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Protein and nucleic acid methylating enzymes: mechanisms and regulation

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Abstract

Protein and nucleic acid methylating enzymes are implicated in myriad cellular processes. These enzymes utilize diverse chemical mechanisms ranging from nucleophilic substitution-displacement to a novel radical-based reaction found in bacterial iron-sulfur cluster proteins. Within the cell, methylation activity is governed by interactions with endogenous molecular machinery. Of particular interest are the observations that methylating enzyme activity can be allosterically controlled by regulatory binding partners. Recent advances and emerging trends in the study of methylating enzyme mechanisms and regulation highlight the importance of protein and nucleic acid methylation in cellular physiology and disease.

Introduction

Protein and nucleic acid methylation play diverse roles in cellular signaling and regulation of macromolecular function [1–4]. Protein arginine methyltransferases (PRMTs) and protein lysine methyltransferases (PKMTs) are the predominant enzymes that catalyze *S*-adenosylmethionine (SAM)-dependent methylation of protein substrates. Broadly, these enzymes promote a nucleophilic substitution-displacement reaction: methyl addition to an arginine or lysine side chain nitrogen atom concomitant with displacement of *S*-adenosylhomocysteine (SAH). Additional protein methyltransferases — those that target various other peptidyl side chains (glutamate, glutamine, and histidine) or *N*-termini and *C*-termini — use a similar polar mechanism [5–9]. In particular, the recent discovery of NRMT, responsible for eukaryotic *N*-terminal methylation has renewed attention on the biochemical role of this modification [8,10]. Unlike protein methyltransferases, nucleic acid methylating enzymes employ various chemical mechanisms to install methyl groups on their DNA and RNA substrates. The activities of RNA methylating enzymes are especially notable given their diversity of substrates (mRNA, tRNA, and rRNA) and modification sites (*N*-methylation, *O*-methylation, and *C*-methylation) [11].

Together, protein and nucleic acid methylation expands the repertoire of functions available to their modified substrates. This review focuses on chemical, regulatory, and physiological

mechanisms of protein arginine and lysine methyltransferases as well as nucleic acid methylating enzymes.

Signature features of PRMT catalysis

In humans, there are nine canonical protein arginine methyltransferase paralogs (PRMT1-9) [1,12]. Beyond the commonly accepted PRMTs, 34 genes share sequence homology [13]. Structural evidence and mutational analysis from representative members of the PRMT family have informed a spatial model of the active site, in which arginine is juxtaposed with the *S*-methylsulfonium of SAM along one face of the enzyme cavity (Figure 1a,b) [14]. By structural inference, a pair of conserved glutamate residues opposite the interface between SAM and the methyl acceptor is thought to polarize the guanidino group of arginine, thus priming nucleophilic attack [14]. Another proposed feature is a proton relay, consisting of one or more amphoteric residues thought to facilitate arginine deprotonation [15].

PRMTs are classified by product selectivity

In vivo, methylarginine exists in three major forms: mono *N*-methylarginine, symmetric *N,N*-dimethylarginine, and asymmetric *N,N*-dimethylarginine (Figure 1d) [1]. The PRMT paralogs are defined as type I or type II based on the capacity to form either asymmetric or symmetric dimethylated arginine, respectively. Both PRMT types are able to form monomethylarginine as a reaction intermediate *en route* to the dimethylated states. Structural and sequence-based comparison between members of the two PRMT types has identified a conserved, type-specific residue that controls product selectivity [16]. For the type II enzyme PRMT5, substitution at Phe379 to a methionine residue, characteristic of type I PRMTs, produces a mutant that catalyzes both symmetric and asymmetric dimethylation. Proximity of SAH to the determinant residue Phe379, revealed in a co-crystal structure, suggests that product selectivity may be partly controlled by restricted methyl donor conformations relative to the substrate nucleophile. Two minor PRMT subclasses, type III and type IV, have also been reported to exclusively mono-methylate arginine on the terminal or internal nitrogen atom, respectively [1].

