**Review**

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# Pterin function in bacteria

DOI 10.1515/pterid-2016-0012
Received December 12, 2016; accepted March 1, 2017; previously published online April 19, 2017

**Abstract:** Pterins are widely conserved biomolecules that play essential roles in diverse organisms. First described as enzymatic cofactors in eukaryotic systems, bacterial pterins were discovered in cyanobacteria soon after. Several pterin structures unique to bacteria have been described, with conjugation to glycosides and nucleotides commonly observed. Despite this significant structural diversity, relatively few biological functions have been elucidated. Molybdopterin, the best studied bacterial pterin, plays an essential role in the function of the Moco cofactor. Moco is an essential component of molybdoenzymes such as sulfite oxidase, nitrate reductase, and dimethyl sulfoxide reductase, all of which play important roles in bacterial metabolism and global nutrient cycles. Outside of the molybdoenzymes, pterin cofactors play important roles in bacterial cyanide utilization and aromatic amino acid metabolism. Less is known about the roles of pterins in nonenzymatic processes. Cyanobacterial pterins have been implicated in phenotypes related to UV protection and phototaxis. Research describing the pterin-mediated control of cyclic nucleotide metabolism, and their influence on virulence and attachment, points to a possible role for pterins in regulation of bacterial behavior. In this review, we describe the variety of pterin functions in bacteria, compare and contrast structural and mechanistic differences, and illuminate promising avenues of future research.

**Keywords:** cofactor; metabolism; pteridines; redox; regulation.

## Introduction

Pterins are ubiquitous compounds produced by organisms in all domains of life. These essential molecules play important roles in diverse biological activities ranging from immune system modulation, cellular signaling, coloration, and metabolism. Pteridine rings are also important biosynthetic building blocks, forming the backbone of several fundamental molecules including folic acid. Several excellent reviews [1, 2] exist on the essential roles of folate in cell maintenance, nucleic acid synthesis, and single-carbon metabolism; therefore, this review will focus on the roles of nonfolate pterin species. Despite a great deal of research on pteridine structure, synthesis, and function, many open questions exist in the field of pterin biology. This is especially true in bacteria because much of the early research into pterins was performed studying eukaryotic systems.

## Early pterin research

Pterins were first discovered in the late 19th century during the examination of yellow and blue fluorescent pigments isolated from butterfly wings [3]. These reports were soon followed by observations of xanthopterin and leucopterin present in fluorescent granules located in gastrointestinal endocrine cells [4, 5]. The first example of a biological requirement for pterins was provided when *Crithidia fasciculata*, a trypanosomatid parasite of mosquitoes, was shown to require a "Crithidia factor" for proper growth in laboratory culture [6]. Originally thought to be a folic acid derivative, further work showed that the "Crithidia factor" was an assemblage of structurally distinct pteridine molecules that were required along with folate for proper growth [7]. This pteridine requirement of *Crithidia* sp. for proper growth has been subsequently used to probe various bacterial families for the presence of pterin molecules [8].

Although it is not the focus of this review, a brief mention of the roles of pterins in eukaryotic organisms is warranted. After the initial discoveries listed above, a considerable amount of research has been conducted to determine the structure, biosynthesis, and function of various pterin molecules (thoroughly reviewed elsewhere [9–15]). Oxidized pterins such as neopterin have been shown to modulate the oxidative burst of reactive oxygen and nitrogen species in the human immune system [9, 16].

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In addition, the immune system transcription factor NF-κβ is directly regulated by several pterin species in mammals [17, 18]. Pterins also act as essential cofactors in several eukaryotic redox enzymes, a function that is shared with many bacterial systems.

In bacteria, pterins were first reported in the Cyanobacteria. Chromatographic fractionation of cell-free supernatants from *Anabaena*, *Anacystis*, and *Nostoc* led to isolation of several fluorescent molecules that exhibited characteristics consistent with the pteridine ring structure [19]. Soon after, detailed biochemical characterization defined a biopterin glucoside from *Anacystis nodulans* [20]. In addition, several noncyanobacterial genera of photosynthetic bacteria such as *Rhodospirillum* and *Rhodopseudomonas* were shown to produce pterin molecules that increased in abundance when these organisms were grown in the light [21]. The discovery of xanthopterin in *Escherichia coli* was among the first reports of pteridines from nonphotosynthetic organisms [22].

