Mechanistic Insights into Cytosine-N3 Methylation by DNA Methyltransferase DNMT3A

Michael Dukatz[1,†], Cristina E. Requena[2,3,†], Max Emperle[1], Petra Hajkova[2,3], Peter Sarkies[2,3] and Albert Jeltsch[1]

1 - Department of Biochemistry, *Institute of Biochemistry and Technical Biochemistry, University Stuttgart, Allmandring 31, 70569 Stuttgart, Germany*
2 - MRC London Institute of Medical Sciences, *Du Cane Road, London W12 0NN, UK*
3 - Institute of Clinical Sciences, *Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK*

Correspondence to Peter Sarkies and Albert Jeltsch:*Correspondence to P. Sarkies, MRC London Institute of Medical Sciences, Du Cane Road, London W12 0NN, UK.Correspondence to A. Jeltsch, Department of Biochemistry, Institute of Biochemistry and Technical Biochemistry, University Stuttgart, Allmandring 31, 70569 Stuttgart, Germany. psarkies@imperial.ac.uk, albert.jeltsch@ibtb.uni-stuttgart.de*
https://doi.org/10.1016/j.jmb.2019.06.015
Edited by Dan Tawfik

Abstract

Recently, it has been discovered that different DNA-(cytosine C5)-methyltransferases including DNMT3A generate low levels of 3mC [Rosic et al. (2018), *Nat. Genet.*, **50**, 452–459]. This reaction resulted in the co-evolution of DNMTs and ALKB2 DNA repair enzymes, but its mechanism remained elusive. Here, we investigated the catalytic mechanism of DNMT3A for cytosine N3 methylation. We generated several DNMT3A variants with mutated catalytic residues and measured their activities in 5mC and 3mC generation by liquid chromatography linked to tandem mass spectrometry. Our data suggest that the methylation of N3 instead of C5 is caused by an inverted binding of the flipped cytosine target base into the active-site pocket of the DNA methyltransferase, which is partially compatible with the arrangement of catalytic amino acid residues. Given that all DNA-(cytosine C5)-methyltransferases have a common catalytic mechanism, it is likely that other enzymes of this class generate 3mC following the same mechanism.

Introduction

DNA cytosine-C5 methylation at CpG sites is a major chromatin regulator essential for development in mammals [1–3]. It functions in concert with other epigenome modifications, most prominently histone tail modifications, and represents one important part of the epigenome network [4,5]. Aberrant DNA methylation has several connections to diseases including cancer [6–8]. DNA methylation is introduced by the family of DNA methyltransferases (DNMTs) in humans comprising DNMT1, DNMT3A, and DNMT3B [9–11]. Structurally, all these DNMTs consist of two main parts, a large N-terminal part containing domains with regulatory functions, and a C-terminal catalytic domain, which mediates the transfer of the methyl group from the cofactor *S*-adenosyl-L-methionine (AdoMet) to the C5 position of cytosine residues in DNA. This part has a conserved methyltransferase fold that is shared among prokaryotic and eukaryotic DNA-(cytosine C5)-methyltransferases and contains 10 conserved amino acid motifs [11–13]. It consists of seven β-strands and one to two α-helices on either side of the central β-sheet. Binding of the AdoMet is mediated by the motifs I (F640-X-G-X-G, numbering refers to human DNMT3A), II, and III. The conserved amino acid motifs number IV (P-C710-Q/N**)**, VI (E756-N-V), and VIII (R790-X-R792) form the active site and provide the amino acid residues involved in catalysis. The catalytic domains of DNMT3A and DNMT3B are active in isolated form [14], and structures of the DNMT3A catalytic domain in complex with the catalytically inactive stimulatory factor DNMT3L were solved in the absence [15] and presence of DNA [16]. The structure with bound DNA revealed details about the active-site structure

