG sites in the 4359 bp
plasmid. DNMT3A shows processive catalysis on
both substrates with an r value of 0.62 for pBR322
and 0.86 for EFS1 at 90 mins. Modeling the non-
chase data showed the same trend as the chase
data. EFS1 has a processive value (p) of 0.90 and
an n½ of 6.6 and pBR322 has a p value of 0.88 and
an n½ of 5.3.

The impact of supercoiling was also
examined with the human p21 promoter (CpG
island with 39 CpG sites in a 530 bp) amplified
from HeLa genomic DNA then inserted into the
pCpGL (p21-CpG L) plasmid in the linear and
supercoiled form. As shown in FIGURE 1E, the
supercoiled plasmid shows a decrease in activity
shortly after the addition of the chaser DNA (r =
0.53) compared to the linear plasmid (r = 0.89)
FIGURE 1D. Modeling showed the enzyme is
twice as processive on linear DNA ($n^{1/2}$ of 8.1 compared to a $n^{1/2}$ of 4.2) than the supercoiled form. This is caused by the enzyme dissociating from the substrate twice as quickly on the supercoiled DNA, a $k_{off}$ of $0.22 \pm 0.02$ h$^{-1}$ compared to a $k_{off}$ of $0.14 \pm 0.03$ h$^{-1}$ for the linear DNA. The rate of methylation ($k$) remains largely unaltered, $1.2 \pm 0.03$ h$^{-1}$ for supercoiled compared to $1.6 \pm 0.08$ h$^{-1}$ for linear DNA (TABLE 3).

The increase in $k_{cat}$ with the number of CpG sites is consistent with processive catalysis

A processive enzyme does not dissociate from its product between catalytic turnovers which results in rate enhancement when the reaction rate is limited by product dissociation steps. Therefore, substrates with larger numbers of recognition sites have higher $k_{cat}$ values for a processive enzyme. For example, DNMT3A is most active with poly-dIdC (multiple site substrate) and has significantly lower $k_{cat}$ values on a single site substrate (TABLE 1). To systematically examine the relationship between $k_{cat}$ and the number of CpG sites we investigated ten human promoters (TABLE 1). These promoters contain CpG islands with varying densities of CpG sites. FIGUREs 2C, D and E show representative Michaelis-Menten graphs. A positive linear correlation was found between $k_{cat}$ values and the number of CpG sites in the promoters with an $R^2$ value of 0.85 (FIGURE 2A). This correlation is not seen when comparing the length of DNA to $k_{cat}$ values, where $R^2$ is equal to 0.45 (FIGURE 2B). This is further evidence that DNMT3A is a processive enzyme, where the rate of product formation increases with the number of CpG's sites in the DNA substrate.

Commitment to processive catalysis

We sought to determine if DNMT3A processivity requires an initial period to assemble an enzyme-DNA complex which displays processive catalysis (commitment). The addition of chaser DNA after only 5 and 10 minutes of DNMT3A catalysis results in very little processive catalysis. In contrast, when chaser DNA was added at 15 minutes there was an increase in processive catalysis but still less then when chaser DNA was added at least 20 minutes into the reaction. These experiments involved the preincubation of enzyme with AdoMet, but similar results were obtained by preincubating with DNA followed by AdoMet addition (data not shown). DNMT3A requires an initial period with AdoMet and DNA to assemble a processive complex as diagramed in FIGURE 3B. The data shows the enzyme does not show the same processive behavior if challenged before one turnover occurs. DNMT3A requires an initial period to assemble a processive complex.

The DNMT3A catalytic domain is processive

The N-terminal domain of DNMT3A is not required for enzyme activity (32). We recently showed that the N-terminal PWWP domain in DNMT3A binds DNA, which may play a role in the enzyme’s processive mechanism (33). The DNMTs are diagramed in FIGURE 4B. The truncated enzyme lacking 525 residues of the N-terminus shows processive catalysis (FIGURE 4A). The processivity of the catalytic domain compares to the full length protein, with an $n^{1/2}$ of 30 turnovers compared to 27 for the full length protein. Previous studies found DNMT1 and DNMT3B were also processive without the full N-terminal domain (18, 32).

