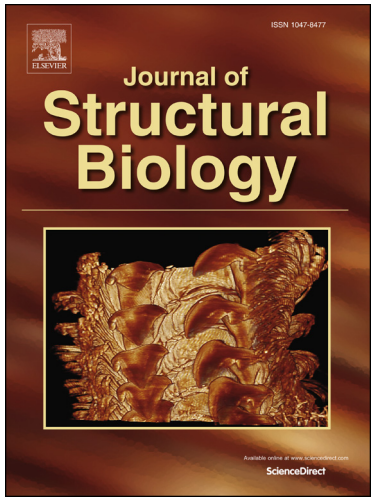

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Structural basis for recognition of histone H3K36me3 nucleosome by human *de novo* DNA methyltransferases 3A and 3B

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ABSTRACT

DNA methylation is an important epigenetic modification involved in chromatin organization and gene expression. The function of DNA methylation depends on cell context and is correlated with histone modification patterns. In particular, trimethylation of Lys36 on histone H3 tail (H3K36me3) is associated with DNA methylation and elongation phase of transcription. PWWP domains of the *de novo* DNA methyltransferases DNMT3A and DNMT3B read this epigenetic mark to guide DNA methylation. Here we report the first crystal structure of the DNMT3B PWWP-H3K36me3 complex. Based on this structure, we propose a model of the DNMT3A PWWP domain-H3K36me3 complex and build a model of DNMT3A (PWWP-ADD-CD) in a nucleosomal context. The trimethylated side chain of Lys36 (H3K36me3) is inserted into an aromatic cage similar to the "Royal" superfamily domains known to bind methylated histones. A key interaction between trimethylated Lys36 and a conserved water molecule stabilized by Ser270 explains the lack of affinity of mutated DNMT3B (S270P) for the H3K36me3 epigenetic mark in the ICF (Immunodeficiency, Centromeric instability and Facial abnormalities) syndrome. The model of the DNMT3A-DNMT3L heterotetramer in complex with a dinucleosome highlights the mechanism for recognition of nucleosome by DNMT3s and explains the periodicity of *de novo* DNA methylation.

Keywords: DNA methylation, methyltransferases, DNMT3A, DNMT3B, PWWP, H3K36me3, Structure, Nucleosome

1. Introduction

DNA methylation occurs at CpG dinucleotides in mammalian cells and is catalyzed by DNA methyltransferases (DNMTs). DNMT3A and DNMT3B are both involved in *de novo* methylation establishing the methylation pattern in genome during embryogenesis while DNMT1 maintains the pattern during chromosome replication. DNA methylation is essential for cell differentiation and development but is also involved in pathologies like cancer (Bird, 2002). The function of DNA methylation depends on cell context and is correlated with histone modification patterns (Jones, 2012). In particular, trimethylation of Lys36 on histone H3 tail (H3K36me3) is associated with gene body methylation in embryonic stem (ES) cells and elongation phase of transcription (Baubec et al., 2015; Hahn et al., 2011; Lee and Shilatifard, 2007). Both DNMT3A and DNMT3B PWWP domains read H3 tails containing H3K36me3 to guide DNA methylation (Baubec et al., 2015; Dhayalan et al., 2010). A point mutation in the DNMT3B PWWP domain (S270P) leads to loss of recognition of this epigenetic mark with H3K36me3-modified nucleosome (Baubec et al., 2015; Ge et al., 2004) and a decrease in DNA methylation at pericentromeric satellite repeat II as observed for ICF syndrome (Chen et al., 2004; Shirohzu et al., 2002).

DNMT3A and DNMT3B possess a C-terminal catalytic domain (CD) and an N-terminal part with a regulatory function mediated by the ADD (ATRX–DNMT3–DNMT3L) domain and a nucleosome recognition PWWP fold. Similar to the chromodomain, MBT and Tudor domains, the PWWP domain is a member of the "Royal" superfamily domains which recognize methylated histone tails through a conserved aromatic cage (Qin and Min, 2014). This H3K36me3-binding ability was established for the PWWP domains of BRPF1 (Vezzoli et al., 2010), DNMT3A (Dhayalan et al., 2010), DNMT3B (Baubec et al., 2015), PSIP1, MSH6, ZMYND11 + H3.3K36me3 (Qin and Min, 2014), LEDGF (Pradeepa et al., 2012), and Tudor domains of PHF1 and PHF19 (Ballaré et al., 2012; Musselman et al., 2012). The PWWP domain contains an anti-parallel β-barrel-like fold formed by five β-strands (β1-β5) (Fig. 1A), where a short 3_{10} helix is found between β4 and β5 (η2), an insertion motif of different lengths between β2 and β3 (η1), and a C-terminal helix bundle of 1-6 α-helices (Qiu et al., 2002). PWWP domains of DNMT3A and DNMT3B are characterized by a short motif insertion (η1) and five α-helices following the β-barrel. The conserved Pro-Trp-Trp-Pro (PWWP) motif becomes SWWP and is found in the β2 strand. DNMT3A and DNMT3B PWWP domains would synergistically bind both histone and DNA through their conserved aromatic cage for the recognition of H3K36me3 epigenetic mark, and a positively charged surface that interacts with DNA (Qin and Min, 2014; Qiu et al., 2002). Recently, DNMT3B was shown to be involved in the selective targeting of transcribed genes in mouse stem