## Pterin synthesis, structure, and general functions

The core pterin molecule is a nitrogen heterocycle composed of fused pyramidine and pyrazine rings with both substituted keto and amino groups on the pyramidine ring (Figure 1). The synthesis of pterins in bacteria has been well examined and a simplified bacterial biosynthetic pathway is presented in Figure 2. The biosynthetic precursor to all pterin molecules is the purine nucleotide guanosine triphosphate (GTP). In bacteria, GTP cyclohydrolase (EC 3.5.4.16, FolE in *E. coli*) converts GTP to dihydroneopterin triphosphate ($H_2$NPt-$P_3$) [23], which acts as the biochemical precursor to folate, neopterin (NPt), monapterin (MPt), and biopterin (BPt) [24]. These three types of pterins differ from each other by the side chain at position 6 of the pteridine ring (Figure 1). A recent report [25], characterizing a novel family of GTP cyclohydrolase enzymes, emphasizes the diversity of pterin metabolism in divergent bacterial taxa. Further modification of NPt, MPt, and BPt is catalyzed by distinct enzymatic pathways. BPt species can be synthesized from $H_2$NPt-$P_3$ through a 6-pyruvoyltetrahydropterin (P-$H_4$-Pt) intermediate. For NPt and MPt, an epimerization reaction, that may alternately occur at different points in the pathway, determines which species of pterin is produced [24]. Molybdopterin, the ubiquitous enzymatic cofactor that also contains a pteridine ring is synthesized directly from GTP via its own distinct pathway (Figure 2), requiring the function of the *moa and moe* class of biosynthetic genes [26]. Transcriptional control of molybdopterin biosynthetic and transport genes, although outside the scope of this review, can be an important aspect of their production [27–29].

Since the discovery of pterins in bacteria almost 60 years ago, intensive research into the function of these molecules has exposed the varied roles that they play in bacterial ecosystems and environments. As with eukaryotes, the role of pterins as redox cofactors was the primary focus of the majority of early bacterial pterin research. More recent research has illuminated the role of pterin-containing enzymes in aerobic and anaerobic metabolism, global nutrient cycles, detoxification of harmful compounds, pigment production, and utilization of non-canonical carbon and nitrogen sources. Pterins can also play biological roles in addition to serving as enzymatic cofactors, mediating processes including protection from UV damage and intracellular signaling.

## Molybdoenzymes and the Moco cofactor

Redox enzymes such as sulfite oxidase, nitrate reductase, dimethyl sulfoxide (DMSO) reductase, and xanthine dehydrogenase were first characterized in eukaryotes and mediate oxidation-reduction reactions involving a variety of different substrates [10, 26]. Redox enzymes, such as those listed above, mediate oxygen transfer between substrate and water. All molybdoenzymes contain the Moco cofactor in their active site, which facilitates electron transport during the redox reaction. Moco is composed of a molybdenum ($Mo^{6+}$) ion, which is conjugated within the enzyme active site via interactions through a molybdopterin molecule and amino acid functional groups of the cognate enzyme. Molybdopterin both modulates the oxidation state of the Mo ion and enables electron transfer to other redox centers such as heme, iron-sulfur centers, or flavins [10]. The requirement for a molybdopterin-containing Moco cofactor is universal for all molybdoenzymes except nitrogenase, which contains only Mo.

The core sulfur-containing, triple-ring structure of molybdopterin is conserved (Figure 1); however, in bacteria molybdopterin can be found conjugated to guanine, cystosine, adenine, or hypoxanthine dinucleotides [30–32]. As mentioned earlier, molybdopterin is synthesized via a multistep enzymatic process [26] directly from a GTP precursor. This pathway is distinct from other known pterin biosynthetic pathways in bacteria (Figure 2).

