S-Adenosylmethionine: jack of all trades and master of everything?

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Abstract
SAM (S-adenosylmethionine, also known as AdoMet) is well known as the methyl donor for the majority of methyltransferases that modify DNA, RNA, histones and other proteins, dictating replicational, transcriptional and translational fidelity, mismatch repair, chromatin modelling, epigenetic modifications and imprinting, which are all topics of great interest and importance in cancer research and aging. In total, 15 superfamilies of SAM-binding proteins have been identified, with many additional functions varying from methylation of phospholipids and small molecules such as arsenic to synthesis of polyamines or radical formation. SAM is regenerated from demethylated SAM via the methionine cycle, which involves folate. Imbalance of this cycle in humans, e.g. through folate shortage via dietary insufficiency, alcohol abuse, arsenic poisoning or hereditary factors, leads to depletion of SAM and human disease. In addition to its role as a methyl donor to modification enzymes that protect bacterial DNA against cognate restriction, SAM also serves as a co-factor for nucleases such as the type I restriction enzyme EcoKI, which is unable to restrict DNA in the absence of SAM. Finally, on a completely different tack, SAM can bind to certain RNA structures called riboswitches that control transcription or translation. In this way, expression of multiple genes can be regulated in a SAM-dependent manner, an unexpected finding that opens up new avenues into gene control. This minireview discusses some of these diverse and amazing roles of this small metabolite.

Introduction
Giulio Cantoni discovered SAM (S-adenosylmethionine, also known as AdoMet) in 1953 [1]. It is a conjugate of methionine and the adenosine moiety of ATP, formed in a reaction catalysed by MAT (methionine adenosyltransferase, also known as SAM synthetase). The nature of protection of bacterial DNA by modification enzymes against cognate restriction, i.e. methylation, was established in the 1960s by Werner Arber and his colleagues, while, in 1968, Bob Yuan and Matt Meselson first showed that, unexpectedly, the restriction enzyme EcoKI also needed SAM for nuclease activity [2,3]. This intriguing enzyme of Escherichia coli K-12 is a large pentameric combined restriction–modification complex that distinguishes non-methylated DNA from hemi-methylated DNA. Binding of EcoKI to the DNA is SAM-dependent, and the co-factor alters the DNA contacts of the MTase (methyltransferase) subunit. If the DNA is hemi-methylated, EcoKI acts as a MTase and modifies the second strand. However, if the DNA is unmethylated, EcoKI acts as a restriction enzyme. It undergoes a large conformational change, translocates the DNA past itself, creating large loops, and cuts up to 5 kb away from the recognition site [2,3]. It is likely that this mechanism is also employed by evolutionarily related mammalian enzymes, but, so far, no data on this interesting topic have emerged.

All parts of the SAM molecule are used as a source for a bewildering variety of biochemical reactions, making it one of the most frequently used enzyme substrates after ATP. No less than 15 superfamilies have been identified with different structural domains and folds that perform all of these chemical reactions. Surprisingly, even within each family of SAM-binding proteins, the protein sequences themselves are not conserved [4–7]. It has been claimed that SAM played this diverse role in the LUCA (last universal common ancestor) of Bacteria, Archaea and Eukarya [5]. LUCA was probably able to synthesize SAM de novo and use it to (i) methylate RNA and proteins, thus affecting translation, (ii) decarboxylate SAM [producing dSAM (decarboxylated SAM)] during the synthesis of polyamines, and (iii) generate SAM radicals. It is estimated that 95% of SAM is used for methylation and 3–5% for the generation of dSAM [8]. In humans, 85% of all of these methylation reactions and 50% of all methionine metabolism takes place in a single organ, the liver [9]. In this minireview, some of these aspects will be highlighted.

