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ENGINEERING DNMT1 FOR CATALYTIC ACTIVITY WITH SYNTHETIC ADOMET ANALOGS

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Cytosine methylation (5mC) is the most common epigenetic modification conserved in mammals. DNA methyltransferases use cofactor S-Adenosyl-L-methionine (AdoMet) as a methyl group donor to covalently modify genomic DNA [1]. In mammals, DNA methylation patterns are established by Dnmt3a and Dnmt3b and maintained by Dnmt1. DNA methylation is significant for embryonic development, gene regulation, suppression of transposable elements, genomic imprinting, and X chromosome inactivation. Regulation patterns of individual methyltransferases are still not clearly understood [2].

This study aimed to determine the ability of Dnmt1 to use synthetic AdoMet analogs, where the carboxyl group is changed into the hydroxyl group, *in vitro*. Hence, vectors containing mouse *Dnmt1* gene with desirable mutations were constructed and inserted into *Pichia pastoris* strain yeast cells by electroporation. Clones resistant to antibiotic G418 were selected and protein expression was induced using methanol. Dnmt1 mutants were purified from a soluble fraction of lysed *P. pastoris* cells via immobilized metal ion affinity chromatography. Purified proteins were used to label DNA using synthetic AdoMet analog SAMol-N_3 *in vitro* and click chemistry was applied to tag azidohex-2-ynyl groups with fluorescent dye. It has been confirmed that Dnmt1 mutants can use SAMol-N_3 to label DNA. Furthermore, HPLC-MS analysis revealed that purified Dnmt1 proteins were able to use synthetic AdoMet analogs (SAMol-N_3), although only in the absence of AdoMet.

[1] Lyko, F. (2018). The DNA methyltransferase family: A versatile toolkit for epigenetic regulation. Nature Reviews Genetics, 19(2), 81–92. https://doi.org/10.1038/nrg.2017.80

[2] Chen, T. (2011). Mechanistic and Functional Links Between Histone Methylation and DNA Methylation. Progress in Molecular Biology and Translational Science (T. 101, p. 335–348). Elsevier. https://doi.org/10.1016/B978-0-12-387685-0.00010-X