5-Deoxyadenosine Metabolism: More than “Waste Disposal”

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Abstract

5-Deoxyadenosine (5dAdo) is a by-product of many radical SAM enzyme reactions in all domains of life, and an inhibitor of the radical SAM enzymes themselves. Hence, pathways to recycle or dispose of this toxic by-product must exist but remain largely unexplored. In this review, we discuss the current knowledge about canonical and atypical 5dAdo salvage pathways that have been characterized in the last years. We highlight studies that report on how, in certain organisms, the salvage of 5dAdo via specific pathways can confer a growth advantage by providing either intermediates for the synthesis of secondary metabolites or a carbon source for the synthesis of metabolites of the central carbon metabolism. Yet, an alternative recycling route exists in organisms that use 5dAdo as a substrate to synthesize and excrete 7-deoxysedoheptulose, an allelopathic inhibitor of one enzyme of the shikimate pathway, thereby competing for their own niche. Remarkably, most steps of 5dAdo salvage are the result of the activity of promiscuous enzymes. This strategy enables even organisms with a small genome to synthesize bioactive compounds which they can deploy under certain conditions to gain a competitive growth advantage. We con-

clude emphasizing that, unexpectedly, 5dAdo salvage pathways seem not to be ubiquitously present, raising questions about the fate of such a toxic by-product in those species. This observation also suggests that additional 5dAdo salvage pathways, possibly relying on the activity of promiscuous enzymes, may exist. The future challenge will be to bring to light these “cryptic” 5dAdo recycling pathways.

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Introduction

S-Adenosyl-L-methionine (SAM or AdoMet) is an essential cofactor and cosubstrate of various biological reactions in all domains of life (see Fontecave et al. [2004] for an overview). SAM is formed from the condensation of ATP and the amino acid methionine by the activity of the SAM synthetase/methionine adenosyltransferase (EC 2.5.1.6) [Chou and Talalay 1972; Tallan and Cohen 1976]. SAM is the major methyl-group donor for the methylation of proteins, nucleic acids, carbohydrates and lipids, which results in the release of the by-product S-adenosylhomocysteine (SAH/AdoHcy) [Cantoni 1975; Chiang et al., 1996] (Fig. 1 a). Furthermore, SAM is a source of aminoalkyl groups during the synthesis of polyamines, the bacterial quorum sensing signal *N*-acylhomoserine lac-

tone (Autoinducer 1) [Nealson and Hastings 1979], the plant hormone ethylene [Yang and Hoffman 1984] and phytosiderophores, which are excreted by plant roots for increasing metal ion availability [Negishi et al., 2002]. During these reactions, methylthioadenosine (MTA) is formed as a by-product (Fig. 1 a). Additionally, SAM is a source of 5-deoxyadenosyl radical, which takes part in various reactions that are catalyzed by the radical SAM enzyme superfamily [Sofia et al., 2001]. Over 100,000 homologous enzymes belong to this superfamily [Holliday et al., 2018], and they all generate a radical species, the 5-deoxyadenosyl radical (5dAdo^{*}), by the reductive cleavage of SAM using an unusual Fe-S cluster [Sofia et al., 2001; Wang and Frey 2007]. Prominent members of the family take part in the biosynthesis of vitamins and cofac-

tors (i.e., biotin and thiamine), but also in that of complex secondary metabolites like antibiotics [Sofia et al., 2001]. In most of these reactions, 5-deoxyadenosine is released as a by-product (Fig. 1 a). A scheme illustrating 5-deoxyadenosine radical formation in radical SAM enzymes can be found in reference [Fontecave et al., 2004].

SAH, MTA and 5dAdo are by-products which have to be removed because they are product inhibitors [Chaland et al., 2009; Parveen and Cornell 2011]. Since elimination via export would lead to a loss of valuable carbon, nitrogen, and sulphur, they are preferentially recycled. SAH and MTA salvage pathways are well characterized. The sulphur form of SAH is rescued via the two- or three-step methionine cycle (also called activated methyl cycle), which is present in almost all organisms (except some ob-

Fig. 1. a S-Adenosylmethionine-derived metabolites. **b** Overview of possible 5-deoxyadenosine cleavage steps.