DNMT3L increases DNMT3A processivity

DNMT3L, a catalytically inactive homolog of DNMT3A is necessary for methylation of imprinted genes (34). This is thought to involve the recruitment and activation of DNMT3A. DNMT3L activates DNMT3A in vitro four fold (16, 25) (FIGURE 5A). How DNMT3L activates DNMT3A is not understood, in spite of high resolution structures of the intact DNMT3L, and a complex of the catalytic domains of DNMT3L and DNMT3A (15). One possible mechanism for DNMT3L activation is through increasing DNMT3A processivity, which would increase the efficiency of multiple methylations.

The mechanism of DNMT3L activation of DNMT3A was tested by comparing the activation on a single site substrate (GCbox2) to multiple sites substrate (poly-dIdC). As shown in FIGURE 5B, DNMT3L activates DNMT3A 1.5-2 fold when on a single site substrate and 4-5 fold on the multiple sites substrate. The effect of DNMT3L on DNMT3A processivity was further tested by using the pulse-chase processivity assays (FIGURE 5C, D). The majority of DNMT3A remains bound to the initial substrate, staying associated through the 240 minutes of the reaction, over 40 turnovers.
DNMT3A with DNMT3L on poly-dIdC has an \( r \) of 0.84 ± 0.06 h\(^{-1}\) compared to an \( r \) of 0.63 ± 0.03 h\(^{-1}\) for DNMT3A by itself. Modeling the non-chase reaction of DNMT3L with DNMT3A on poly-dIdC shows 91 turnovers would occur before 50% of the enzyme is dissociated from the substrate, compared to 27 for the DNMT3A reaction in the absence of DNMT3L. The effect of DNMT3L on DNMT3A processivity was further shown using p21-pCpGL as the substrate, which showed an \( n^{1/2} \) of 13 when DNMT3L is present compared to an \( n^{1/2} \) of 4.2 with only DNMT3A (FIGURE 5D). Thus, DNMT3L increases DNMT3A processivity by 3-fold on both substrates tested. DNMT3L in addition activates 2-3-fold more on a multiple site substrate compared to a single site substrate. DNMT3L increases \( k_{\text{cat}} \) for DNMT3A on both substrates, and decreases \( k_{\text{off}} \) selectively with poly-dIdC (TABLE 3).

**DISCUSSION**

How new epigenetic marks such as DNA methylation and histone modifications are acquired remains a fundamental unanswered question. A functional understanding of the key enzymes known to carry out these processes is needed to fully appreciate how \textit{de novo} changes occur. The mechanism investigated here is whether DNMT3A has the ability to methylate multiple CpG sites upon binding to DNA (diagramed in FIGURE 6). Processive methylation could explain how methylation spreading occurs on newly integrated viral DNA (35). Similarly, the recruitment of DNMT3A to a particular promoter followed by processive catalysis ensures that multiple CpG sites undergo methylation to induce transcriptional silencing (36). The regulation of processive methylation by interacting proteins or changing the form of the DNA provides another mechanism to control \textit{de novo} methylation.

We provide three independent pieces of evidence that the full length DNMT3A is processive. First, the excellent correlation between the number of CpG sites and \( k_{\text{cat}} \) (FIGURE 2) is expected for a processive enzyme where product release is rate limiting; in contrast, a distributive enzyme will not show this behavior. Second, processivity experiments without any chase DNA (FIGURES 1A-E) show the anticipated curvature in product formation resulting from the partitioning of enzyme following each cycle of methylation. Kinetic modeling of this data was used to determine the relative contributions towards processivity deriving from the dissociation rate versus catalytic events (TABLE 3). This analytical approach, originally developed for helicase processivity studies (30, 31), was used to show that the maintenance DNMT1 is highly processive (19). The full length DNMT3A is shown to be processive on diverse substrates using this assay (FIGURE 1A-E). On human promoters between 5-8 methylation events are observed per binding event. The DNMT3A catalytic domain shows similar levels of processivity as the full length protein, suggesting that the PWWP domain and other parts of the N-terminal domain are not essential for processivity (FIGURE 4).