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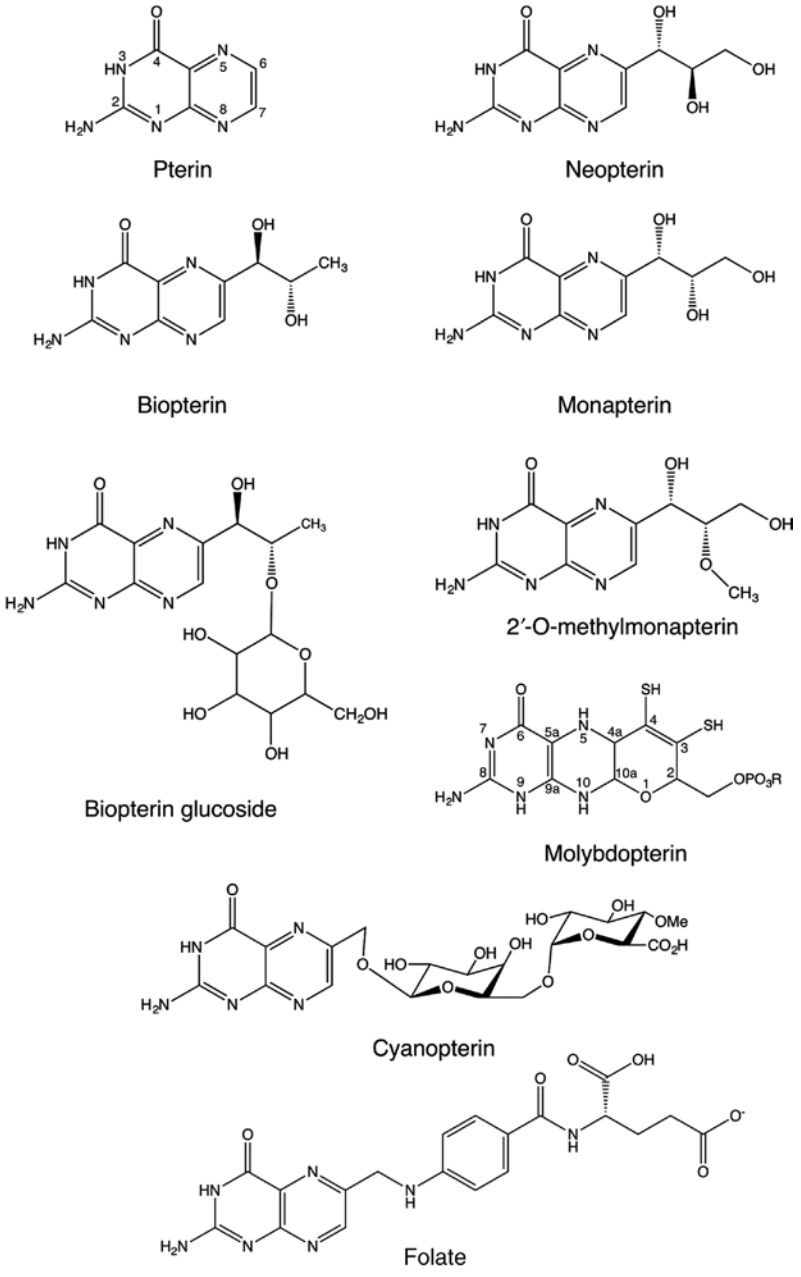


**Figure 1:** Pterin chemical structures. Structures of the pteridine ring and select bacterial pterin species as described in the text. R group on molybdopterin can be either a hydrogen atom or guanine, cystosine, adenine, or hypoxanthine dinucleotides. Structures drawn using ChemDraw Professional v.15.1 (Perkin Elmer Informatics).

Molybdopterin biosynthesis can be regulated at the transcriptional level in response to both levels of molybdate and molybdopterin present inside the cell [26, 29].

## Sulfite oxidase

Sulfites are naturally occurring compounds in the environment that can arise as decomposition products of reduced sulfur compounds such as thiosulfate, sulfonates, and polythionate [33]. Sulfite ($SO_3^{2-}$) can also arise from sulfur dioxide dissolving in water or from the intracellular breakdown of metabolic products including sulfur-containing amino acids. The high toxicity of sulfite and its derivatives is due to both its high nucleophilicity and strong reducing capacity, which can interact adversely with DNA and protein molecules within the cell [34]. One of the roles of sulfite oxidase activity is to detoxify sulfite

