In humans, some 60 enzymes are thought to belong to this class including proline/lysine hydroxylases, but also histone demethylases. The physiological roles they exert range from gene control (DNA repair, DNA modifications, regulation of transcription factors) over oxygen sensing to metabolic processes. From an organic chemical point of view, the nature of the transformations achieved by 2-OG; that is, regio- and stereoselective manipulation of unactivated C-H bonds is highly attractive. This holds true also particularly for microorganismal 2-OG enzymes, such as the penicillin synthases and halogenases.

Kessler and Schofield and colleagues reveal in this issue and for the first time the design and application of 2-OG probes in biological systems (Rotili et al., 2011). They explored two ABPs (Figure 1B), one (compound **1**) based on the 2-oxoglutarate analog oxalylglycine and the other (compound **2**) on the known 2-OG inhibitor 8-hydroxyquinoline. Both compounds are equipped with a phenyl azide as the photoreactive group and a biotin for identification purposes. Of the two probes, the oxalylglycine derivative proved unsuitable for use in ABPP. The hydroxyquinoline derivative, however, met with more success. In a key experiment (Figure 1C), they treated nuclear protein extracts with the ABP compound **2**, followed by streptavidin pulldown and SDS PAGE analysis. One of the resulting bands proved to correspond to the histone demethylase FBXL11. Compound **2** is a first-of-its-kind ABP, with which 2-OG proteins can be identified and enriched from complex mixtures using ABPP. In a series of related experiments, the authors demonstrate that hypoxia-related 2-OGs can also be enriched, which led them to show that expression levels of the target 2-OGs is dependent on the oxygen levels. Functional chemical biology studies involving activity-based 2-OG profiling are now realistic.

Obviously, there is room for improvement. As the authors state, the hydroxyquinoline is not likely to become the reagent of choice for broad-spectrum ABPP of 2-OGs. Somewhat surprisingly, the oxalylglycine derivative failed to give the desired result, but the attachment of the affinity label and biotin might have added considerable bulk to the 2-oxoglutarate scaffold. Possibly, and also noted by the authors, two-step bioorthogonal labeling may come to the rescue here (Ovaa et al., 2003). Overall, the paper not only adds to the growing list of enzymes amenable for ABPP studies, but also sets the stage for future research, both in the development of improved 2-OG ABPs and in applying these in physiological studies.

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Methylations: A Radical Mechanism

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DOI 10.1016/j.chembiol.2011.05.001

On the basis of labeling experiments, Grove et al. (2011) have shown how an electrophilic carbon (from an RNA adenosine) can be methylated by S-adenosylmethionine-dependent methyltransferases though an original radical mechanism.

The diversity of proteins and nucleic acids rests on the combination of a very limited number of chemical bricks, 20 amino acids and 4 nucleotides, respectively. Even though this leads to a huge number of combinations, in order to extend this diversity further living organisms have evolved additional selective mechanisms which allow the direct and site-specific incorporation of chemical groups into these macromolecules (Walsh, 2006; Grosjean, 2009). These modifications

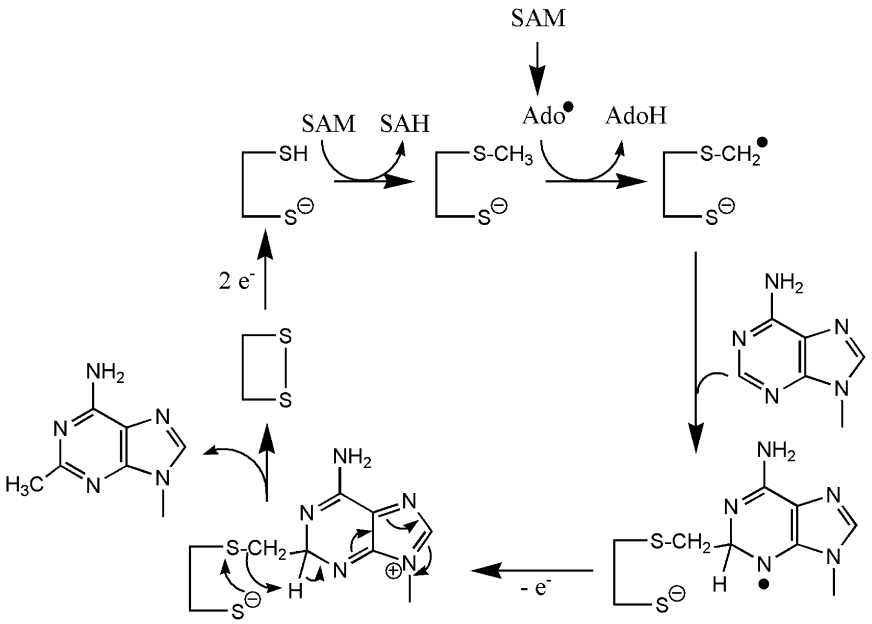

Figure 1. Proposed Mechanism for the Radical C2-Methylation of Adenosine Catalyzed by the SAM-Dependent Methyltransferase RlmN
SAH, S-adenosyl-homocysteine; AdoH, 5′-deoxyadenosine.

provide them with additional local functionalities and conformations, resulting in novel catalytic and recognition properties, respectively.