Fig. 2. Overview of different 5dAdo salvage pathways and universal methionine salvage pathway. **a** 5dAdo salvage via the DHAP shunt. This pathway is either conducted by promiscuous enzymes of the MSP pathway (MtnP/MtnN and MtnK, MtnA, MtnB), e.g. in *R. rubrum*, or by an additional set of paralogous enzymes (DrdK, DrdI, DrdA), e.g. in *B. thuringiensis* [Beaudoin et al., 2018; North et al., 2020]. **b** In the unicellular cyanobacterium *S. elongatus* PCC 7942 5dAdo can be metabolized into 5-deoxyribose and the bioactive deoxy-sugar 7-deoxysedoheptulose. Depending on the environmental conditions, 5dAdo is presumably also metabolized via

the DHAP shunt [Rapp et al., 2021]. **c** 5dAdo salvage by the formation of DKFP in the methanogenic archaeon *M. jannaschii* [Miller et al., 2014; Miller et al., 2018b]. **d** First steps of the universal methionine salvage pathway [Sekowska and Danchin 2002; Sekowska et al., 2004]. 5dAdo, 5-deoxyadenosine; 5dR, 5-deoxyribose; 5dR-1P, 5-deoxyribose 1-phosphate; 5dRu-1P, 5-deoxyribulose 1-phosphate; 5dI, 5-deoxyinosine; DKFP, 6-deoxy-5-keto-fructose 1-phosphate; DHK-MTPene, 1,2-dihydroxy-3-keto-5-(methylthio)pent(1)ene; KMTB, 2-keto-4-(methylthio)butyric acid.

(For figure see next page.)

a 5dAdo salvage via the DHAP shunt

b 5dAdo salvage in *Synechococcus elongatus*

c 5dAdo salvage by the formation of DKFP in *Methanocaldococcus jannaschii*

d Universal methionine salvage pathway

ligate endosymbionts) [Vendeville et al., 2005; North et al., 2020]. The sulphur of MTA is rescued via the methionine salvage pathway (MSP, also MTA cycle or Yang cycle in plants). This pathway is well characterized in various bacterial species like *Klebsiella pneumoniae* [Wray and Abeles 1995] and *Bacillus subtilis* [Sekowska and Danchin 2002], but also in the rat liver [Wray and Abeles 1995]. The canonical MSP consists of six to eight enzymatic steps catalyzed by the nucleosidase/kinase (MtnN/MtnK) or the phosphorylase (MtnP), an isomerase (MtnA), a dehydratase (MtnB), an enolase/phosphatase (MtnC or MtnW/MtnX), a dioxygenase (MtnD), and a transaminase (MtnE) (see Fig. 2 d). MTA is thereby converted into adenine, formate, and L-methionine by consuming inorganic phosphate, molecular oxygen, and a suitable amino acid as an amine donor [Sekowska et al., 2004]. Apart from the canonical, universally oxygen-dependent MSP [see Sekowska et al. [2004]; Albers [2009]; Sekowska et al. [2018]], different alternative pathways have been described in the last years. For example, the aerobic/anaerobic “MTA-isoprenoid shunt” [Erb et al., 2012], the anaerobic “DHAP-ethylene shunt” [North et al., 2017], the aerobic “DHAP-methanethiol shunt” [Miller et al., 2018a], the “bifunctional DHAP shunt” [North et al., 2020] and a modified anaerobic MSP in methanogenic archaea [Miller et al., 2018b]. A nice overview of the different MSP pathways was recently provided by North et al. [2020] and Miller et al. [2018a]. In this review, we therefore focus on the metabolism of 5-deoxyadenosine, a nearly universal metabolite.

Overview of 5dAdo Salvage Pathways

While MTA salvage has been intensively studied in the last decades, 5dAdo metabolism has received little attention. This is quite surprising because MTA is only released in some reactions, whereas 5dAdo is the by-product of a multitude of enzymatic reactions. Some authors have suggested that 5dAdo is metabolized by enzymes of the MSP that display broad substrate specificity, and that the 5dAdo salvage pathway is paralogous to the MSP [Sekowska et al., 2018]. The utilization of different substrates by one enzyme has been referred to as enzyme promiscuity [Copley 2003]. In some studies, promiscuous enzymes are distinguished from broad-specificity or bifunctional enzymes in that the former catalyze a fortuitous reaction for which they did not evolve [Khersonsky and Tawfik 2010]. However, it is very unlikely that enzyme reactions in a physiological context are ever fortu-

itous, and therefore, we will use the term enzyme promiscuity in the same manner as bifunctional/broad-specificity enzymes. In the past, 5dAdo salvage was often only discussed as a side topic of MTA salvage. Earlier literature only focused on 5dAdo cleavage, but not on its further metabolism [Savarese et al., 1981; Plagemann and Wohlhueter 1983; Choi-Rhee and Cronan 2005]. Only recently some 5dAdo salvage pathways have been experimentally confirmed in different bacterial species [Beaudoin et al., 2018; North et al., 2020; Rapp et al., 2021] (Fig. 2 a, b). Recently, the first steps of 5dAdo salvage in the methanogenic archaeon *Methanocaldococcus jannaschii* were confirmed, but the later steps still remain hypothetical [Miller et al., 2018b] (Fig. 2 c). Interestingly, little is known about 5dAdo salvage in humans.