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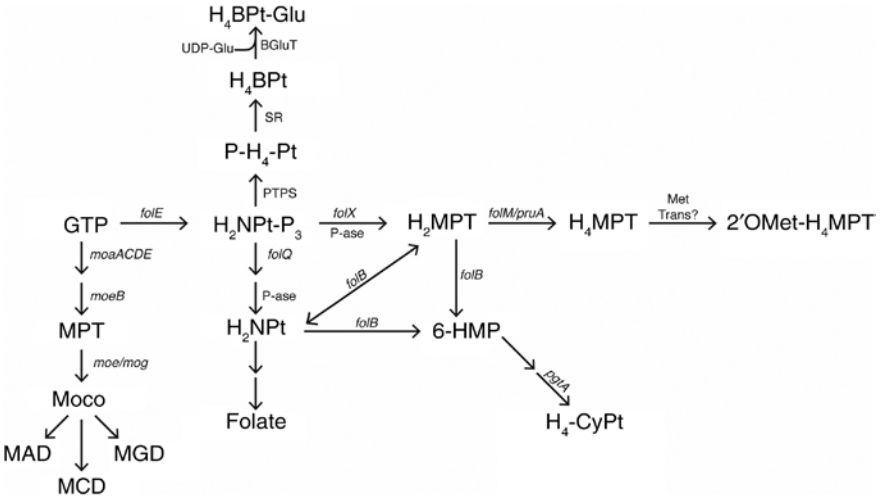


**Figure 2:** Pterin biosynthetic pathways. Generalized bacterial pterin biosynthetic pathways compiled from several distinct bacterial taxa. Gene names in italics next to pathway arrows. Nonitalicized text next to pathway arrow denotes a general enzymatic activity. MPT, molybdopterin; Moco, molybdenum cofactor; MAD, molybdopterin adenine dinuncleotide; MCD, molybdopterin cytosine dinucleotide; MGD, molybdopterin guanine dinucleotide; $H_2NPT$, dihydroneopterin; $H_2NPT\text{-}P_3$, dihydroneopterin triphosphate; $P\text{-}H_4\text{-}Pt$, 6-pyruvoyltetrahydropterin; $H_4BPt$, tetrahydrobiopterin; $H_4BPt\text{-}Glu$, biopterin glucoside; $H_2MPT$, dihydromonapterin; $H_4MPT$, tetrahydromonapterin; 2′OMet-$H_4MPT$, 2′-O methyltetrahydromonapterin; 6-HMP, 6-hydroxymethyl dihydropterin; $H_4$-CyPt, tetrahydrocyanopterin; PTPS, 6-pyruvoyltetrahydropterin synthase (EC 4.2.3.12); BGluT, biopterin glucosyltransferase (EC 2.4.1); SR, sepiapterin reductase (EC 1.1.1.325); Met Trans, methyltransferase (EC 2.1.1). P-ase, phosphatase (EC 3.1.3).

compounds, thereby preventing harmful damage of cellular components.

Sulfite oxidase enzymes (EC 1.8.3.1) catalyze the direct oxidation of sulfite ($SO_3^{2-}$) to sulfate ($SO_4^{2-}$) using a multistep electron flow pathway [35, 36]. Two electrons from sulfite are transferred to the Mo reaction center, generating a reduced ($Mo^{4+}$) ion. These electrons are then passed to either another redox-active center (Fe-S cluster, heme, etc.) or directly to a final electron acceptor such as $O_2$ or cytochrome *c* [37]. This process results in the regeneration of the oxidized ($Mo^{6+}$) form of the molybdate ion. The molybdate ion is coordinated in the enzyme active site in part by three thiol groups: two provided by the molybdopterin cofactor and one provided by a conserved cysteine residue on the sulfite oxidase enzyme. Molybdopterin itself is anchored in the active site through a network of hydrogen bonding to the cognate enzyme [36].

The majority of sulfite oxidase sequence diversity resides in bacteria [34, 38], and although the general structural and molecular features discussed above are present in all members of the sulfite oxidase family, several differences exist. There are two main recognized classes of sulfite oxidizing enzymes in prokaryotes. Sulfite oxidases can transfer electrons directly to oxygen, while sulfite dehydrogenases can transfer electrons only to acceptors other than oxygen, such as cytochromes or other anaerobic acceptors [35]. The subcellular localization of sulfite oxidizing enzymes can vary, with examples being isolated from membrane [39], periplasmic [40], and cytoplasmic [41] fractions. Sulfite oxidases also exhibit diversity in subunit structure [34], with many enzymes adopting a monomeric form [42], while some enzyme complexes display a heterodimeric arrangement with separate enzymatic and heme/cytochrome-binding domains.