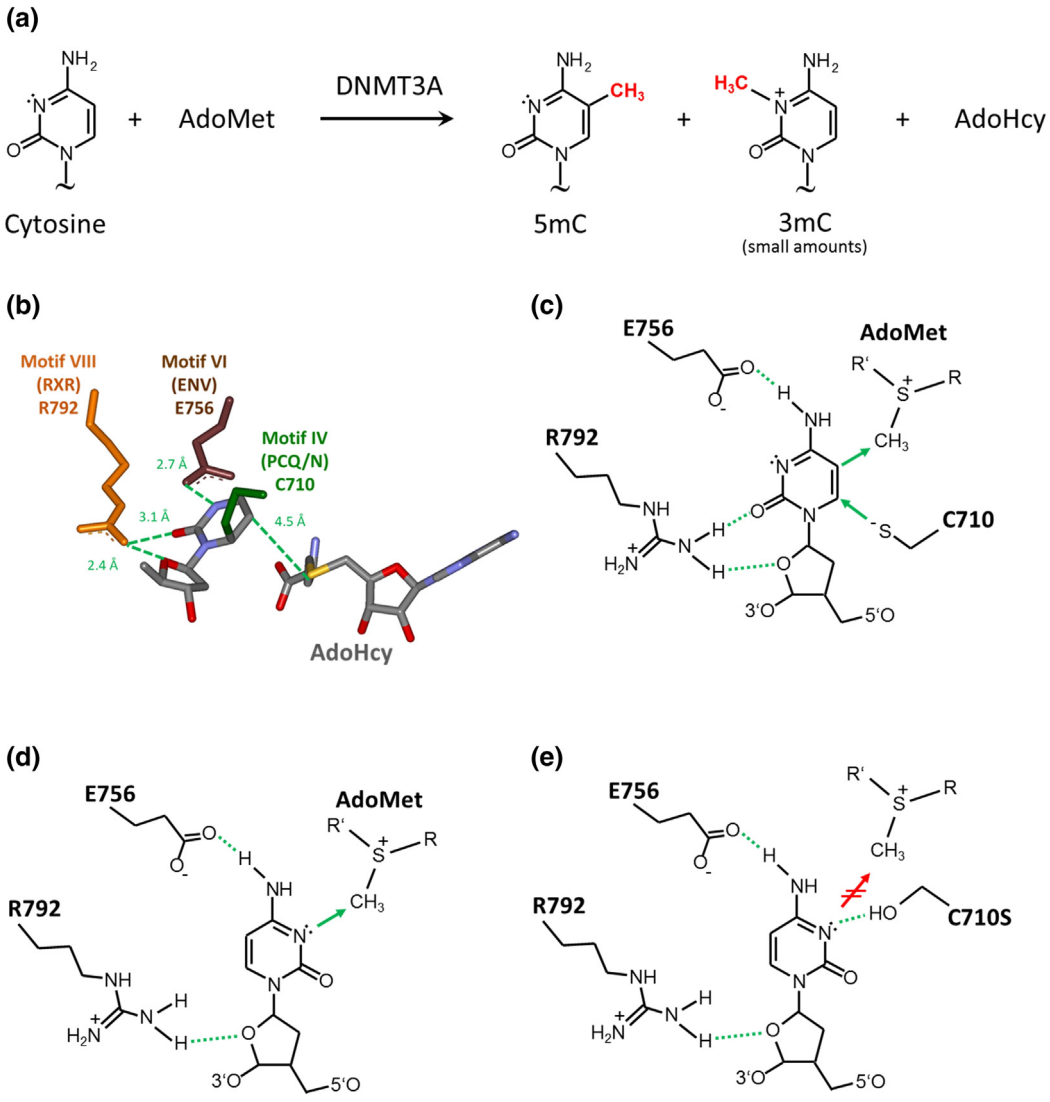

Fig. 1. Catalytic mechanism of DNA-(cytosine C5)-methyltransferases. (a) General reaction of cytosine C5 and N3 methylation by DNMT3A studied here. AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine. (b) Structure of the active site of DNMT3A with a bound Zebularine base (a cytidine analog lacking the amino group in position 4) and adenosyl-L-homocysteine (AdoHcy), the cofactor product after methyl group transfer (PDB: 6F57) [16]. (c) Schematic picture of the first catalytic steps of covalent complex formation and methyl group transfer. (d) Catalytic mechanism of N3 methylation, proposed on the basis of the results of this study. The flipped target base is bound into the active-site pocket of DNMT3A in an inverted orientation, which leads to a close approximation of the N3 atom and the methyl group bound to AdoMet allowing for methyl group transfer. (e) Model explaining the loss of 3mC catalysis after the C710S exchange. S710 could form a hydrogen bond to N3, thereby preventing methyl group transfer.

of DNMT3A showing the positions of several catalytic residues involved in the methylation reaction (Fig. 1a and b).

All DNA-(cytosine C5)-methyltransferases follow a common catalytic mechanism for transferring a methyl group from AdoMet to the target cytosine base [10–12] (Fig. 1c). The reaction is initiated by the flipping of the target cytosine out of the DNA helix into a hydrophobic pocket in the catalytic domain of the enzyme, where it is hydrogen bonded to E756 and R792. Afterward, a nucleophilic attack of the catalytically active cysteine C710 occurs on the C6-position of the aromatic ring, which leads to the formation of a covalent bond between the enzyme and the target cytosine. This reaction is supported by an electron shift and protonation of N3, which is stabilized by E756. Covalent complex formation is followed by methyl group transfer to the C5-position and resolution of the covalent enzyme–DNA complex by deprotonation of the C5 by an unknown base and elimination of the thiol group of C710 [9,12]. As mentioned above, the amino acid motifs containing these catalytic residues were

identified in amino acid sequence alignments and found to be conserved in all enzymes of this type [12,13]. The corresponding residues C710 [17–19], E756 [17–19], and R792 [16,18,20] have been studied in DNMT3A showing that their mutation leads to strong reductions in activity.