5dAdo Formation

Various authors have shown that 5-deoxyadenosine accumulation strongly inhibits the activity of radical SAM enzymes [Choi-Rhee and Cronan 2005; Challand et al., 2009; Farrar et al., 2010; Palmer and Downs 2013]. *Escherichia coli* mutants impaired in processing 5dAdo displayed a strong growth phenotype, which could be overcome by the addition of biotin and lipoate [Choi-Rhee and Cronan 2005]. These coenzymes are formed by radical SAM enzymes [Sofia et al., 2001; Berkovitch et al., 2004] and their synthesis is therefore inhibited by the accumulation of 5dAdo. The number of genes encoding radical SAM enzymes is quite diverse in different organisms. In *M. jannaschii*, 2% of the genome encodes radical SAM enzymes (35 of 1,811 protein-encoding genes), whereas other organisms possess less radical SAM enzymes (*Synechococcus elongatus*: 18 of 2,714 genes, $\pm 0.66\%$; *Bacillus thuringiensis*: 15 of 6,334 genes, $\pm 0.24\%$; *Rhodospirillum rubrum*: 25 of 3,916 genes, $\pm 0.64\%$, *Homo sapiens*: 8 of $\sim 23,000 \pm 0.035\%$) [Beaudoin et al., 2018; North et al., 2020; Rapp et al., 2021]. A large part of radical SAM enzymes is only active under anaerobic conditions [Challand et al., 2010; Beaudoin et al., 2018], but 5dAdo formation was also observed under aerobic conditions in *S. elongatus* [Rapp et al., 2021] and in *R. rubrum* [North et al., 2020]. We assume that under aerobic conditions, 5dAdo mostly derives from radical SAM enzymes which are involved in essential cofactor biosynthesis like the biotin synthase or the lipoic acid synthetase. Biotin is an essential cofactor, e.g. for the acetyl-CoA-carboxylase, whereas lipoic acid is an essential cofactor of the pyruvate dehydrogenase complex. In *R. rubrum* the amount of

5dAdo was however increased 75-fold under anaerobic conditions (see $\Delta mtnP$ mutant [North et al., 2020]).

5dAdo Cleavage

The first step of 5dAdo metabolism, the cleavage of 5dAdo, is well characterized and confirmed by various in vitro and in vivo studies (Fig. 1 b). 5-Deoxyadenosine can be cleaved in a phosphate-dependent manner by the activity of the MTA phosphorylase (MtnP, MTAP, EC 2.4.2.28), which results in the release of adenine and 5-deoxyribose 1-phosphate (5dR-1P) (Fig. 1b, middle part) [Savarese et al., 1981]. Alternatively, 5dAdo is cleaved by a MTA nucleosidase (MtnN, Pfs, EC 3.2.2.9), releasing 5dR and adenine [Choi-Rhee and Cronan 2005; Challand et al., 2009]. Subsequently, the adenine molecule is directed towards the purine salvage pathway [Nygaard 1993], while 5dR is phosphorylated by the MTR kinase (MtnK, 2.7.1.100), resulting also in the formation of 5dR-1P (Fig. 1 b, upper part) [Beaudoin et al., 2018]. With the exception of plants, most eukaryotic cells use the phosphorylase, whereas about 50% of all sequenced bacteria [North et al., 2020], as well as protozoa, use the two-step mechanism with the nucleosidase and kinase [Zappia et al., 1988; Albers 2009]. Other bacteria ($\approx 30\%$ [North et al., 2020]), like *Pseudomonas* and most cyanobacteria, but also trypanosomes [Ghoda et al., 1988] and most archaea use the phosphorylase. The enzymes involved in these first metabolic steps are well known for their promiscuous activity. The MTA nucleosidase of commensal *E. coli* is a variable SAH/MTA/5dAdo nucleosidase which catalyzes the cleavage of these by-products into S-ribosylhomocysteine, 5-methylthioribose and 5-deoxyribose with almost similar efficiency [Choi-Rhee and Cronan 2005; Challand et al., 2009; Parveen and Cornell 2011; North et al., 2020], although MTA is a slightly preferred substrate. Furthermore, the homologous nucleosidase of *Mycobacterium tuberculosis* has a preference for 5dAdo, but is also able to process MTA and SAH, albeit with less efficiency [Namanja-Magliano et al., 2016]. Because of the fact that mammals do not possess a MTA nucleosidase, this enzyme is an attractive target for antimicrobial drugs [Lee et al., 2001; Li et al., 2003]. The MTA kinase MtnK is promiscuous as it catalyzes 5dR and MTR phosphorylation with equal efficiency, whereas S-ribosylhomocysteine is a very poor substrate suggesting that SAH is preferentially processed by the specific SAH hydrolase [North et al., 2020]