Sulfite oxidase enzymes are relevant in a wide variety of bacterial lifestyles [43], ranging from aerobic lithoautotrophs, anaerobic photoautotrophs, to microaerophillic chemoheterotrophic human pathogens, several of which are discussed below. In addition to detoxification of harmful sulfite compounds, bacteria can also utilize sulfite oxidase enzymes as part of a dissimilatory or assimilatory enzymatic process to utilize reduced sulfur compounds as electron sources for cellular respiration [44] or for anabolic metabolism, respectively. The oxidation of sulfur compounds often releases large amounts of $H^+$ into the bacterial periplasm, augmenting the proton-motive-force that can be utilized for ATP production [45].

Sulfite oxidation is common in several classes of non-oxygenic phototrophic bacteria, which can use compounds such as hydrogen sulfide, thiosulfate, or tetrathionate as electron donors for photosynthetic $CO_2$ reduction [44, 45]. Green-sulfur bacteria belonging to the family Chlorobiaceae are obligate anaerobes that utilize sulfide compounds as electron donors, producing sulfate as an oxidative by-product. Purple-sulfur bacteria belonging to the families Chromatiaceae and Ectothiorhodospiraceae

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are extremophiles who can utilize hydrogen sulfide from sulfur springs, generating elemental sulfur globules [45].

Several other notable cases of pterin-dependent sulfite oxidase activity occur in heterotrophic bacteria. The chemoheterotrophic ε-proteobacterial human pathogen *Campylobacter jejuni* employs a SorA-type sulfite oxidase to utilize sulfite as an electron donor [46]. In addition to a respiratory role, *C. jejuni* sulfite oxidase is hypothesized to detoxify sulfite radicals released by neutrophils of the mammalian immune system in response to bacterial infection and, thus, perhaps plays a role in immune evasion [46]. Sulfite oxidases in the α-protobacterial plant symbiont *Sinorhizobium melioti* play a role in the breakdown of the organosulfate taurine for use as a carbon and sulfur source [47]. The β-proteobacterial soil-dwelling *Comamonas acidovorans* can use linear alkanesulfonates as carbon, sulfur, and electron sources [41].

## Nitrate reductase

Nitrate reductase enzymes catalyze the direct reduction of nitrate ($NO_3^-$) to nitrite ($NO_2^-$) using a multistep electron flow reaction similar to sulfite oxidases. Several types of nitrate reductases have been characterized (see below), but general principles of the core reaction mechanism have emerged [48, 49]. Electrons from an electron donor such as NADH or the quinone pool are transferred to the Mo reaction center (through a possible redox-center intermediate), generating reduced molybdate ($Mo^{4+}$), which coordinates a nitrate molecule in the enzyme active site [50]. Nitrate is then reduced, utilizing electrons from molybdate and solution $H^+$ ions, to produce nitrite and water in the process regenerating oxidized $Mo^{6+}$. The Mo ion is coordinated in the active site by five thiol groups [50]: four provided by dual molybdopterin cofactors (Figure 1) conjugated with guanine nucleotides (bis-MGD) and one by a cysteine residue from the nitrate reductase enzyme. As with sulfite oxidase, the molybdopterin cofactors are anchored in the enzyme active site by a network of hydrogen bonds with specific amino acid residues [50].

Three classes of nitrate reductases have been described in bacteria [49], all with significant differences in subcellular location, gene organization, regulation, and the eventual fate of reduced nitrogen. Early characterization of nitrate reductase activity in bacteria [51, 52] was centered on utilization of nitrate as an electron acceptor for anaerobic respiration, with special focus on the biochemical isolation and enzymatic characterization of this class of molybdopterin-containing enzymes [53–55] from Gram-negative species such as *E. coli* and *Pseudomonas aeruginosa*. Further research led to the realization that different types of nitrate reductases exist [48, 49], with each class being encoded by distinct genetic loci and playing unique biological roles [56].

Bacterial assimilatory nitrate reductases (EC 1.7.1.1, NAS) generate reduced nitrogen species such as ammonium ($NH_4^+$), which are incorporated into essential molecules such as amino acids. Nitrate reductase produces nitrite ($NO_2^-$), which is then further reduced by nitrite reductase enzymes to $NH_4^+$. Assimilatory nitrate reductases are cytoplasmic enzymes, with most examples containing both a bis-MGD cofactor and one or more Fe-S clusters [48]. Two classes of NAS enzymes exist: the NADH-dependent reductases (*nas* genes) such as those found in species of *Klebsiella* and *Rhodobacter*, and the ferredoxin-dependent reductases (*nrt* genes), often found in the cyanobacteria [49]. NAS enzymes often are multimeric complexes, with distinct enzymatic and redox-active subunits.