SAM-binding proteins
The SAM domain/Rossmann fold
Despite the lack of sequence identity, the majority of SAM-dependent MTases share a common conserved fold despite the lack of sequence identity, the Rossmann fold [4–7]. Although the fold itself is conserved in evolution, the residues that
Scheme 1 | Simplified diagram of SAM metabolism

The top half of the Scheme shows the methionine cycle, in which SAM is generated from methionine by MAT. SAM is converted into SAH by many MTases, in which process it donates the methyl group. SAH is hydrolysed to homocysteine. The latter is the substrate for methionine synthase, which uses a derivative of folate, MTHF, as a methyl donor to regenerate methionine, producing THF (tetrahydrofolate). The bottom half of the Scheme shows the role of SAM in the polyamine pathway. SAM is first decarboxylated by SAMDC. The methionine backbone of dcSAM is used by spermidine synthetase to convert putrescine into spermidine. A second molecule of dcSAM is used by spermine synthase to convert spermidine into spermine (back-conversion routes not shown).

Contact SAM are not, making it a chemist’s bonanza. Classification of this large superfamily is based on substrate specificity (e.g. DNA, RNA, protein, lipid and small molecules such as arsenic) and on the atom targeted for methylation (e.g. N, O, C or S) [4–6]. Not all proteins with this fold, however, are active as MTases. Two interesting examples are DNMT2 (DNA MTase 2), which binds DNA tightly and structurally resembles the well-characterized modification enzyme M.HhaI, and the de novo MTase-like DNMT3L (DNA MTase 3-like), which is required for methylation of imprinted genes in germ cells [6]. Both are inactive as MTases, but the latter appears to accelerate the binding of DNA and SAM to other MTases. Another interesting example is spermidine synthase, an important enzyme in the polyamine pathway. The polyamines (putrescine, spermidine and spermine) are small positively charged molecules in the cell that bind tightly to DNA, RNA, proteins, phospholipids and many other negatively charged molecules. In this way, they can affect DNA bending and transition from B to Z DNA, cause frameshifts and other infidelities at the RNA level, and modulate signal transduction [10–13]. Overexpression of enzymes in this pathway is implicated in cancer, while homozygous knockout of the enzymes in mice proves lethal [11]. Spermidine synthase is 70% identical with putrescine N-methyltransferase, yet lacks MTase activity. Instead, it fuses the methionine backbone of dcSAM to putrescine [6].

Other SAM-binding domains

SAM-radical enzymes form a completely different class of SAM-binding enzymes that possess the TIM barrel domain, named after triose phosphate isomerase [5]. These enzymes use SAM to generate methionine and a 5′-deoxyadenosyl radical that can be used to generate further radicals on the same protein or on a coupled enzyme. One important example of this class is SAMDC (SAM decarboxylase), the enzyme that provides the precursor dcSAM for spermidine synthesis mentioned above (Scheme 1). The structure of this enzyme and its manifold control of synthesis is a true lesson in gene control, and the reader is referred to excellent reviews on this topic [5,10–13].

A third class of important SAM-dependent enzymes contains the SET domain. This domain was discovered as a conserved domain shared by the chromatin remodelling proteins suppressor of variegation 3–9 [Su(var)3–9], enhancer of zest and trithorax. These enzymes affect chromatin function and transcription by methylating lysines in, for
example, histones and p53, the importance of which is obvious [5].

Several enzymes in the methionine cycle have unusual or even unique folds [5]. MATs, the all-important enzymes of de novo SAM biosynthesis of ancient LUCA origin, are still closely related at the sequence level in all Kingdoms, with unique wedge-shaped structures. The fold of methionine synthetase, which uses MTHF (methylated tetrahydrofolate) as a co-factor to regenerate methionine, is rare, while the repressor of the methionine operon MetJ, which uses SAM as a co-repressor, is the only known SAM-binding protein with a RHH (ribbon–helix–helix) domain, again an evolutionarily ancient class of DNA-binding proteins. It is tempting to speculate that such crucial enzymes with their rare folds may get priority access to SAM when methionine/SAM levels in the cell become dangerously low.