Recently, an unexpected side reaction of DNMTs has been discovered by showing that different DNMTs (bacterial M.SssI and mouse DNMT3A catalytic domain) also generate low levels of 3mC (Fig. 1a) [21]. This reaction introduces DNA damage, which interferes with base pairing and thus results in a strong replication block. The 3mC lesion can be directly repaired by ALKB2 family enzymes in an oxidative demethylation process [22,23]. Consequently, it has been found that ALKB2 enzymes coevolve with active DNMTs in many species [21], an observation that underscored the importance of the presence of this DNA repair system in species with active cytosine-C5 methyltransferases. While the evolutionary connection of DNMTs and ALKB2 enzymes indicates that the aberrant methylation of cytosine rings at position N3 had a strong impact in nature, the catalytic mechanism leading to this side-reaction of cytosine-C5 methyltransferases is unknown. It has been investigated here in a mutational study of catalytically relevant amino acids in DNMT3A revealing a plausible mechanism for this reaction.

Results

It was the aim of this study to explore the catalytic mechanism of DNMT3A for methylation of the cytosine base at position N3 generating 3-methylcytosine (3mC) instead of its preferred reaction generating 5-methylcytosine (5mC) (Fig. 1a). To this end, we mutated several active site residues in DNMT3A and determined the methylation rates of cytosine at C5 and N3 by DNMT3A wild type and mutants using liquid chromatography linked to tandem mass spectrometry (LC–MS/MS). In our previous study, we have shown that the F640A mutation in motif I resulted in a loss of methylation activity at C5 and N3 [21], which was expected, because this amino acid exchange abrogates AdoMet binding [18]. Here, we mutated the active site cysteine C710 in motif IV (to Ala and Ser), the glutamate E756 in motif VI (to Ala), and the motif VIII arginine residue R792 (to Ala and His). In addition, the C710S/E756A double mutant was prepared and analyzed.

The catalytic domain of the wild type DNMT3A and all mutant DNMT3A enzymes were overexpressed in *Escherichia coli* as His-tagged proteins and purified by affinity chromatography (Fig. 2a). We first measured conventional 5mC DNA methylation activities of DNMT3A wild type and mutants using a radioactive assay where all mutants showed strongly reduced activities (Fig. 3a). The results of these experiments were largely consistent with previous measurements [16–20], although we note that the relative activities of E756A and C710A observed here slightly differ from previous results, where C710A showed a higher and E756A showed a lower residual activity. This difference could be due to the different substrates and assay conditions used in the previous publication [17].

Next, methylation was investigated using plasmid DNA as substrate that was isolated from bacteria lacking endogenous 5mC. The purified DNMT3A enzymes were incubated with the plasmid DNA in the presence of AdoMet, the cofactor of the methylation reaction. These reactions were conducted at higher enzyme and AdoMet concentrations and longer incubation times than the radioactive assays, to support the 3mC detection in the later stage. Afterward the DNA was purified and degraded to nucleosides, and the levels of 5mC and 3mC were determined by LC–MS/MS (Fig. 2b and c). All experiments were conducted in duplicates. In our experiments, the rates of 3mC and 5mC generation of each mutant were determined simultaneously. This set-up is ideal to compare the functional roles of catalytic residues in 3mC and 5mC generation, because potential effects of the mutations on DNA and AdoMet binding will be equal. Under the conditions of this assay, all mutants showed higher relative 5mC activities than in the radioactive kinetics. The E756A mutant had around 10-fold reduced 5mC activity compared to WT, and all other mutants showed around 100-fold reduced activity (Fig. 3b). 3mC generation of wild type DNMT3A was comparable to the published data [21]. It was reduced about 4-fold for E756A and R792A and 10-fold for C710A and R792H. 3mC generation was reduced below detectable limits for C710S mutants (Fig. 3c).

To explore the implications of these differences for the mechanism of 3mC generation, we investigated the ratio of 3mC/5mC relative to the WT enzyme (Fig. 3d). Importantly, our data show that the mutations in the catalytic domain affected 3mC and 5mC differentially, with some mutants showing increased and some mutants decreased 3mC/5mC ratios. Based on their effects, the mutations could be assigned to three groups: (1) E756A showed a 2-fold increase in the 3mC/5mC ratio, when compared to wild type DNMT3A; (2) R792A/H and C710A showed a 10 to 15-fold relative increase of the 3mC/5mC ratio; and (3) the C710S mutation affected 3mC more than 5mC generation, because both C710S mutants (either alone or in conjunction with E756A) showed 5mC levels similar to the C710A mutant, but 3mC was below detectable limits in the C710S mutants.