Considering the strict requirement for an optimized fidelity of the polymerization machineries involved in DNA synthesis, DNA transcription into RNA, and RNA translation into proteins, it is not surprising that the modification chemistry most often applies to the polymers themselves rather than to the monomers before polymerization. On the other hand, this raises the problem of reaction selectivity because of the large number of sites with similar reactivity within the same macromolecule.

The chemistry at work in these modifications was considered, until recently, to be exclusively electrophilic in nature (Walsh, 2006; Grosjean, 2009). However, with the discovery of the "Radical-SAM" (SAM = S-adenosylmethionine) enzyme family in particular, a number of protein and RNA modification reactions proved to proceed through radical mechanisms (Atta et al., 2010). The strong reactivity of the intermediate 5′-deoxyadenosyl radical (Ado°), generated through reductive cleavage of SAM, allows practically any site of the target substrate to be activated, by hydrogen atom abstraction, for modification. The flip side is that the selectivity of the reaction is much more difficult to control. The mechanistic and structural investigation of these fascinating radical-based enzymes is currently a very active field of research as it may help to understand: (1) how primary free radicals can be generated in one protein and directed to specific sites into a macromolecular target, (2) how intermediate free radicals are controlled to generate the desired product, and (3) how all these radicals avoid deleterious redox quenching reactions.

Methylation reactions are one of the most common selective modifications of biological macromolecules. In most cases, the mechanism involves SAM as a donor of an electrophilic methyl group to a specific nucleophilic atom in amino acid side chains or nucleotide bases and sugars. In a recent work, S. J. Booker and collaborators have studied two fascinating SAM-dependent methyltransferases, RlmN and Cfr, which proceed through radical intermediates instead and discovered an original reaction mechanism (Grove et al., 2011). These enzymes introduce a methyl group at C2 and C8 of adenosine 2503 of ribosomal 23SrRNA, respectively. The C2 modification is suggested to allow fine-tuning of the translation, whereas the methylation of the C8 position provides bacteria with increased resistance to antibiotics (Toh et al., 2008). One easily understands why a different mechanism should apply in that case since these aromatic sp^2-hybridized carbon atoms are electrophilic and cannot be directly methylated by SAM in a standard S_N2 reaction. Another mechanistic complication comes from the fact that both enzymes belong to the "Radical-SAM" enzyme family and thus SAM serves not only as the methyl group donor but also as a source of a 5′-deoxyadenosyl radical (Ado°), as previously established by in vitro studies (Yan et al., 2010). The requirement, during each catalytic cycle, for two molecules of SAM used in different chemistries is a property shared by methylthiotransferases, another subgroup of "Radical-SAM" enzymes, which catalyzes the incorporation of methylthio groups into ribosomal proteins and tRNAs, such as RimO and MiaB/MtaB, respectively (Atta et al., 2010).

Using labeled SAM and labeled enzymes and characterizing the products of in vitro one-turnover reactions by mass spectrometry, S. J. Booker et al. have nicely shown that the same reaction mechanism applies to RlmN and Cfr and reported evidence that (1) methyl transfer from SAM takes place with exchange of one hydrogen atom; (2) Ado° does not abstract the H atom from C2 or C8, in agreement with the expected very high activation barrier (>25 kcal. mol^{-1}, unpublished results) required for the generation of an energetically unfavorable σ-radical, but instead an H atom from a protein-bound methyl group; (3) two absolutely conserved cysteines play an essential role during catalysis, one transiently carrying the methyl group and the other used to liberate the former cysteine at the last step of the catalytic cycle.

The proposed mechanism thus involves the following steps (Figure 1): (1) methylation of a specific conserved cysteine through a standard S_N2 reaction with SAM; (2) abstraction of one hydrogen atom from that cysteine-bound methyl group by Ado° derived from a second molecule of SAM; (3) attack of C2 (or C8) by the resulting protein-bound radical, thus forming the required C-C bond at C2 (or C8). After still ill-defined electron and proton transfer steps, the attack of the thiolate of the second cysteine onto the sulfur atom of the alkylated cysteine destroys the crosslink between the

protein and the methylated RNA, yielding a disulfide bond, which remains to be firmly established.

At about the same time, D. G. Fujimori and collaborators, also studying RlmN and Cfr enzymes, reported a series of original experiments, using a RNA substrate containing adenosines specifically labeled with deuterium at C2 (or C8), which demonstrated that the label was not recovered into 5′-deoxyadenosine, in agreement with S. J. Booker's data, but into the methyl group incorporated into the modified RNA product (Yan and Fujimori, 2011). This provides additional information, namely, that the hydrogen atom at C2 (or C8) is not exchanged with the solvent but recovered in the methyl group of the product. The concerted mechanism shown in Figure 1 takes this information into account and is thus an original version combining data from both studies. The mechanism proposed in the *Science* paper, implying a deprotonation at C2 of the crosslinked intermediate by a basic site B^- is valid only if the resulting BH does not exchange protons with the solvent.

There are still a number of questions to address for complete characterization of this mechanism, in particular, with regard to intriguing electron transfer steps. For example, if loss of one electron is required, what is the electron acceptor and furthermore what is the hydrogen donor for the reduction of the postulated disulfide bridge that closes the cycle? Nevertheless, this work illustrates how nature performs chemically challenging reactions, here methylation of an electrophilic carbon, using very simple reactants, here SAM.

ACKNOWLEDGEMENTS

I am grateful to M. Atta and E. Mulliez for their useful comments.

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