**SAM and human disease**

The folate derivative MTHF is active in the methionine cycle, in which SAM is regenerated after donating its methyl group in MTase reactions (Scheme 1). Demethylation of SAM generates SAH (5-adenosylhomocysteine), which is converted into homocysteine, in turn, the substrate for methionine synthase, which uses MTHF as a methyl donor. As mentioned above, methionine is converted back into SAM by MAT, which uses the adenosine moiety of ATP. Low levels of folate in serum, high levels of homocysteine in plasma, and/or a polymorphism in the MTHF receptor (MTHFR C677T) have been implicated in SAM depletion and a wide range of diseases in humans: colon cancer, breast cancer, coronary disease, atherosclerosis, (alcoholic) liver disease, pathogenic brain function (depression, Alzheimer’s disease, Down’s syndrome, AIDS dementia) and development (neural tube defect) [14–20]. SAM supplements may be helpful in at least some cases, e.g. SAM may protect against deleterious effects of TNFα (tumour necrosis factor α) in liver disease [21].

From the above, it can be gathered that the ability to synthesize SAM is crucial to life. Hence, few organisms are known to lack MAT. One such organism is the parasite *Pneumocystis*, which needs to obtain SAM from its host [8]. This fungus is of clinical importance as it affects immunsuppressed individuals, e.g. transplantation or HIV patients and people being treated with anticancer drugs. The SAM homologue sinefungin effectively competes with SAM in vitro and inhibits the fungus in the laboratory. However, this drug is too toxic for use in humans, but a new application is suggested based on the observation that pneumonia relapse due to *Pneumocystis* in HIV patients is low in smokers. In line with this, nicotine treatment of immunosuppressed rats apparently suppresses the fungus [22], which may be good news for some smoking addicts.

**SAM and riboswitches**

Finally, on a completely different tack, SAM is capable of binding RNA [23,24]. Riboswitches, first discovered in *Bacillus subtilis*, are highly structured RNA regions within the 5′-UTR (untranslated region) of certain mRNAs that are metabolite (e.g. thiamine, purine, glycine, SAM)-sensing structures and control gene expression (Figure 1). Binding of the metabolite alters secondary-loop formation of this region, thus affecting transcription or translation. Formation of alternative loops may obscure the ribosome-binding site and inhibit translation, or it may create a transcription terminator site. The original SAM riboswitch in *B. subtilis* has a very complex structure, but, recently, a completely different and amazingly simple and active one has been described in *Agrobacterium tumefaciens* [25]. SAM riboswitches are involved in, e.g., sulphur metabolism [22].

Currently, novel riboswitches are being engineered in the laboratory to control gene expression (http://aptamer.icmb.utexas.edu). One example is a tetracycline-dependent riboswitch that can be used to regulate translation of a reporter gene in yeast [26]. This riboswitch responds to the presence of tetracycline in the cell in a reversible manner.

Last, but not least, riboswitches are not confined to Gram-positive bacteria. Thiamine riboswitches have also been.

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**Figure 1 | Diagram of the effects of SAM binding to a riboswitch**

(A) Inhibition of translation by SAM. SAM binds the structured 5′-UTR of some mRNAs (riboswitch), which results in the replacement of stem-loops (1–2) and (3–4) by new loops (2–3) and (4–5), thus obscuring the ribosome-binding site (RBS) that is present in region 5.

(B) Termination of transcription by SAM. A polyU stretch ahead of the RBS is ignored because of its position relative to the anti-terminator loop. The SAM-dependent riboswitch located upstream of this anti-terminator within the 5′-UTR of the mRNA acts in cis after binding SAM. The stem-loops (1–2) and (3–4) are replaced by stem-loops (2–3) and (4–5), and result in termination of transcription of the target gene at the polyU stretch immediately downstream of stem-loop (4–5).
described in plants and fungi [23,24]. In the light of the simple SAM riboswitch of *A. tumefaciens* mentioned above, this suggests that SAM riboswitches may be just around the corner from our human RNA world.

**Conclusion**

SAM is one of the most versatile compounds in life, second perhaps only to ATP in a myriad of biochemical processes, many of which date back to the early origins of life. In this way, SAM can be considered as a spider in a biochemical web.

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**References**


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