Discussion

In this study, we investigated the catalytic mechanism of 3mC generation by DNMT3A. We generated several mutant enzymes with amino acid

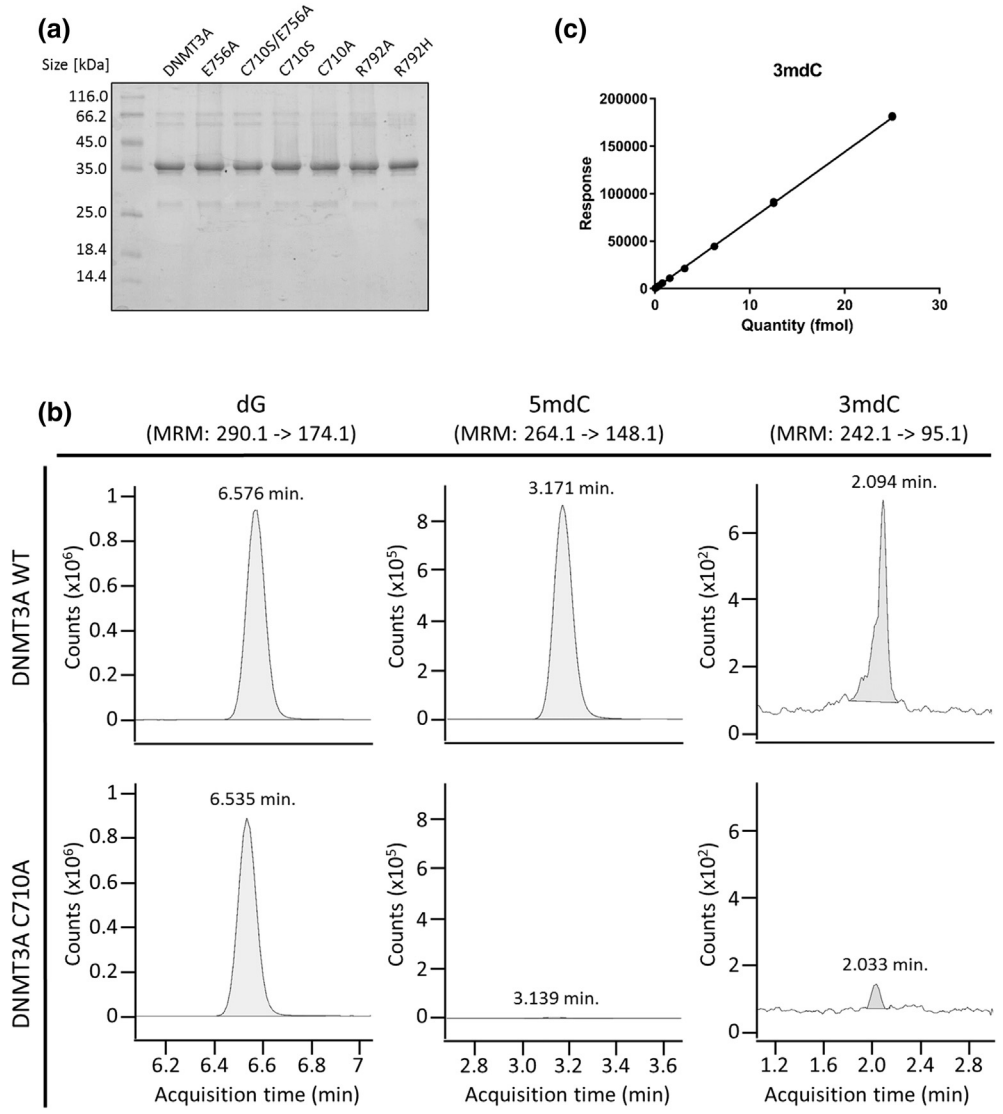

Fig. 2. Protein purification and mass spectrometry. (a) Purified His-tagged catalytic domains of DNMT3A wild type and mutants after loading on an SDS-PAGE and staining with Coomassie BB. (b) Example of primary LC–MS/MS data. (c) Example of the 3mC standard curve used in the LC–MS/MS analyses.

exchanges in the catalytic center and studied their ability to methylate cytosine at the C5 and N3 positions. All mutations led to a strong reduction in 5mC formation in agreement with their highly conserved role in the catalytic mechanism of DNA–(cytosine C5)-methyltransferases. On the basis of the changes in the relative rates of 5mC and 3mC formation, we propose a mechanism for 3mC formation by DNMT3A in which the substrate cytosine is flipped out of the DNA helix similarly to in 5mC catalysis, but it is bound into the catalytic pocket in an inverted conformation rotated by ~180 (Fig. 1d). Then, the N3 atom of cytosine would occupy the place of the C5 atom in the regular arrangement. Hence, the N3 atom would approach the methyl group of the AdoMet close enough for methyl group transfer to occur.