Respiratory membrane-bound nitrate reductases (EC 1.7.5.1, NAR) are dissimilatory enzymes involved in the utilization of nitrate as an electron acceptor for cellular respiration. These enzymes are often found as three-subunit complexes that are composed of a membrane-bound bi-heme-containing, quinol-oxidizing subunit, a cytoplasmic-facing Fe-S cluster-containing subunit, and a Moco-containing catalytic subunit [49]. NAR enzymes (*nar* genes) are associated with both denitrification and dissimilatory nitrate reduction to ammonium (DNRA) [48, 49] and contribute to the generation of proton-motive force (PMF) for ATP production.

Denitrification converts nitrate ($NO_3^-$) to dinitrogen gas ($N_2$) with nitric oxide (NO) and nitrous oxide ($N_2O$) as intermediates, with the participation of downstream nitrite reductases (*nir* genes) [57]. Denitrification plays a prominent role in the terrestrial nitrogen cycle, as $N_2O$ is easily lost from the soil [58] and can act as a potent greenhouse gas. Denitrification is responsible for large global losses of fixed nitrogen (which limits primary productivity), with some aquatic oxygen minimal zones producing >90% of total $N_2$ emissions through this process [58]. Denitrifying bacteria, belonging primarily to the proteobacterial group, are commonly found in activated sludge from sewage treatment plants [59]. Denitrification also plays an important role in removing human-derived inorganic nitrogen from aquatic environments such as estuaries [57], reducing the potential for harmful eutrophication.

DNRA involves the Moco-dependent reduction of nitrate to ammonium, with nitrite as an intermediate [49], mediated by the *nrf* genes. Ammonium produced by DNRA is retained in terrestrial and aquatic environments where

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it can be assimilated into plant biomass and promote primary productivity [58]. In certain terrestrial ecosystems DNRA is responsible for more than 50% of the turnover of the nitrate pool [58]. Dissimilatory nitrate reduction is also prominent in the human intestinal microbiota. Dietary nitrate can be utilized as an electron acceptor by enteric commensal bacteria such as *E. coli* and *Lactobacillus* sp. [60]. In addition, nitrate-respiring, facultative anaerobes can gain a competitive advantage in the inflamed GI tract by utilizing reactive nitrogen compounds originating from the host inflammatory response [61].

The third and final class of Moco-dependent nitrate reductases are the dissimilatory periplasmic nitrate reductases (EC 1.9.6.1, NAP) [62]. NAP enzymes primarily eliminate excess reducing agents ($NADH/FADH_2$) in the bacterial cytoplasm through their redox reactions in the periplasm [48], and therefore do not contribute to the PMF. Periplasmic nitrate reductases utilize the quinol pool as electron donors and are usually composed of a heterodimeric structure containing a Moco catalytic subunit and a cytochrome *c*/bi-heme subunit [49].

### DMSO reductase

DMSO is a component of the biological sulfur cycle that can be produced via photooxidation of dimethyl sulfide (DMS) in the atmosphere [63]. DMS is produced by natural sources such as marine algae, plants, and soil [64] and is also released by industrial point sources such as paper mills [63]. DMS is hypothesized to play a significant role in the regulation of global temperature, as sulfate aerosols resulting from DMS oxidation can act as cloud condensation nuclei [65]. Increases in these nuclei result in increased cloud albedo, which can decrease solar energy penetration, reduce global temperatures, and decrease primary productivity.

DMSO reductase enzymes (EC 1.8.5.3) are a major source of global DMS, as they directly catalyze the reduction of DMSO to DMS. Early research hypothesized that nitrate reductase enzymes were responsible for this activity, but subsequent work demonstrated that DMSO reductase was a distinct enzyme complex [66]. DMSO reductase enzymes contain a single or dual MGD cofactor [30, 67] and exhibit a similar electron flow mechanism [67] as described above. Two electrons are donated from NADH or quinol, passed to a molybdate ion, and eventually to DMSO after transfer through additional redox-active centers such as cytochrome *c* or Fe-S clusters. Molydenum is anchored in the active site by MGD thiol groups and cysteine/serine residues from the apoenzyme [67, 68].