Allosteric modulation of PRMT activity via multimerization

Seminal structural investigations revealed that PRMTs form multimeric complexes composed of repeating homo-dimer units [14,15,17]. It was proposed that the proximity of active sites in PRMT dimers promotes dimethylation through a quasi-processive mechanism, in which the paired enzymes each add a methyl group to a single arginine residue. However, for at least a subset of enzymes and their substrates, biochemical data support distributive or partially processive mechanisms [18–20]. While the role of dimerization in processivity remains inconclusive, PRMT homo-dimers are essential to catalysis. Mutations that abrogate homo-dimerization concomitantly eliminate methylation activity, which has been attributed to disruption of the SAM-binding surface proximal to the dimer interface [14,15,17]. Moreover, a study of PRMT1 catalytic activity as a function of multimerization showed that apparent turnover rate correlates with oligomer formation, with both metrics reaching a maximum at enzyme concentrations in the range of 0.5–1.0 μM [19]. These observations support a model in which catalytic activity is dependent upon multimerization of a minimal dimer unit.

Studies of PRMT hetero-multimers suggest a regulatory function. For example, both PRMT1 and PRMT2 function as transcriptional co-activators with the ability to methylate histone H4 [21]. Through co-immunoprecipitation and fluorescence microscopy experiments, it was shown that these paralogs physically interact [22]. Furthermore, this association increases PRMT1 activity independent of the catalytic capacity of PRMT2,

suggesting stimulation through allostery. In another case, PRMT5 and regulatory accessory proteins coexist in a large heterogeneous complex responsible for depositing methyl marks necessary during proper spliceosome assembly [23]. Among these complex partners is pICLn, a protein that restricts the inherently promiscuous substrate specificity of PRMT5 [23]. Together, these examples demonstrate that hetero-multimers — whether among PRMTs or with other proteins — can regulate catalytic behavior.

Signature features of PKMT catalysis

The SET domain-containing enzyme family, named after *Drosophila* Su(var), E(z) and Trithorax histone lysine methyltransferases, shares sequence homology with 51 human genes [13]. The ~130-residue SET domain comprises non-contiguous, conserved regions known as nSET/SET-N and cSET/SET-C, denoting proximity to respective primary sequence termini [24,25]. Separating these regions is a variable iSET/SET-I region, which is thought to influence substrate specificity and catalytic activity. Together, these regions comprise the active site, which organizes SAM and the peptidyl lysine substrate at opposing ends of a narrow channel that accommodates the substrate side chain (Figure 1b,c) [26]. Along with the canonical lysine methyltransferases, there are at least two non-SET domain PKMTs: the histone methyltransferase DOT1L that possesses seven- α - β -strand architecture and a multi-subunit methyltransferase (WDR5, RbBp5, Ash2L, and DPY-30) derived from the MLL1 core complex that targets histone H3 [27,28].

Transition state modeling of the SET7/9 homolog suggests a collinear arrangement of the lysyl ϵ -nitrogen and the *S*-methylsulfonium bond of SAM, consistent with a substitution-displacement mechanism [29]. At physiological pH, the side chain of lysine exists primarily as an ammonium species, which requires deprotonation for nucleophilic addition into SAM. Dynamical simulations of a composite active site, derived from LSMT, vSET, and SET7/9 structures, indicate the existence of a water channel that relays protons to bulk solvent [30]. Therefore, it is expected that at high pH, facile deprotonation drives formation of the nucleophilic amine species. Accordingly, PKMT catalysis is pH-dependent, reaching near-maximal activity at pH ~9 [31,32]. In agreement with a nucleophilic substitution-displacement mechanism, deprotonation is a critical step that activates the lysyl amine for methyl transfer.

Substrate binding site influences degree of product methylation by PKMTs

In vivo, protein lysine methylation results in monomethylated, dimethylated, or trimethylated species (Figure 1d) [33]. Because specific physiological functions are dependent upon methylation status, product selectivity is precisely regulated to preclude spurious signaling [34,35]. The methyl acceptor binding site architecture is a major determinant that governs the extent of product methylation. Analysis of the DIM-5 methyltransferase indicated that an acceptor site mutation at Phe281 influences methylation multiplicity. In particular, the F281Y mutation causes a product distribution shift towards lower-order methylated states [35,36]. The reciprocal mutation in SET7/9 (Y305F) imparts an additional capacity to form trimethylated product. From this work, a Phe/Tyr switch model was developed to explain PKMT product selectivity. For enzymes containing a phenylalanine determinant residue, higher-order methylation products are expected. A corresponding tyrosine residue, conversely, is predicted to limit methylation extent. Consistent with this model, available structural evidence from methyltransferases with a phenylalanine determinant residue shows sterically permissive methyl acceptor binding sites capable of accommodating partially methylated substrates [37]. With tyrosine as the determinant residue, imposition of a phenolic hydroxyl group precludes binding of

intermediate methylated species; thus, these enzymes are restricted to production of lower-order methylated products [37].