The MTA phosphorylase MtnP is also known for its promiscuity [Savarese et al., 1981; Plagemann and Wohlueter 1983; North et al., 2020], but it can only process MTA and 5dAdo with similar affinities, whereas SAH is cleaved by the SAH hydrolase in mammals [La Haba and Cantoni 1959; Parveen and Cornell 2011]. In *R. rubrum*, MtnP is a promiscuous MTA and 5dAdo phosphorylase, but is not able to use SAH as a substrate [North et al., 2020]. Likewise, the phosphorylase of *S. elongatus* appears to be a MTA/5dAdo phosphorylase, because a knockout mutant of this gene excretes 5dAdo as well as MTA [Rapp et al., 2021]. Defects in the activity of the MTA phosphorylase in human cells are furthermore strongly correlated with cancer development [Kamatani and Carson 1980; Christopher et al., 2002; Berasain et al., 2004; Bertino et al., 2011].

A third and less common mechanism of 5dAdo processing is present in anaerobic organisms like the methanogenic *M. jannaschii* where 5dAdo is first deaminated by a MTA/SAH deaminase (DadD, EC 3.5.4.31/28), which results in the formation of 5-deoxyinosine (5dI) [Miller et al., 2014; Miller et al., 2018b]. Subsequently, a methylthioinosine phosphorylase (MTIP, EC 2.4.2.44) cleaves the molecule, resulting in the release of hypoxanthine and 5dR-1P (Fig. 1 b, lower part). In *M. jannaschii*, the methylthioinosine phosphorylase (MTIP) only accepts hypoxanthine-containing MTI and 5dI with similar affinity (it is unable to use MTA, 5dAdo, adenosine) [Miller et al., 2018b]. It has also been suggested that MTIP plays a broader role in the general salvage of hypoxanthine-containing purine nucleosides.

Although the cleavage of 5dAdo is well characterized, the further fate of 5dR/5dR-1P is more diverse and only poorly characterized, as discussed in the following paragraphs.

5dAdo Salvage via the DHAP (Dihydroxyacetone Phosphate) Shunt

In the last years, various authors have shown that 5dR/5dR-1P is metabolized via the “DHAP” shunt, named by North and colleagues [Beaudoin et al., 2018; North et al., 2020]. In the DHAP shunt, 5dR/5dR-1P is metabolized by the activity of a kinase (only for 5dR) and/or an isomerase, leading to 5dRu-1P. The subsequent activity of an aldolase results in the release of dihydroxyacetone phosphate (DHAP) and acetaldehyde which can be metabolized via primary metabolism (Fig. 2 a). This pathway is similar to L-fucose and L-rhamnose metabolism in

Fig. 3. Variants of the 5dAdo salvage pathway via the DHAP shunt (see Fig. 2 a). The molecules are processed by the activity of a phosphorylase/kinase, an isomerase, and an aldolase. **a** Fucose metabolism in *E. coli* [Chen et al., 1987]. **b** Fluoroacetaldehyde formation in *Actinomycetes* [Onega et al., 2007]. Fluoroacetaldehyde is the precursor molecule for the toxic fluorometabolites fluoroacetate and fluorothreonine which are produced by various *Actinomycetes* like *S. cattleya* [O'Hagan et al., 2002].

E. coli where these two molecules are processed by the activity of an isomerase, a kinase and an aldolase, leading to the formation of L-lactaldehyde and DHAP [Chen et al., 1987] (Fig. 3 a).

5dAdo salvage via the DHAP pathway can be either conducted by promiscuous enzyme activity of the first enzymes of the MSP pathway (e.g., *R. rubrum*) [North et al., 2020] or by paralogous genes (e.g., *B. thuringiensis*) [Beaudoin et al., 2018] (Fig. 2 a). By using comparative genomics, a specific gene cluster was first identified in *B. thuringiensis* for 5dR metabolism and subsequently verified its involvement biochemically [Beaudoin et al., 2018]. The gene cluster encodes the deoxyribose disposal (Drd) kinase DrdK, the isomerase DrdI and the aldolase DrdA. This gene cluster is present in this organism in addition to the gene cluster for the methionine salvage pathway (MSP), and are hence considered to be paralogous genes. Specific gene clusters for the DHAP shunt are present in at least six different bacterial phyla and occur in more than 10% of the sequenced species [North et al., 2017; Beaudoin et al., 2018; North et al., 2020]. Interestingly, some organisms possess a specific gene cluster for the DHAP