Our proposed mechanism of cytosine N3 methylation is supported by the results of mutations in the active site of DNMT3A. First, our data show that the mutation of C710 to alanine affects C5 methylation more strongly than N3 methylation (equivalent to an increase 3mC/5mC ratio). This indicates that, in contrast to C5 methylation, there is no direct catalytic role for formation of a C710-mediated covalent complex in N3 methylation. The observation of a small reduction in the absolute rate of 3mC generation by the C710A mutant may be explained by the close proximity of C710 to the AdoMet cofactor where the mutation of Cys to Ala may lead to slight perturbations of the conformation. Our model also explains the pronounced loss of 3mC generation resulting from mutation of C710 to serine,

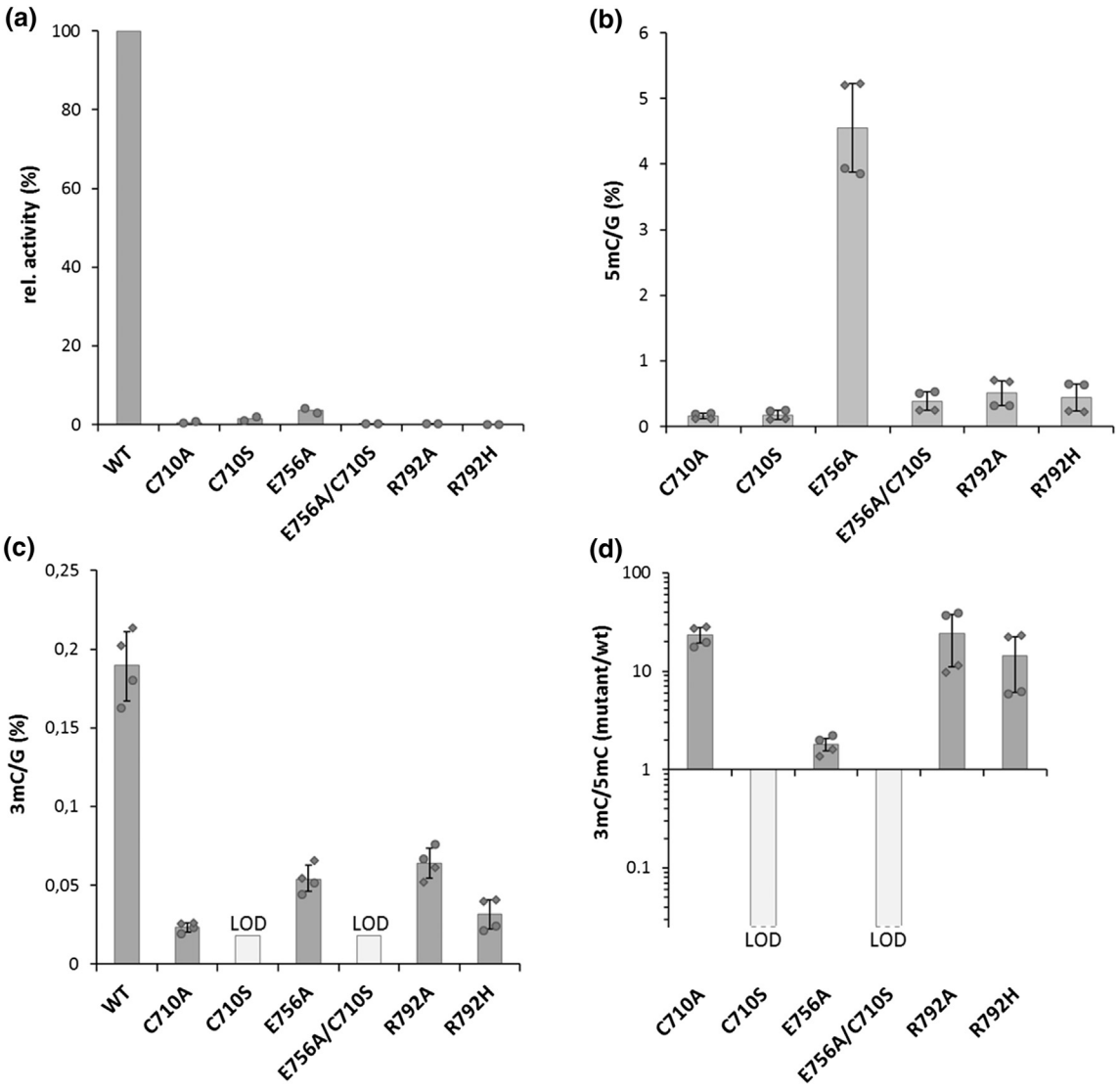

Fig. 3. Activity analysis of the DNMT3A mutants. (a) DNA methylation determined using the radioactive DNA methylation assay. (b) 5mC generation determined by LC–MS/MS. 5mC generation displayed as ratio of 5mC and G. (c) 3mC generation determined by LC–MS/MS. 3mC generation displayed as ratio of 3mC and G. LOD, limit of detection. (d) Relative 3mC generation displayed as ratio of 3mC and 5mC indicated as relative value compared mutant and wild type. In panels a–c, data are shown for two experiments (diamonds and circles) with two technical repeats in panels b and c. In panel a, data were derived by linear regression of reaction progress curves over 2–15 min. Average values are shown by the columns, and error bar displays the upper and lower quartile of the data. The ratios for C710S and E756A/C710S are upper estimates based on the limits of detection (LOD) for 3mC.

because a Ser at the place of C710 would be ideally positioned to donate a hydrogen bond to the cytosine N3 atom after binding of the cytosine in the inverted arrangement thus blocking N3 methylation (Fig. 1e).

Second, during 5mC generation, R792 forms a hydrogen bond to the O3 atom (Fig. 1c), thereby stabilizing the flipped base in the correct orientation for 5mC formation. In our proposed mechanism for 3mC generation, this interaction would not occur (Fig. 1d). Consistent with this, the exchange of R792 to A or H leads to a strong increase in the 3mC/5mC ratio. The reduction in the absolute rate of 3mC formation of these mutants when compared to wild type DNMT3A can be explained by the loss of the second H-bond of R792 to the deoxyribose (Fig. 1c and d), which may support base flipping in both mechanisms.

Third, in our proposed mechanism of 3mC catalysis, E756 could form the same H-bond to the exocyclic N4 amino group as in 5mC generation (Fig. 1c and d), which would anchor the flipped base and indicate that this residue has an important role in 5mc and 3mC generation. However, a potential second bidentate H-bond of E756 to the N3 protonated during the reaction cycle would be lost,