DMSO reductase activity has been observed in cell lysates from *E. coli*, *Klebsiella* sp, *P. aeruginosa*, *Bacillus subtilis*, and *Proteus* sp. [69, 70]. DMSO can act as an electron acceptor for anaerobic growth in several Proteobacteria [71], including the photosynthetic purple nonsulfur bacteria *Rhodopseudomonas capsulata* [72]. DMSO reduction can be coupled to an outward flow of protons, creating a PMF that can be utilized for ATP production [73]. DMSO reductase enzymes are membrane-anchored membrane complexes [74], that are often composed of distinct Moco-catalytic, cofactor/Fe-S cluster-binding, and membrane-anchored subunits that are encoded by the *dms* operon [75].

## Pterin cofactors

Molybdopterins play an essential role in the function of several important molybdoenzymes. However, outside of their role as a moiety of the Moco coenzyme functioning as a redox cofactor, pterins themselves play critical roles in other bacterial processes, both enzymatic and non-enzymatic. These processes span diverse bacterial taxa and have important functions in a variety of ecosystems and environmental niches.

### Cyanide utilization

Cyanides are compounds which contain a cyano moiety, with a triple bonded carbon and nitrogen ($C\equiv N^-$). Cyanides are naturally produced by a wide variety of plants and fungi [76], and in large quantities from industrial processes such as electroplating, mining, and acrylic fiber production [77]. The toxicity of cyanide arises from its ability to disrupt electron transport chains via inhibition of cytochrome *c* oxidase [78]. High levels of toxic cyanide compounds can have profound effects on fish, bird, and mammalian populations [77]. Therefore, the biological breakdown of cyanide is an attractive bioremediation strategy in comparison to chemical remediation approaches that produce harmful by-products. Enzymes responsible for cyanide conversion utilize pterin cofactors.

Microbial cyanide utilization was first reported for a sewage isolate and subsequently characterized for *Pseudomonas fluorescens* isolates from river mud. These isolates converted KCN to ammonia ($NH_3$) before assimilation into biomass [79, 80]. Cyanide utilization required NADH and was primarily an aerobic process [80]. Species

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of *Burkholderia* and *Bacillus* have also been reported utilize a wide array of cyanide compounds (potassium thiocyanide, aliphatic and aromatic nitriles, etc. [81, 82]).

Almost 40 years after the discovery of bacterial cyanide degradation, it was demonstrated that cyanide oxygenase was responsible for most, if not all, of cyanide degradation in *P. fluorescens* [83]. Cyanide oxygenase, with the contribution of a yet-unidentified dehydrogenase protein, converts cyanide compounds to $NH_3$ and $CO_2$. The enzyme is cytoplasmically localized and requires both oxygen and NADH for optimal activity. Additional biochemical characterization led to the finding that a pterin cofactor was required for the *P. fluorescens* cyanide oxygenase activity [84]. The pterin requirement seems to be structurally flexible, as BPt, MPt, or NPt species proved to be equivalent cofactors [85]. Recent findings have demonstrated that cyanide oxygenase activity in *P. fluorescens* is actually a multienzyme complex composed of four distinct activities: NADH oxidase (EC 1.6.3.3), NADH peroxidase (EC 1.11.1.1), cyanide dihydratase (EC 4.2.1.66), and carbonic anhydrase (EC 4.2.1.1) [86]. Currently, a detailed structure of cyanide oxygenase is not available, and therefore outstanding questions exist on the precise enzymatic mechanisms, functions of the individual complex members, and the role of the pterin cofactor.

## Aromatic amino acid hydroxylases

Aromatic amino acid hydroxylases are widespread enzymes with several unique roles in both prokaryotic and eukaryotic organisms. In animals, tryptophan and tyrosine hydroxylases are required for the synthesis of the neurotransmitters serotonin [87] and dopamine [12], respectively. Phenylalanine hydroxylase deficiency is associated with the human metabolic disorder phenylketonuria [88], in which excess phenylalanine can cause severe intellectual disabilities. In bacteria, phenylalanine and tryptophan hydroxylases have diverse roles, several of which are examined below.