shunt but lack genes for the MSP pathway (*Myxococcus xanthus*, *Clostridium botulinum*, various extraintestinal pathogenic *E. coli* (ExPEC) strains), whereas others, for example many *Bacillus* species, have both paralogous gene clusters [North et al., 2020]. It was also shown that the kinase, isomerase and aldolase that are responsible for 5dR cleavage (DrdK, DrdI, DrdA) are phylogenetically distinct from homologues that are responsible for canonical methionine salvage or fucose/rhamnose metabolism ([Beaudoin et al., 2018] supplementary information). It is very likely that, in organisms that do not possess a specific gene cluster for 5dAdo salvage, 5dR/5dR-1P can be metabolized by the promiscuous activity of enzymes of the MSP pathway. This is supported by the evidence that the MTR-1P isomerase (MtnA, EC 5.3.1.23) from the universal MSP of *B. subtilis*, which catalyzes the isomerization of MTR-1P to MTRu-1P, is able to isomerize 5dR-1P into 5dRu-1P, albeit with poor efficiency [North et al., 2020]. Furthermore, the next enzyme of the MSP, the MTRu-1P dehydratase MtnB (EC 4.2.1.109), can exhibit promiscuous aldolase activity on 5dRu-1P leading to the formation of DHAP and acetaldehyde. This promiscuity

was experimentally shown for DEP1 of *Arabidopsis thaliana* ([Beaudoin et al., 2018] supplementary information) and for MtnB of *B. subtilis* [North et al., 2020]. We have also suggested that *S. elongatus* is able to use the promiscuous enzymes of the MSP for 5dAdo salvage via the DHAP shunt under certain conditions [Rapp et al., 2021] (Fig. 2b, DHAP shunt).

Interestingly, the enzymes of the DHAP shunt can also be used to metabolize MTR, an intermediate of the MTA salvage pathway. In *R. rubrum*, MTR can be metabolized via the DHAP shunt leading to the formation of DHAP and (2-methylthio) acetaldehyde under both aerobic and anaerobic conditions [North et al., 2020]. The possibility of using the DHAP shunt for MTR salvage in *R. rubrum* is essential as this organism lacks most of the genes of the canonical MSP [Erb et al., 2012]. Furthermore, the canonical MSP, by using the dioxygenase MtnD, is strictly dependent on the presence of oxygen. Therefore, under anaerobic conditions the bifunctional DHAP shunt can be used for MTA salvage. This is underlined by the fact that DHAP shunt genes are enriched in anaerobic and facultative anaerobic bacteria. Nevertheless, also the aerobic *B. thuringiensis* can use the enzymes of the DHAP shunt (DrdK, DrdI, DrdA) for MTR metabolism [Beaudoin et al., 2018]. Furthermore, it was shown that the enzymes from the MSP are so promiscuous that they can even metabolize ribose into the homoserine precursor 2-keto-4-hydroxybutyrate (KHB) in *B. subtilis* [Nakano et al., 2013].

5dAdo Salvage in *Synechococcus elongatus* Leading to Bioactive Deoxy-Sugar Synthesis

Only recently we identified an unusual 5dAdo salvage pathway in the unicellular cyanobacterium *S. elongatus* PCC 7942, which has a small and stream-lined genome [Rapp et al., 2021]. In this organism, 5dAdo salvage can result in the excretion of 5-deoxyribose and 7-deoxysedoheptulose under certain conditions [Brilisauer et al., 2019; Rapp et al., 2021] (Fig. 2 b). In this pathway, 5dAdo is cleaved by promiscuous MTA/5dAdo phosphorylase (*Synpcc7942_0923*, EC 2.4.2.28), leading to the formation of 5dR-1P. During growth at high CO₂ concentrations, 5dR-1P is then dephosphorylated by a presumably promiscuous phosphatase belonging to the HAD-like hydrolase superfamily (*Synpcc7942_1005*, EC 3.1.3.10), resulting in the formation of 5dR. This is quite surprising, as in other 5dAdo salvage pathways 5dR must be phosphorylated for further metabolism. 5dR is immediately ex-