The chase assays provide further evidence for the inherent processivity of the full length and catalytic domain of DNMT3A (FIGURE 1, 3 and 4). Addition of chaser DNA after one turnover results in less than 25% decrease in product formation, whereas addition of the same chaser at the start of the reaction causes more than 90% of the activity to be lost. This data indicates the majority of enzyme is not dissociating from the substrate, and carries out multiple rounds of catalysis on the same piece of DNA. However, no processivity is observed when DNMT3A was challenged by chaser DNA before one turnover (FIGURE 3), showing that DNMT3A requires an initial period to assemble a processive complex.

Our demonstration that DNMT3A is processive with human promoters is consistent with several whole genome methylation analyses (37-39), which showed human promoters have bimodal distributions of methylated and unmethylated sequences for total and individual subclones. The majority of promoters were either less than 10% methylated or greater than 80% methylated (37). Processive methylation by DNMT3A could certainly contribute to this bimodal distribution.

Our findings show DNMT3A processivity can be modulated by DNA topology (FIGURE 1B-E) and protein-protein interactions (FIGURE 5) which provide new avenues whereby \textit{de novo} methylation may be regulated in the cell. The compaction of DNA has been proposed to alter DNMT function, and nucleosomes, a topologically strained form of DNA, are poor DNMT substrates (13, 40-42). We suggest that alterations to DNA
topology regulate DNMT3A processivity which in turn controls which CpG sites are methylated. This model is supported by chromatin remodelers being essential for DNA methylation in many model organisms (14, 43).

The number of proteins shown to interact with DNMT3A (and DNMT3B) continues to expand (44), although in only rare cases (16, 45, 46) have these interactions been shown to be direct. DNMT3A interacting proteins includes chromatin regulators (NCAPG, SMARCA and ZNF2238) and ATP dependent chromatin remodeling enzyme (LSH and hSNF2H)(45, 46). LSH is proposed to facilitate DNMT processivity on nucleosomal DNA (46, 47).

The consequences on DNMT3A function resulting from protein-protein interactions remain poorly understood. Despite offering insight into the physical interaction between DNMT3L and DNMT3A, the co-crystal structure evokes no insight into how DNMT3L results in a 3-5 fold activation of DNMT3A activity (15). DNMT3L does not enhance the binding of DNMT3A to DNA (16) but causes a slight increase in AdoMet affinity (25). Recent work suggests that DNMT3L does not play a significant role in guiding DNMT3A to unmodified or modified nucleosomes (48). Knockout of DNMT3L is lethal in mice when transmitted through the maternal germ line and mutant males show reactivation of retrotransposons and extreme meiotic defects (49).

Our results (FIGURE 6) provide evidence that DNMT3L is a processivity factor for DNMT3A. The underlying mechanism involves both an enhancement of catalysis, and a stabilization of the DNMT3A-DNA complex. Our data support previous models that protein interactions increase the processivity of de novo methyltransferases (47). The activation by DNMT3L (FIGURE 5A) is less pronounced on a single site substrate compared to a multisite substrate because the former is incapable of revealing processive catalysis. The DNMT3A-DNMT3L interface is suggested to stabilize cofactor binding (16) as well as an active-site loop conformation (15). We suggest that the increase in stability of the DNMT3A-DNA complex in the presence of DNMT3L (Figure 6, Table 3) may derive from alterations in the DNMT3A-DNA interface. It is intriguing to consider that the lethality of the DNMT3L knockouts may in part derive from changes in DNMT3L modulation of DNMT3A processivity.

The modulation of processivity by interacting proteins occurs with many enzymes (50-53). Eukaryotic DNA polymerases become highly processive through their interactions with processivity factors, including clamp protein PCNA that encircles duplex DNA and interacts with the DNA polymerase (54). To our knowledge, all known processivity factors interact with the substrate of the enzyme (50-53). The mechanism of DNMT3L mediated enhancement of DNMT3A processivity is not known, but appears to be novel, since DNMT3L is not thought to interact directly with DNA. The heterotetrameric cocrystal structure of the carboxy-terminal domains of DNMT3A and DNMT3L lacks DNA, however DNA from the M.HhaI-DNA cocrystal structure has been modeled into the DNMT3A structure and shows neither of the DNMT3L subunits are likely to directly interact with DNA (15).