The Phe/Tyr switch phenomenon had been ascribed to a single structurally homologous site until the identification of several disease-associated mutations in a gene encoding EZH2, a histone H3 methyltransferase [38,39^{**}]. Discovered in a screen of non-Hodgkin's lymphoma samples, substitutions at Tyr641 (Y641F/N/S/H) were originally thought to abrogate methyltransferase activity on non-methylated substrates. However, subsequent work using heterogeneous extracted nucleosomes indicates catalytic competence, attributed to the preference of mutant EZH2 for partially methylated substrates (*i.e.* monomethylated and dimethylated) [39^{**}]. Because of their complementary substrate specificities, these enzymes coordinate to produce H3K27Me3 (histone H3, lysine 27, trimethyl) through sequential methyl transfers — initially by wild-type EZH2 then by a Y641F/N/S/H mutant (Figure 2) [39^{**}]. Similar to the structural alterations caused by canonical determinant residue substitution, homology modeling of the EZH2 active site suggests that mutations at Tyr641 exhibit an analogous reduction of steric hindrance [40].

Allosteric modulation of PKMT activity

In an example of allosteric regulation, H3K27 trimethylation by EZH2 is modulated by protein–protein interactions. EZH2 is the enzymatic subunit of the polycomb repressive complex 2 (PRC2) responsible for gene silencing [41]. Another subunit of PRC2 is EED, which was shown to preferentially bind H3K9Me3 and H3K27Me3 using a peptide binding screen and coprecipitation with methyllysine analog-modified nucleosomes (Figure 3) [41,42]. On the basis of the respective subunit functions, PRC2 has the dual-capacity to generate and bind H3K27Me3. Steady-state kinetic analysis of PRC2 in the presence of H3K27Me3 peptide revealed a ~7-fold increase in apparent maximal reaction rate, while the substrate concentration necessary for half-maximal velocity remained unchanged. These findings support a model of allosteric feed-forward regulation, whereby H3K27Me3 interaction with EED stimulates EZH2 to methylate nearby unmodified H3K27. This mechanism is consistent with a proposed spreading process that explains the contiguous distribution of repressive histone marks along chromatin [43].

DNA methyltransferases

Enzymatic DNA methylation in prokaryotes yields several species: N⁶-methyladenosine, N⁴-methylcytosine and 5-methylcytosine. Among eukaryotes, DNA is exclusively methylated on C5 of cytosine [44]. This epigenetic modification is commonly associated with loci-specific repressive chromatin states. Deposition of this modification is performed by DNA methyltransferases (DNMTs), which utilize SAM as the methyl donor [45]. Common among these enzymes is the ability to flip their target cytosine out of helical DNA before nucleophilic addition of a catalytic cysteine at C6 [46]. The resultant localization of negative charge on C5 promotes reactivity with SAM. Subsequent pyrimidine re-aromatization drives β -elimination of the appended cysteine, thus regenerating the resting-state enzyme and releasing the methylated cytosine (Figure 4a). Investigations into the regulatory mechanisms that control DNMT catalytic activity have revealed that some DNMTs are sensitive to the post-translational modification status of their chromatin substrates. For example, the ADD domain of murine Dnmt3a is necessary for efficient DNA methylation on *in vitro* reconstituted oligo-nucleosomes lacking post-translational modification; while, DNMT activity on the corresponding substrate harboring H3K4 methylation is reduced by three to fivefold — consistent with the physiological distribution of these mutually exclusive epigenetic marks [47]. In another example of DNMT regulation,

human DNMT3A forms homomultimers and heteromultimers that are necessary for processive methylation of multiple cytosine bases along a DNA strand [48].

