BIOCHEMISTRY AND MOLECULAR BIOLOGY SEMINAR SERIES

Rebecca L. Switzer

Assistant Professor of Chemistry, Bucknell University

Investigating the Impact of Disease-Associated Mutations on DNA Methyltransferase 1

In eukaryotes, the most common epigenetic DNA modification is methylation of the 5-carbon of cytosines, predominately in CpG dinucleotides. Methylation patterns are established and maintained by a family of proteins known as DNA methyltransferases (DNMTs). DNA methylation is an important epigenetic mark associated with gene repression, and disruption of the normal methylation pattern is known to play a role in several disease states. Methylation patterns are primarily maintained by DNMT1, which possess specificity for methylation of hemimethylated DNA. DNMT1 is a multidomain protein with a C-terminal methyltransferase domain and a large N-terminal regulatory region. The Replication Focus Targeting Sequence (RFTS) domain, found in the N-terminal regulatory region, is a key regulator of DNMT1 function in vivo. The RFTS domain acts as an endogenous inhibitor of DNMT1 by binding to the active site and preventing DNA binding. In addition, the RFTS domain is hub for important protein-protein interactions that serve to localize DNMT1 to particular sites in the genome and active DNMT1 for catalysis. These intra- and intermolecular RFTS-mediated interactions are critical for proper maintenance of the methylation pattern in cells. Over the past decade, several mutations in the RFTS domain have been shown to be casual for two adult onset neurodegenerative disorders; however, little is known about the impact of these mutations on the structure and function of DNMT1. We are expressing and purifying these mutant DNMT1 proteins to investigate the impact of the mutations on the intra- and intermolecular RFTS-mediated interactions that regulate DNMT1 activity. We have recently shown that two disease-associated mutations - G589A and V590F - decrease the thermal stability of DNMT1 and result in increases in DNA binding affinity and DNA methylation activity. These data suggest that disease-associated RFTS mutations decrease protein stability and, at least partially, relieve normal RFTS-mediated autoinhibition of DNMT1.

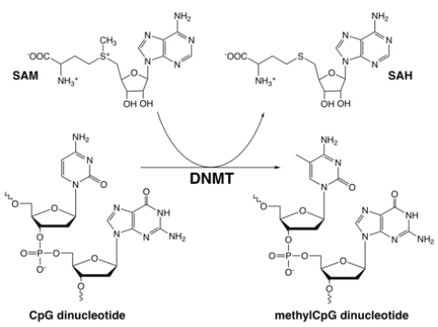

Figure 1: Reaction catalyzes by DNMTs

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Bowen Auditorium (McCreary 115)

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