Two prior reports suggested that the murine form of Dnmt3a does not act processively (5, 32). However, these studies were carried out in ways that preclude such interpretation. The initial report using full length Dnmt3a reports a $k_{cat}$ of 0.05 hr$^{-1}$, yet the processivity studies were limited to time regimes under 90 minutes. Without the opportunity to carry out multiple rounds of catalysis, an enzyme’s processivity cannot be revealed. In subsequent work proffered as further evidence of a distributive mechanism using the catalytic domain of the murine Dnmt3a, the processivity assays used 50 nM DNA and 2 µM enzyme. Such single turnover conditions are incapable of revealing enzymatic processivity since multiple enzymes are binding to the same DNA molecules. The level of sequence relatedness of the murine and human enzymes (95% identity), suggests that the murine enzyme, like the human DNMT3A, is also processive.

Our finding that DNMT3A acts processively extends our understanding of this critical developmental regulator and biomedically relevant enzyme. Furthermore, the modulation of DNMT3A’s processivity by DNMT3L, DNA topology, or interactions with other cellular proteins or nucleic acids, considerably expands the enzyme’s potential for regulation.
Acknowledgements

We are grateful to Zeljko Svedruzic for his help in modeling the processivity data. We thank Michael Arensman for his help in creating p21-pCpG^+. We are also thankful to Douglas Matje for his critical review of the manuscript. The pCpG^+ was kindly provided by Michael Rehli and the pTYB1-3L used to express hDNMT3L was kindly provided by Frederic Chedin. This work was supported by funds from the Cancer Research Coordinating Committee (University of California).
References

1. Bird, A. (2002) *Genes & Dev* **16**, 6-2
2. Reik, W., Dean, W. & Walter, J. (2001) *Science* **293**, 1089-1093
3. Li, E. (2002) *Nat. Rev. Genet.* **3**, 662-673
4. Robertson, K. (2005) *Nature Reviews Genetics* **6**, 597-610
5. Gowher, H. & Jeltsch, A. (2001) *J. Mol. Biol.* **309**, 1201-1208
6. Toth, M., Müller, U. & Doerfler, W. (1990) *J. Mol. Biol.* **214**, 673-683
7. Bestor, T.H. (2000) *Hum. Mol. Genet.* **9**, 2395-2402
8. Chen, Z. & Riggs, A.D. (2005) *Biochem. Cell Biol.* **83**, 438-448
9. Hsieh, C.L. (1999) *Mol. Cell. Biol.* **19**, 8211-8218
10. Fatemi, M., Hermann, A., Pradhan, S. & Jeltsch, A. (2001) *J. Mol. Biol.* **309**, 1189-1199
11. Pradhan, S., Bacolla, A., Wells, R.D. & Roberts, R.J. (1999) *J. Biol. Chem.* **274**, 33002-33010
12. Howell, C.Y., Steptoe, A.L., Miller, M.W. & Chaillet, J.R. (1998) *Mol. Cell. Biol.* **18**, 4149-4156
13. Takeshima, H., Su etake, I., Shimahara, H., Ura, K., Tate, S. & Tajima, S. (2006) *J Biochem* **139**, 503-515
14. Cedar, H. & Bergman, Y. (2009) *Nat. Rev. Genet.* **10**, 295-304
15. Jia, D., Jurkowska, R.Z., Zhang, X., Jelt sch, A. & Cheng, X. (2007) *Nature* **449**, 248-251
16. Svedruzić, Z.M. & Reich, N.O. (2005) *Biochemistry* **44**, 14977-14988
17. Klug, M. & Rehli, M. (2006) *Epigenetics* **1**, 127-130
18. Rickwood, D. (1993) DNA Topology,Oxford University Press, Oxford UK,
19. Purdy, M.M., Holz-Schietinger, C. & Reich, N.O. (March 12, 2010) *Arch Biochem Biophys.*
20. Kareta, M.S., Botello, Z.M., Ennis, J.J., Chou, C. & Chédin, F. (2006) *J. Biol. Chem.* **281**, 25893-25902
21. Gill, S.C. & von Hippel, P.H. (1989) *Anal. Biochem.* **182**, 319-326
22. Su etake, I., Miyazaki, J., Murakami, C., Takeshima, H. & Tajima, S. (2003) *J Biochem* **133**, 737-744
23. Peterson, S.N. & Reich, N.O. (2006) *J. Mol. Biol.* **355**, 459-472
24. Aoki, A., Su etake, I., Miyagawa, J., Fujio, T., Chijiwa, T., Sasaki, H. et al. (2001) *Nucleic Acids Res.* **29**, 3506-3512
25. Porter, D.J., Short, S.A., Hanlon, M.H., Preugschat, F., Wilson, J.E., Willard, D.H.J. et al. (1998) *J. Biol. Chem.* **273**, 18906-18914
26. Eckhardt, F., Lewin, J., Cortese, R., Rakyan, V.K., Attwood, J., Burger, M. et al. (2006) *Nat. Genet.* **38**, 1378-1385
27. Grunau, C., Hindermann, W. & Rosenthal, A. (2000) *Hum. Mol. Genet.* **9**, 2651-2663
28. Strichman-Almashanu, L.Z., Lee, R.S., Onyango, P.O., Perlman, E., Flam, F., Frieman, M.B. et al. (2002) *Genome Res.* **12**, 543-554
TABLE Legends