which might explain the mild increase in 3mC/5mC generation with E756A.

We conclude that our data suggest that the side-reaction of DNMT3A leading to the methylation of N3 instead of C5 most likely is caused by an inverted binding of the flipped cytosine target base into the active-site pocket, which is partially compatible with the arrangement of catalytic amino acids residues. Given that all DNA-(cytosine C5)-methyltransferases have a common mechanism of 5mC generation, one can extrapolate that other enzymes of this class can generate 3mC also following the mechanism described here for DNMT3A. However, despite the fact that critical catalytic residues are conserved within the family of DNA-(cytosine C5)-methyltransferase, active sites differ between individual enzymes. Therefore, additional future work will be needed to determine to what degree other 5mC DNMTs have a 3mC side-activity and whether this activity is indeed mediated by the same "inverted base flipping" mechanism as described here for DNMT3A.

Experimental Procedures

Site-directed mutagenesis, protein expression, and purification

Mutagenesis was performed using the megaprimer site-directed mutagenesis method [24] and confirmed by restriction marker analysis and DNA sequencing. The His-tagged C-terminal domain of human DNMT3A (Q9Y6K1.4) (amino acids 612–912) and the C710A, C710S, E756A, R792A, R792H, and C710S/E756A mutants were overexpressed in BL21 (DE3) Codon+ RIL *E. coli* cells (Stratagene) and purified as described [19]. The purity of the preparations was estimated to be ≫95% from Coomassie stained SDS gels. The concentrations of the proteins were determined by UV spectrophotometry and confirmed by densitometric analysis of Coomassie stained SDS–polyacrylamide gels.

Radioactive methyltransferase activity assay

The methyltransferase activity of DNMT3A and the mutant was measured using 100 nM of a biotinylated 509mer containing 58 CpG sites using the following primers (Bt-AGA TTA GGG AAG GGG GTG TG and AAG ATC CTT TCA AGG CCT CAG) as described [19]. Briefly, DNA methylation was measured by the incorporation of tritiated methyl groups from radioactively labeled AdoMet (PerkinElmer) into the biotinylated substrate, using an avidin–biotin methylation plate assay [25]. The methylation reactions were carried out in methylation buffer [20 mM Hepes (pH 7.5), 1 mM EDTA, 50 mM KCl, 0.05 mg/ml bovine serum albumin] at 37 °C using 2 μM DNMT3AC wild type or mutant enzyme, and the reaction was started by adding 0.76 μM AdoMet. The initial slope of the enzymatic reaction was determined by linear regression.