creted into the supernatant because accumulation of 5dR is toxic for the producer strain *S. elongatus* [Rapp et al., 2021]. It is unclear where and how 5dR exerts its toxic effects, but the addition of 5dR is sufficient to strongly affect the growth of *B. thuringiensis* or also *S. elongatus* in high concentrations [Rapp et al., 2021]. *S. elongatus* is able to take up exogenously added ¹³C₅-labelled 5dR and excrete endogenously produced, unlabelled 5dR at the same time [Rapp et al., 2021]. Therefore, 5dR is continuously exported out of and imported into the cells. At the transition to stationary growth, a portion of the 5dR is converted into the unusual and bioactive deoxy-sugar 7-deoxysedoheptulose (7dSh). This reaction is catalyzed by the promiscuous transketolase (*Synpcc7942_0538*, EC 2.2.1.1), which transfers a C₂-unit onto 5dR [Brilisauer et al., 2019; Rapp et al., 2021]. It is known that transketolase enzymes can transfer a C₂-ketol unit (e.g., from hydroxypyruvate or xylulose 5-phosphate) onto an acceptor aldehyde while accepting various aldehyde substrates with 3S, 4R-configuration [Kobori et al., 1992]. Accordingly, 5dR was used for the in vitro synthesis of 7dSh by the *S. elongatus* transketolase in the presence of hydroxypyruvate as artificial ketol-donor [Brilisauer et al., 2019]. However, it turned out that the affinity of the *S. elongatus* transketolase for 5dR is much lower than for the native substrate ribose 5-phosphate [Brilisauer et al., 2019]. Under ambient CO₂ conditions, *S. elongatus* only accumulates very small quantities of 5dR, and no detectable amounts of 7dSh [Rapp et al., 2021]. Since neither the levels of 5dAdo nor MTA were enhanced under high carbon conditions, we suggested that 5dAdo is presumably rescued via the DHAP-shunt under ambient carbon conditions by the promiscuous activity of the first enzymes of the MSP pathway [Rapp et al., 2021]. Despite its small genome size, *S. elongatus* possesses the whole set of genes of the MSP, whereas other cyanobacteria (for example *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7502, *Synechococcus* sp. PCC 6312, *Anabaena* sp. PCC 7120, *Anabaena variabilis* ATCC 29413) only possess the first two enzymes (MtnP, MtnA) [see Rapp et al. [2021], supporting information]. Significantly, these other cyanobacteria do not excrete 5dR or 7dSh [Rapp et al., 2021]. Therefore, it is unclear how these organisms perform 5dAdo salvage. It could be speculated that they use MtnP and MtnA for the first steps resulting in 5dRu-1P formation. After that, 5dRu-1P may be cleaved by an aldolase, which are in general also known for their promiscuity [Fessner et al., 1991; Laurent et al., 2018]. The presence of a complete, canonical MSP is not necessary in the above-mentioned cyanobacteria as they probably do not pro-

duce MTA. Spermidine synthesis via the spermidine synthase (EC 2.5.1.16) plays a major role in MTA formation, but spermidine can also be synthesized via the carboxyspermidine decarboxylase (CASDC, EC 1.5.1.7) and carboxyspermidine dehydrogenase (CASDH, EC 4.1.1.96), in a series of reactions that do not lead to MTA excretion. *Synechocystis* sp. PCC 6803, for example, uses CASDC and CASDH for spermidine synthesis [Zhu et al., 2015].

5dAdo Salvage via 6-Deoxy-5-Keto-Fructose 1-Phosphate Formation, a Precursor Molecule for the Synthesis of Aromatic Amino Acids in Methanogens

Miller and coworkers [Miller et al., 2014; Miller et al., 2018b] suggested a special pathway for 5dAdo salvage in the methanogenic archaeon *M. jannaschii*, leading first to the formation of methylglyoxal and then of 6-deoxy-5-keto-fructose 1-phosphate (DKFP) (Fig. 2 c). The authors even proposed that this pathway is essential for the synthesis of aromatic amino acids in archaea, which use DKFP for the synthesis of 3-dehydroquinate, a precursor of aromatic amino acids that is generally produced via the shikimate pathway. However, archaea do not possess the enzymatic equipment for the oxidative pentose phosphate pathway, which provides the precursor molecule for the canonical shikimate pathway, erythrose-4-phosphate [Grochowski et al., 2005]. Furthermore, in the genome of methanogenic archaea, the first two enzymes of the canonical shikimate pathway (DAHP synthase and dehydroquinate synthase) are missing. Therefore, archaea have evolved a different strategy to synthesize aromatic amino acids which starts with the condensation of DKFP and L-aspartate semialdehyde into 3-dehydroquinate, a common metabolite in the shikimate pathway [White 2004; Gulko et al., 2014]. As mentioned above, the first step of 5dAdo metabolism is conducted by a deaminase, followed by a phosphorylase reaction, leading to 5dR-1P which is then isomerized by MTRI to 5dRu-1P as in the DHAP shunt (Fig. 2 c). 5dRu-1P is then hypothesized to be further processed by the activity of an epimerase and a transketolase into lactaldehyde, which is reduced by the promiscuous reductase Mer [Miller et al., 2017] to methylglyoxal. Although this metabolite is quite toxic, it was shown that methylglyoxal is an intermediate in the synthesis of DKFP [White and Xu 2006], where the molecule is used in a transaldolase reaction with fructose 1,6-bisphosphate catalyzed by the DKFP synthase (EC 2.2.1.11), leading to the formation of glyceraldehyde 3-phosphate and DKFP. In contrast to that, North and

coworkers hypothesized that, although archaea do not possess a homolog of an aldolase for the DHAP shunt, they might also use an analogous aldolase and thereby also apply the DHAP shunt [North et al., 2020].

5dAdo Salvage to Gain Growth Advantage

In some organisms, 5dAdo is more than a toxic by-product, and the 5dAdo salvage pathway more than simply a means of “waste disposal.” As a matter of fact, some species can use “unique” 5dAdo salvage pathways to gain a growth advantage over other members of a microbial community either by using 5dAdo as a substrate for the production of antimicrobial/bioactive compounds or as a sole carbon source in nutrient-limited habitats. In both scenarios, 5dAdo confers a fitness and colonization advantage to those organisms endowed with the ability to differently “recycle” this potentially toxic metabolite.