RNA methylating enzymes

Methylation of RNA is a widespread post-transcriptional modification that expands structural and functional diversity [11]. The RNA substrates contain nucleophilic sites, which methylating enzymes target for reaction with the methyl group of SAM. These sites include various nucleoside heteroatoms as well as an enolate of uracil produced via conjugate-addition by an enzyme-derived cysteine residue [49–51]. However, these reactions are not compatible with C2 and C8 methylation of adenosine [52[•],53[•],54[•],55[•]]. Methylation of these electrophilic sites is achieved by the bacterial radical SAM enzymes RlmN and Cfr, which employ a unique methyl synthase mechanism. These enzymes assemble methyl groups on their substrate from a methylene fragment and a hydrogen atom at the site of methylation [53[•]]. Essential to catalysis is the utilization of SAM in two distinct ways: First, the methyl group of SAM is transferred to a cysteine residue within the enzyme that serves as a methylene precursor [54[•],55[•]]. Secondly, another molecule of SAM is homolytically cleaved to produce a 5'-deoxyadenosyl radical, which abstracts a hydrogen atom from the methylated cysteine. The resultant thiomethylene radical subsequently adds into the substrate (Figure 4b) [52[•],55[•]]. A proposed series of acid-base steps resolves the enzyme-substrate adduct to yield methyladenosine. Because 2-methyladenosine formed by RlmN resides in the peptidyltransferase center (PTC) of the ribosome, it is probable that this modification fine-tunes translation activity [56]. The Cfr paralog evolved an additional capacity to methylate C8, which decreases the effectiveness of many PTC-targeting antibiotics [57]. The folate-dependent and flavin-dependent RNA methylating enzyme TrmFO utilizes an atypical mechanism to install methyl groups [58,59]. Although the catalytic mechanism is yet to be fully elucidated, it is proposed that the enzyme-activated uridine substrate reacts with the electrophilic carbon of methylene-tetrahydrofolate. The resulting adduct is then reduced by flavin to yield 5-methyluridine (Figure 4c).

Conclusion

While significant advancements have been made to elucidate the underlying chemistry of methylating enzyme catalysis, there remains much to be revealed about their regulation. Many methylating enzymes participate in large protein complexes, suggesting the possibility of allosteric regulation. Moreover, the factors that govern substrate recognition are largely unknown. For example, both protein and nucleic acid methylating enzymes target chromatin, which harbors myriad chemical modifications that may affect enzyme–substrate interactions. Furthermore, pharmacological inhibition of methylation activity offers the potential to dissect methylation-independent functions of multi-protein methylating complexes, while maintaining the quaternary structure — often perturbed by genetic knockdown/knockout methods. As demonstrated by the body of work compiled in this review and elsewhere, chemical biological techniques have proven to be powerful tools for the investigation of mechanisms underlying protein and nucleic acid methylating enzymes.

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Figure 1.

Substitution-displacement reaction mechanism of PRMTs and PKMTs. **(a)** PRMT1 dimer model (1OR8; colored green) with reaction scheme. The substrate residue (colored black) and catalytic residues (colored gray) are shown. The SAM and methyl acceptor binding sites are identified with magenta and red arrows, respectively. **(b)** PRMTs and PKMTs utilize *S*-adenosylmethionine as a methyl group donor. The methyl group (colored red) is transferred to the substrate residue concomitant with *S*-adenosylhomocysteine formation. **(c)** SET7/9 (2F69; colored yellow) with substrate, catalytic residues, and catalytic water (colored blue). **(d)** Substrates and methylated products of protein methyltransferases.

Figure 2.

Complementary product selectivities of wildtype and mutant EZH2 underlie the hyper-trimethylation phenotype among heterozygotes. Wildtype EZH2, a subunit of PRC2, monomethylates and dimethylates H3K27. Mutant EZH2 (Y641F/N/S/H), with its product selectivity shifted to higher-order methylation products, has the capacity to form H3K27Me3.

Figure 3.

Allosteric activation of PRC2 methyltransferase activity via EED interaction with H3K27Me3. PRC2 is a multi-subunit complex that establishes the repressive H3K27Me3 mark. **(a)** PRC2 binds non-methylated chromatin to install H3K27Me3 via EZH2 activity. **(b)** EED binds H3K27Me3, which causes allosteric activation of EZH2. With its improved catalytic efficiency, EZH2 methylates chromatin regions adjacent to the founding H3K27Me3 mark. **(c)** Nucleosomes modified with methyllysine analogs were used to assay EED substrate recognition and allostery.

Figure 4. Chemical mechanisms of nucleic acid methylation. **(a)** DNA methylation at C5 of cytosine. **(b)** Proposed mechanism of rRNA methylation by the radical SAM methyl synthase RlmN. **(c)** Proposed mechanism of the folate/FAD-dependent tRNA methylating enzyme TrmFO. The transferred methyl group or its component parts are colored red. R' = (*p*-aminobenzoyl)-glutamate and R'' = adenosine-5'-pyrophosphate-ribityl.