TABLE 1. $k_{cat}$ values for DNMT3A with different substrates.

DNMT3A at 50 nM for poly-dIdC, pBR322 and EFS1 and 150 nM for all other substrates. AdoMet at 2 μM, substrate concentration were varied and product formation measured after one hour. The data for each substrate was fit to Michaelis-Menten or substrate inhibition equations, then $k_{cat}$ values were obtained by the $V_{max}$ values divided by the amount of active enzyme.

TABLE 2. Chase Reaction Results.
The difference in product formation between the non-chase and chase experiment is compared using Eq. 1 ($r$). $r$ is the difference in product formation between the chase and non-chase experiment as an indication on how fast the enzyme is dissociating from its substrate.

TABLE 3. Processivity values for DNMT3A from kinetic modeling
Best fit values for methylation rates ($k$), substrate dissociation rates ($k_{off}$), (Eq. 4). All rates are given as the best fit value ± asymptotic standard error. The processivity probability $p$ was calculated according to Equation 5. The $n^{1/2}$ values (Eq. 6) represent a number of processive steps for the given reaction before 50% of the enzyme is dissociated of the substrate DNA.

FIGURE Legends

FIGURE 1. DNMT3A is Processive on a Variety of Substrates using a Pulse Chase Assay A. Poly-dIdC (5 μM bp). B. EFS1 human promoter (10 μM bp) and C. Bacterial supercoiled plasmid pBR322 (10 μM bp). The p21 human promoter in the pCpG+ plasmid, linearized (20 μM bp) (D) and supercoiled (20 μM bp) (E). Substrate was added at time 0 to start the reaction. DNMT3A full length at 50 nM and
AdoMet at 2 μM. ■ = only substrate, ◆ = substrate then 200 μM bp pCpG\textsuperscript{L} at 20 mins, ▲ = substrate and 200 μM bp pCpG\textsuperscript{L} at start of reaction.

FIGURE 2. The Number of CpGs in a Promoter Increases the Rate of Product Formation. The turnover rate was calculated for DNMT3A on substrates with a varying CpG sites. A. \( k_{\text{cat}} \) values were compared to the number of CpG sites in the DNA substrate. Each point is the \( k_{\text{cat}} \) value for the human promoters and synthesized substrate with one CpG site. Inset includes poly-dldC. Regression line shows \( R^2 = 0.85 \), and 0.98 for the insert. B. Comparing length of DNA to \( k_{\text{cat}} \) values, show little correlation \( R^2 = 0.45 \). C, D, E Substrate Inhibition plots for the PCR amplified human promoters, which are representative plots for obtaining \( V_{\text{max}} \) data. \( V_{\text{max}} \) values were divided by the amount of active enzyme in the reaction to obtain \( k_{\text{cat}} \) values.