Methyltransferase activity assay using plasmid DNA

The substrate used to measure methyltransferase activity of DNMT3A was a plasmid (pCAGiGFP, Addgene, No. 11150) prepared from DAM/DCM minus *E. coli* cells (NEB #C2925) to ensure absence of cytosine and adenine DNA methylation. DNA methylation was measured in 20 μl reaction volume containing 20 mM Hepes (pH 7.0), 1 mM EDTA, 50 mM KCl, 25 μg/μl bovine serum albumin, 5% glycerol, and 320 μM AdoMet (Sigma) using 20 μM DNMT3AC wild type or mutant enzyme and 1 μg plasmid DNA. The reaction mixture was incubated for 2 h at 37 °C. Afterward, the DNA was purified using Zymo Research DNA Clean & Concentrator Kit TM5 and analyzed using LC–MS/MS as described below.

Methylation analysis by LC–MS/MS

LC–MS/MS was conducted basically as described [21]. Methylated DNA (500 ng) was digested to nucleosides overnight at 37 °C using a nucleoside digestion mix (NEB No. M0649). The nucleosides were separated on an Agilent RRHD Eclipse Plus C18 2.1 × 100 mm 1.8u column using the HPLC 1290 system (Agilent) and analyzed using an Agilent 6490 triple quadrupole mass spectrometer. Quantification was carried out in multiple reaction monitoring mode by monitoring specific transition pair of *m/z* 250.1/134.1 for dC, 290.1/174.1 for dG, 264.1/148.1 for 5mC, and 242.2/95.1 for 3meC. To calculate the concentrations of individual nucleosides, standard curves were generated (dC and dG from Berry and Associated; 5mdC from CarboSynth and 3mdC from ChemGenes). All samples and standard curve points for dC, dG, and 5mdC were spiked with a similar amount of isotope-labeled synthetic nucleosides (dC 13C15N and dG 13C15N purchased from Silantes, and D3m-dC was obtained from T. Carell, Center for Integrated Protein Science at the Department of Chemistry, Ludwig-Maximilians-Universität München, Germany). dC, dG, and 5mdC were quantified using a 1:100 dilution of the 500 ng used to quantify 3mdC. The threshold for peak detection is a signal-to-noise (calculated with a peak-to-peak method) above 10. Limits of detection were 0.05–50 fmol for 3mdC, 0.005–250 fmol for 5mdC, and 0.5–5000 for dC and dG. Limits of quantification were 0.05–50 fmol for 3mdC, 0.025–250 fmol for 5mdC, and 1–1000 for dC and dG. The detectable limit was calculated by dividing the minimum detected value by the dG or 5mdC level for each sample. All nucleobase abbreviations used in this manuscript refer to the deoxy form.

Acknowledgments

This work has been supported by the Deutsche Forschungsgemeinschaft DFG (Grant JE 252/10 to A.J.), Medical Research Council MRC (grant MC-A652-5PY80 to P.S. and MC-US-A652-5PY70 to P. H.) and ERC (grant ERC-CoG-648879 to P.H.). P.S. is an EMBO Young Investigator.

Author Contributions: A.J. and P.S. devised the project. M.D. and M.E. conducted protein purification, biochemical assays, and protein modeling, and C.E. R. performed the nucleoside quantification by LC/MS and data analysis. A.J., P.H., and P.S. supervised the research. A.J. wrote the manuscript draft. All authors contributed to data interpretation and discussion, read, and approved the final manuscript.

Received 5 March 2019;
Received in revised form 3 June 2019;
Available online 21 June 2019

Keywords:
DNA methylation;
DNA methyltransferase;
enzyme mechanism;
enzyme catalysis
†These authors contributed equally to the work.

Abbreviations used:
DNMT, DNA methyltransferase; AdoMet, *S*-adenosyl-L-methionine; 3mC, 3-methylcytosine; 5mC, 5-methylcytosine; LC–MS/MS, liquid chromatography linked to tandem mass spectrometry.