As mentioned above, the unicellular cyanobacterium *S. elongatus* PCC 7942 is able to excrete 5dR and 7dSh as a consequence of 5dAdo salvage [Rapp et al., 2021]. 7dSh is a competitive inhibitor of the dehydroquinate synthase, the second enzyme in the shikimate pathway, responsible for the synthesis of aromatic amino acids [Brilisauer et al., 2019]. Inhibitors of the shikimate pathway are generally attractive compounds for the development of antibacterial, antifungal, and herbicidal products, as the shikimate pathway is essential for these organisms but absent in mammals. It was shown that 7dSh acts as an allelopathic inhibitor towards other cyanobacteria, for example against the especially sensitive *A. variabilis* strains [Brilisauer et al., 2019]. Furthermore, it was shown that 7dSh can also inhibit the growth of *Saccharomyces cerevisiae* and more importantly, it showed herbicidal activity towards *A. thaliana* seedlings germinating on agar plates, but also on soil [Brilisauer et al., 2019]. Furthermore, 7dSh exhibited no toxic effects on human cell lines [Brilisauer et al., 2019] and did not alter the development of zebrafish (*Danio rerio*) [Schweizer et al., 2019]. Therefore, it could be applied as a harmless herbicide, which explains why the compound has attracted much attention [Brilisauer and Harter, 2020]. With respect to the physiological relevance of 7dSh production by *S. elongatus*, it should be noted that formation of 7dSh, as well as 5dR, is dependent on the cultivation conditions. Both molecules are mainly formed under elevated CO₂ conditions, and 7dSh formation occurs in late growth phases [Rapp et al., 2021]. As the amount of the precursor molecule 5dAdo is unaltered under high CO₂ conditions compared to ambi-

ent CO₂ conditions, we assume that 5dAdo is actively directed towards 5dR/7dSh synthesis under conditions in which competition with other community members, such as in biofilms, becomes important [Rapp et al., 2021]. In its natural habitat *S. elongatus* can form biofilms [Yang et al., 2018; Golden 2019] and tend to excrete exopolysaccharides [Rossi and Philippis 2015], which can be used as a carbon source by heterotrophic members of the microbial community thereby causing locally elevated CO₂ concentrations. It is remarkable that *S. elongatus* with a small, streamlined genome and no known gene cluster for the synthesis of secondary metabolites, can use a “waste product” of primary metabolism, 5dAdo, to synthesize a bioactive compound by promiscuous enzyme activity, thus competing against other species for the colonization of its own niche. This makes *S. elongatus* the archetypal organism able to derive bioactive compounds from primary, rather than the secondary metabolism. Interestingly, 7dSh had previously already been isolated from *Streptomyces setonensis* [Ito et al., 1971], which indicates that 5dAdo salvage via 7dSh excretion is not a feature unique to *S. elongatus*. As the genome of *S. setonensis* is currently unsequenced, it remains speculative whether 7dSh in this organism is also derived from 5dAdo.

Interestingly, it was shown that especially pathogenic strains of several organisms (e.g., *Clostridium tetani*, *C. botulinum*, *B. thuringiensis*, *Bacillus cereus* and *Bacillus anthracis*) possess putative DHAP shunt gene clusters, whereas the non-pathogenic strains of the genera do not [Beaudoin et al., 2018; North et al., 2020]. Furthermore, putative DHAP shunt gene clusters are present in nearly 50% of all extraintestinal pathogenic *E. coli* (ExPEC) isolates, whereas commensal *E. coli* strains neither possess a complete MSP pathway nor the DHAP shunt gene cluster [North et al., 2020]. Also, only 0.1% of intestinal pathogenic *E. coli* isolates harbour putative DHAP gene clusters [North et al., 2020]. In the intestinal environment, nutrients are normally not limiting. In comparison, in the extraintestinal environment (urine, blood, and cerebrospinal fluid) carbon and sulphur sources are often limiting or only present in compounds like urea, organic acids, purines and amino acids [North et al., 2020]. 5dAdo (also as 5-deoxyinosine) and MTA along with the degradation products 5dR/5dR-1P and MTR (methylthioribose), are common metabolites in this extraintestinal environment [Liebich et al., 1997; Lee et al., 2004; North et al., 2020], because mammals only metabolize 5dAdo beyond the phosphorylase step [Plagemann and Wohlhueter 1983; Savarese et al., 1981]. Furthermore, commensal *E. coli*, which do not possess a complete MSP, excrete MTR