FIGURE 3. Commitment for Processivity. A. Processivity assays were performed by adding chaser DNA (pCpG\textsuperscript{L}) at different times into the reaction. Reactions were run with DNMT3A full length at 50 nM, AdoMet at 2 μM, substrate DNA (poly-dldC) at 5 μM and chaser DNA (pCpG\textsuperscript{L}) at 200 μM. The timing of adding chaser DNA varied in each reaction indicated by arrows. Enzyme was preincubated with AdoMet for 3 mins before DNA addition (similar results were seen by preincubating with DNA, data not shown). ■ = poly-dldC 5 μM, ◆ = poly-dldC 5 μM and 200 μM pCpG\textsuperscript{L} at 20 mins, · = poly-dldC 5 μM and 200 μM pCpG\textsuperscript{L} at 15 mins, □ = poly-dldC 5 μM and 200 μM pCpG\textsuperscript{L} at 10 mins, ◊ = poly-dldC 5 μM and 200 μM pCpG\textsuperscript{L} at 5 mins, ▲ = poly-dldC 5 μM and 200 μM pCpG\textsuperscript{L} at start of reaction. B: Schematic of the enzyme’s actions when chaser DNA is added at different times.

FIGURE 4. The N-Terminus of DNMT3A is not needed for Processive Catalysis. A. The pulse-chase processivity assay was performed on the catalytic domain of DNMT3A. Chaser DNA (pCpG\textsuperscript{L}) was added 20 mins into the reaction. Enzyme at 50 nM, AdoMet at 2 μM, substrate DNA (poly-dldC) at 5 μM and chaser DNA at 200 μM. ■ = poly-dldC 5 μM, ◆ = poly-dldC 5 μM and 200 μM pCpG\textsuperscript{L} at 25 mins, ▲ = poly-dldC 5 μM and 200 μM pCpG\textsuperscript{L} at start of reaction. B: Schematic of the domains of DNMT3A, DNMT3A catalytic domain and DNMT3L.

FIGURE 5. DNMT3L is a Processivity Factor of DNMT3A. A. DNMT3L activates DNMT3A, where DNMT3L is varied from 12.4 to 200 nM and DNMT3A is at 50 nM. B. Comparing DNMT3L activation on DNMT3A on a multiple recognition substrate poly-dldC (25 μM) and a single site substrate GCbox2 (25 μM), in saturating conditions. C, D: Processive assay same as in FIGURE 1 but including DNMT3L. DNMT3A and DNMT3L (50 nM each) were preincubated for 1 hr in reaction buffer with AdoMet at 2 μM, followed by DNA addition at time 0. C: substrate, poly-dldC (5 μM), D: The p21-pCpG\textsuperscript{L} plasmid, supercoiled used at the substrate (20 μM) ■ = only substrates, ◆ = substrate then 200 μM pCpG\textsuperscript{L} added at 20 mins ▲ = substrate and 200 μM pCpG\textsuperscript{L} at start of reaction. E, F: average number of catalytic events on a substrate before dissociation (\( n^{1/2} \) value), with and without DNMT3L, error bars are standard deviation between three independent reactions.

FIGURE 6. Model of de novo methylation. A depiction of DNMT3A with DNMT3L carrying out either processive or non-processive catalysis.
### TABLE 1.

| Substrate       | Form     | # of Cpg | Size bp | $k_{cat}$ h$^{-1}$ |
|-----------------|----------|----------|---------|-------------------|
| poly-dldC       | linear   | ~800     | ~1900   | 5.9 ± 0.3         |
| pBR322          | supercoiled | 329     | 4359    | 1.5 ± 0.2         |
| EFS1            | linear   | 85       | 936     | 1.2 ± 0.3         |
| p21-pCpG$^\perp$| linear   | 37       | 4275    | 0.74 ± 0.16       |
| p21-pCpG$^\perp$| supercoiled | 37     | 4275    | 0.42 ± 0.06       |
| $\beta$-actin   | linear   | 3        | 550     | 0.38 ± 0.08       |
| CDC25           | linear   | 3        | 278     | 0.40 ± 0.06       |
| ODC             | linear   | 14       | 1268    | 0.43 ± 0.07       |
| p15             | linear   | 29       | 865     | 0.83 ± 0.04       |
| p21             | linear   | 39       | 808     | 0.92 ± 0.06       |
| Smarca          | linear   | 21       | 516     | 0.59 ± 0.07       |
| Survivin        | linear   | 26       | 918     | 0.52 ± 0.06       |
| Xist            | linear   | 6        | 117     | 0.32 ± 0.05       |
| Gcbox2          | linear   | 1        | 21      | 0.21 ± 0.03       |