References

[1] A. Jeltsch, J. Broche, P. Bashtrykov, Molecular processes connecting DNA methylation patterns with DNA methyltransferases and histone modifications in mammalian genomes, Genes (Basel) (2018) 9.
[2] C. Ambrosi, M. Manzo, T. Baubec, Dynamics and context-dependent roles of DNA methylation, J. Mol. Biol. 429 (2017) 1459–1475.
[3] D. Schubeler, Function and information content of DNA methylation, Nature 517 (2015) 321–326.
[4] C.D. Allis, T. Jenuwein, The molecular hallmarks of epigenetic control, Nat Rev Genet 17 (2016) 487–500.
[5] A.A. Soshnev, S.Z. Josefowicz, C.D. Allis, Greater than the sum of parts: complexity of the dynamic epigenome, Mol. Cell 69 (2018) 533.
[6] S.B. Baylin, P.A. Jones, A decade of exploring the cancer epigenome—biological and translational implications, Nat. Rev. Cancer 11 (2011) 726–734.
[7] Y. Bergman, H. Cedar, DNA methylation dynamics in health and disease, Nat. Struct. Mol. Biol. 20 (2013) 274–281.
[8] L. Yang, R. Rau, M.A. Goodell, DNMT3A in haematological malignancies, Nat. Rev. Cancer 15 (2015) 152–165.
[9] H. Gowher, A. Jeltsch, Mammalian DNA methyltransferases: new discoveries and open questions, Biochem. Soc. Trans. 46 (2018) 1191–1202.
[10] A. Jeltsch, R.Z. Jurkowska, Allosteric control of mammalian DNA methyltransferases—a new regulatory paradigm, Nucleic Acids Res. 44 (2016) 8556–8575.
[11] R.Z. Jurkowska, T.P. Jurkowski, A. Jeltsch, Structure and function of mammalian DNA methyltransferases, Chembiochem 12 (2011) 206–222.
[12] A. Jeltsch, Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases, Chembiochem 3 (2002) 274–293.
[13] X. Cheng, Structure and function of DNA methyltransferases, Annu. Rev. Biophys. Biomol. Struct. 24 (1995) 293–318.
[14] H. Gowher, A. Jeltsch, Molecular enzymology of the catalytic domains of the Dnmt3a and Dnmt3b DNA methyltransferases, J. Biol. Chem. 277 (2002) 20409–20414.
[15] D. Jia, R.Z. Jurkowska, X. Zhang, A. Jeltsch, X. Cheng, Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation, Nature 449 (2007) 248–251.
[16] Z.M. Zhang, R. Lu, P. Wang, Y. Yu, D. Chen, L. Gao, S. Liu, D. Ji, S.B. Rothbart, Y. Wang, G.G. Wang, J. Song, Structural basis for DNMT3A-mediated de novo DNA methylation, Nature 554 (2018) 387–391.
[17] S. Reither, F. Li, H. Gowher, A. Jeltsch, Catalytic mechanism of DNA-(cytosine-C5)-methyltransferases revisited: covalent intermediate formation is not essential for methyl group transfer by the murine Dnmt3a enzyme, J. Mol. Biol. 329 (2003) 675–684.
[18] H. Gowher, P. Loutchanwoot, O. Vorobjeva, V. Handa, R.Z. Jurkowska, T.P. Jurkowski, A. Jeltsch, Mutational analysis of the catalytic domain of the murine Dnmt3a DNA-(cytosine C5)-methyltransferase, J. Mol. Biol. 357 (2006) 928–941.
[19] M. Emperle, A. Rajavelu, R. Reinhardt, R.Z. Jurkowska, A. Jeltsch, Cooperative DNA binding and protein/DNA fiber formation increases the activity of the Dnmt3a DNA methyltransferase, J. Biol. Chem. 289 (2014) 29602–29613.
[20] O.V. Lukashevich, N.A. Cherepanova, R.Z. Jurkovska, A. Jeltsch, E.S. Gromova, Conserved motif VIII of murine DNA methyltransferase Dnmt3a is essential for methylation activity, BMC Biochem. 17 (2016) 7.
[21] S. Rosic, R. Amouroux, C.E. Requena, A. Gomes, M. Emperle, T. Beltran, J.K. Rane, S. Linnett, M.E. Selkirk, P. H. Schiffer, A.J. Bancroft, R.K. Grencis, A. Jeltsch, P. Hajkova, P. Sarkies, Evolutionary analysis indicates that DNA alkylation damage is a byproduct of cytosine DNA methyltransferase activity, Nat. Genet. 50 (2018) 452–459.
[22] L. Shen, C.X. Song, C. He, Y. Zhang, Mechanism and function of oxidative reversal of DNA and RNA methylation, Annu. Rev. Biochem. 83 (2014) 585–614.
[23] B.I. Fedeles, V. Singh, J.C. Delaney, D. Li, J.M. Essigmann, The AlkB family of Fe(II)/α-ketoglutarate-dependent dioxygenases: repairing nucleic acid alkylation damage and beyond, J. Biol. Chem. 290 (2015) 20734–20742.
[24] A. Jeltsch, T. Lanio, Site-directed mutagenesis by polymerase chain reaction, Methods Mol. Biol. 182 (2002) 85–94.
[25] M. Roth, A. Jeltsch, Biotin–avidin microplate assay for the quantitative analysis of enzymatic methylation of DNA by DNA methyltransferases, Biol. Chem. 381 (2000) 269–272.