[Schroeder et al., 1972; Hughes 2006]. *E. coli* ExPEC strains harbouring gene clusters for the DHAP shunt are able to grow on these metabolites as sole carbon and sulphur sources, whereas commensal *E. coli* were not able to grow on these metabolites [North et al., 2020]. The presence of this gene cluster therefore leads to a growth advantage in this ecological niche. Because the putative DHAP shunt gene cluster was present in nearly half of all *E. coli* ExPEC strains, for example in the lineage ST 131, which is multidrug resistant and a pathogen with worldwide distribution, causing urinary tract and blood infections [Petty et al., 2014], the DHAP shunt might be a very suitable target for drug development [North et al., 2020]. Furthermore, a similar pathway to the DHAP shunt is used for the formation of toxic fluorometabolites in *Actinomyces*, for example in *Streptomyces cattleya* [Sanada et al., 1986] (Figure 3 b). In this species, SAM serves as a precursor molecule for 5-fluoro-5-deoxyadenosine, which is formed by a specific fluorinase [O'Hagan et al., 2002]. This molecule is then also processed by a nucleotide phosphorylase [Cobb et al., 2004], an isomerase [Onega et al., 2007], and an aldolase [Moss et al., 2000], leading to fluoroacetaldehyde, the precursor molecule for the toxic fluorometabolites, fluoroacetate and fluorothreonine. These examples illustrate how, by salvaging 5dAdo, some organisms may acquire a growth advantage over others in specific niches and/or environmental conditions.

Export and Import of 5dAdo and Related Metabolites

Little is known about the export and import of 5dAdo and related metabolites. Various authors showed that strains deficient in the activity of the MTA phosphorylase excrete MTA and 5dAdo. This was shown for *S. elongatus* [Rapp et al., 2021], *R. rubrum* [North et al., 2020], *S. cerevisiae* (only MTA) [Chattopadhyay et al., 2006] and also mammalian cell lines (only MTA) [Kamatani and Carson 1980]. Feeding experiments with 5dAdo showed that not only bacterial strains, but also mammalian cell lines are capable of 5dAdo uptake [Rapp et al., 2021; North et al., 2020; Plagemann et al., 1988]. In mammalian cells, for example erythrocytes, which are deficient in de novo purine biosynthesis, 5dAdo and MTA are taken up by a nucleoside transporter with a broad substrate specificity [Plagemann et al., 1988]. 5dAdo uptake in bacteria, to the best of our knowledge, has not been further characterized. In *R. rubrum*, it is assumed that MTA is taken up via

the methionine transport complex (MetINQ) because proteins of this complex are enriched when cells grow on MTA as a sole sulphur source [North et al., 2016]. Furthermore, the MetINQ methionine transport complex is known for its substrate promiscuity [North et al., 2016]. However, it is not known if 5dAdo is also a possible substrate.

It is obvious that several organisms are capable of 5dR uptake. When supplemented into the medium of *B. thuringiensis*, 5dR strongly accumulates intracellularly, thereby causing growth retardation [Beaudoin et al., 2018]. It was also shown that *E. coli* ExPEC strain can use 5dR as a sole carbon source [North et al., 2020]. Interestingly, several organisms harboring gene clusters for the DHAP shunt also contain putative sugar transporters [Beaudoin et al., 2018] with the potential to take up 5dR, which, nonetheless, remains to be demonstrated. While it was shown that in rats 5dR can be reduced to 5-deoxyribose and also be excreted [Ichihara et al., 1985], to our knowledge, 5dR excretion has only been reported for *S. elongatus* [Rapp et al., 2021]. Because *S. elongatus* is capable of 5dR uptake and excretion at the same time, we assume that two transporters are present in *S. elongatus* in analogy to the two different genes (MtrA/YfnA and MtrE) reported to code for efflux and influx transporters of MTR in *B. subtilis* [Borriss et al., 2018]. The authors also identified secondary MTR transporters, including an ABC transporter for ribose (RbsDACB) and a guanosine transporter (NupQ). It is conceivable that 5dR is imported via the same transporter as 7dSh in *S. elongatus*, which is supported by the demonstration that a spontaneous 7dSh-resistant mutant is also resistant towards 5dR treatment, but the mutant excretes as much 5dR and 7dSh as the wild type (Rapp, unpubl. data).

Conclusions

The mechanisms behind 5-deoxyadenosine salvage have mainly been addressed in the last years, and many aspects still remain elusive. Since several, unusual pathways for MTA salvage have recently been discovered, it is very likely that other, yet to be unravelled pathways for 5dAdo salvage exist. The fact that most steps of 5dAdo salvage are conducted by promiscuous enzymes makes it difficult to predict gene clusters with a putative role in this process. However, the use of comparative genomics coupled with biochemical validation promises to be a useful approach to identify 5dAdo recycling pathways.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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Author Contributions

J.R. wrote the manuscript. K.F. supervised manuscript writing.

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