$^a = k_{cat}$ values were obtained by dividing $V_{max}$ values by the amount of active enzyme for each substrate.
| Proteins          | Substrate          | $r$ at 90 mins$^a$ | $r$ at 240 mins$^b$ |
|-------------------|--------------------|-------------------|-------------------|
| DNMT3A            | supercoiled p21    | 0.53              | 0.56              |
| DNMT3A            | linear p21         | 0.89              | 0.67              |
| DNMT3A/DNMT3L     | supercoiled p21    | 0.93              | 0.89              |
| DNMT3A            | EFS1               | 0.86              | 0.77              |
| DNMT3A            | pBR322             | 0.62              | 0.56              |
| DNMT3A            | poly-dIdC          | 0.78 +/- 0.04     | 0.63 +/- 0.03     |
| DNMT3A/DNMT3L     | poly-dIdC          | 0.84 +/- 0.01     | 0.84 +/- 0.06     |

$^a$ = $r$ difference in product formation at 90 mins

$^b$ = $r$ = difference in product formation at 240 mins
### TABLE 3.

| Proteins          | Substrate                     | $k$ (h$^{-1}$)$^a$ | $k_{off}$ (h$^{-1}$)$^b$ | $p$ $^c$ | $n_{1/2}$ $^d$ |
|-------------------|-------------------------------|-------------------|-------------------------|-------|---------------|
| DNMT3A            | pBR322-supercoiled            | 7.4 ± 0.40        | 1.0 ± 0.11              | 0.88  | 5.3           |
| DNMT3A            | EFS1-linear                   | 4.8 ± 0.18        | 0.52 ± 0.05             | 0.90  | 6.6           |
| DNMT3A            | p21-pCpG\(^{-}\)-supercoiled | 1.2 ± 0.03        | 0.22 ± 0.02             | 0.85  | 4.2           |
| DNMT3A            | p21-pCpG\(^{-}\)-linear      | 1.6 ± 0.08        | 0.14 ± 0.03             | 0.92  | 8.1           |
| DNMT3A/DNMT3L     | p21-pCpG\(^{-}\)-supercoiled | 9.0 ± 0.59        | 0.48 ± 0.06             | 0.95  | 13            |
| DNMT3A            | poly-dIdC-linear             | 11 ± 0.58         | 0.29 ± 0.04             | 0.98  | 27            |
| DNMT3A/DNMT3L     | poly-dIdC-linear             | 18 ± 0.66         | 0.14 ± 0.02             | 0.99  | 91            |
| DNMT3A-catalytic domain | poly-dIdC-linear         | 11 ± 0.80         | 0.25 ± 0.06             | 0.98  | 30            |

$^a$ = best fit values for methylation rate ($k$) ± standard error calculated according to Eq. 4

$^b$ = best fit values for substrate dissociation rate ($k_{off}$) ± standard error calculated according to Eq. 4

$^c$ = processivity probability ($p$) was calculated according to Eq. 5

$^d$ = represents the number of processive steps for the given reaction before 50% of the enzyme is dissociated for the substrate DNA ($n_{1/2}$), calculated according to Eq. 6

$^e$ = DNMT3L preincubated with DNMT3A at a 1:1 ratio
FIGURE 1.
FIGURE 2.

A

B

C

D

E
FIGURE 4.

A

B

DNMT3A (912 aa)    
PWWP     PHD       Catalytic

DNMT3A (301 aa)     
Catalytic Domain

DNMT3L (387 aa)     
PHD       Catalytic

- protein and nucleic acid binding
- Methylation, DNA and AdoMet binding
- Zn-finger protein binding
The inherent processivity of the human de novo DNA methyltransferase 3A (DNMT3A) is enhanced by DNMT3L
Celeste Holz-Schietinger and Norbert O. Reich

J. Biol. Chem. published online July 14, 2010

Access the most updated version of this article at doi: 10.1074/jbc.M110.142513

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2010/07/14/M110.142513.DC1