Dynamics of Methylated Cytosine Flipping by UHRF1

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The last decade has seen an explosion in our understanding of the underlying molecular mechanisms that govern gene expression, with epigenetics taking center stage. Epigenetics refer to the heritable phenotypic changes that occur without altering the DNA sequence. Major epigenetic markers include DNA methylation, post-translational modifications of histones, histone variants and nucleosome positioning.[1-3] In eukaryotes, DNA methylation is a heritable cytosine modification, critical for gene expression, is replicated by DNA methyltransferase 1 (DNMT1) and Ubiquitin-like containing PHD and RING Finger domains 1 (UHRF1) proteins. This replication is initiated by the recognition of hemi-methylated CpG sites and further flipping of methylated cytosines (mC) by the Set and Ring Associated (SRA) domain of UHRF1. Though crystallography has shed light on the mechanism of mC flipping by SRA, tools are required to monitor in real time how SRA reads DNA and flips the modified nucleobase. To accomplish this aim, we have utilized two distinct fluorescent nucleobase surrogates, 2-thienyl-3-hydroxychromone (3HCnt) and thienoguanosine (thG), incorporated at different positions into hemi-methylated (HM) and non-methylated (NM) DNA duplexes. Large fluorescence changes were associated with mC flipping in HM duplexes, showing the outstanding sensitivity of both nucleobase surrogates to the small structural changes accompanying base flipping (Fig. 1). Importantly, the nucleobase surrogates marginally affected the structure of the duplex and its affinity for SRA at positions where they were responsive to base flipping, illustrating their promise as non-perturbing probes for monitoring such events. Stopped-flow studies using these two distinct tools revealed the fast kinetics of SRA binding and sliding to NM duplexes, consistent with its reader role. In contrast, the kinetics of mC flipping was found to be much slower in HM duplexes, substantially increasing the lifetime of CpG-bound UHRF1, and thus the probability of recruiting DNMT1 to faithfully duplicate the DNA methylation profile. The fluorescence-based approach using these two different fluorescent nucleoside surrogates advances the mechanistic understanding of the UHRF1/DNMT1 tandem and the development of assays for the identification of base flipping inhibitors.

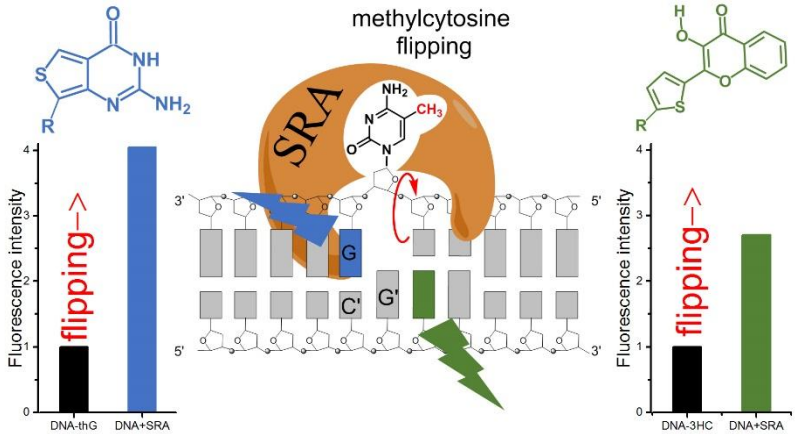

Figure 1. Proposed mechanism for the interaction of SRA with DNA duplexes. SRA interacts with the duplexes through a fast two-step "bind-and-slide" mechanism. At CpG recognition sites, SRA flips the methylated cytosines with rate-limiting kinetics that stabilize the binding of SRA to hemi-methylated CpG sites.

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