### Phenylalanine hydroxylase

Phenylalanine hydroxylase (EC 1.14.16.1, PAH) catalyzes the enzymatic conversion of phenylalanine to tyrosine. In species of *Pseudomonas*, phenylalanine can be used as a sole carbon source, partly through the activity of PAH which converts it to tyrosine [89]. This enzymatic reaction requires a tetrahydropteridine cofactor and provided one of the earliest, if not the first, demonstrations of a pteridine requirement for the activity of a microbial enzyme. Purification of PAH showed that $Fe^{2+}$ and several other divalent cations ($Hg^{2+}$, $Cd^{2+}$, and $Cu^{2+}$) were required for the activity of the enzyme *in vitro* [90]. These studies were among the first to describe L-threoneopterin, which is structurally identical to L-monapterin, as an enzymatic cofactor [91].

Additional research has described phenylalanine hydroxylase activities from a variety of diverse proteobacterial species including *Chromobacterium* sp., *Vibrio* sp., and *Legionella pneumophila* [92–95]. All described PAHs described thus far have been single subunit proteins with an absolute requirement for a tetrahydropterin cofactor. The exact chemical identity of this pterin cofactor is flexible, as MPt, dimethyl pterin, and BPt have all been reported to be sufficient for proper enzymatic activity [24, 96, 97]. The pterin cofactor is coordinated in the enzymatic active site by a network of hydrogen bonds and a conserved aspartate residue on the cognate protein [98]. This aspartate residue is hypothesized to be essential for maintaining proper orientation of the cofactor to support productive catalysis.

In the human pathogen *L. pneumophila*, phenylalanine hydroxylase has been shown to play a role in the production of pyomelanin [95]. Pyomelanin is a pigment that has been shown to be important in virulence, protection from reactive oxygen species, and iron metabolism [99, 100]. Like many of the PAH enzymes mentioned above, *L. pneumophila* PAH is required for growth on media lacking tyrosine and absolutely requires iron for detectable enzymatic activity. The requirement of the *P. aeruginosa* PAH for the tetrahydromonapterin ($H_4$-MPt) cofactor has been utilized to develop a clever genetic assay in an *E. coli* tyrosine auxotroph, in which PAH conversion of phenylalanine is the only source of tyrosine [24]. Mutations that block production of the MPt cofactor at any step prevent PAH activity and these strains therefore require exogenous tyrosine to grow.

### Tryptophan hydroxylase

Tryptophan hydroxylase (EC 1.14.16.4) catalyzes the enzymatic conversion of tryptophan to 5-hydroxytryptophan. The only bacterial tryptophan hydroxylase that has been described is from the β-proteobacteria *Chromobacterium violaceum* [101]. The hydroxylation reaction was shown to be specific to tryptophan [102]. Partial purification of the enzyme demonstrated that enzymatic activity was dependent on the presence of a tetrahydropterin species, with both NPt and BPt proving sufficient for proper hydroxylase activity [103].

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The production of 5-hydroxytryptophan is an intermediate in the synthesis of the pigment violacein [104]. In bacteria, violacein is utilized as a bacterial self-defense mechanism against predators or competitors, having strong toxicity against both nematodes and Gram-positive bacteria [105]. Violacein is also of interest for its potential use as a cancer therapeutic [106]. It is currently unknown whether tryptophan hydroxylase enzymes exist in other bacterial species and what other biological roles this enzyme or its products may possess.

## Cyanobacterial pterins

Cyanobacteria are an essential component of life on earth, with marine phytoplankton accounting for approximately half of global primary productivity [107]. As mentioned earlier in this review, the first reports of bacterial pterins were from cyanobacterial species such as *Anacystis*, *Anabaena*, and *Nostoc* [19, 20]. More recent biochemical characterizations have isolated novel pterin derivatives such as 6-threo BPt [108] and previously uncharacterized pterin glycosides, pterin forms which are restricted almost exclusively to cyanobacteria and green-sulfur bacteria [109–111]. Despite these discoveries, sparse evidence exists describing the function of pterin molecules in cyanobacteria.

One of the first demonstrations of the role of pterins in cyanobacteria was an experiment screening different cyanobacterial isolates for resistance to UV-A radiation [112]. This approach revealed an *Oscillatoria* species that exhibited similar growth rates under both UV-A irradiation and white light exposure. Organic extractions led to the isolation of a BPt glucoside (Figure 1), of which intracellular concentrations were shown to greatly increase shortly following the initiation of UV exposure. The authors hypothesized that the BPt glucoside may act as a photoprotective pigment that can shield the photosynthetic apparatus, which is damaged by UV light. Similarly, in the cyanobacteria *Spirulina platensis*, a biopterin glucoside was shown to protect the components

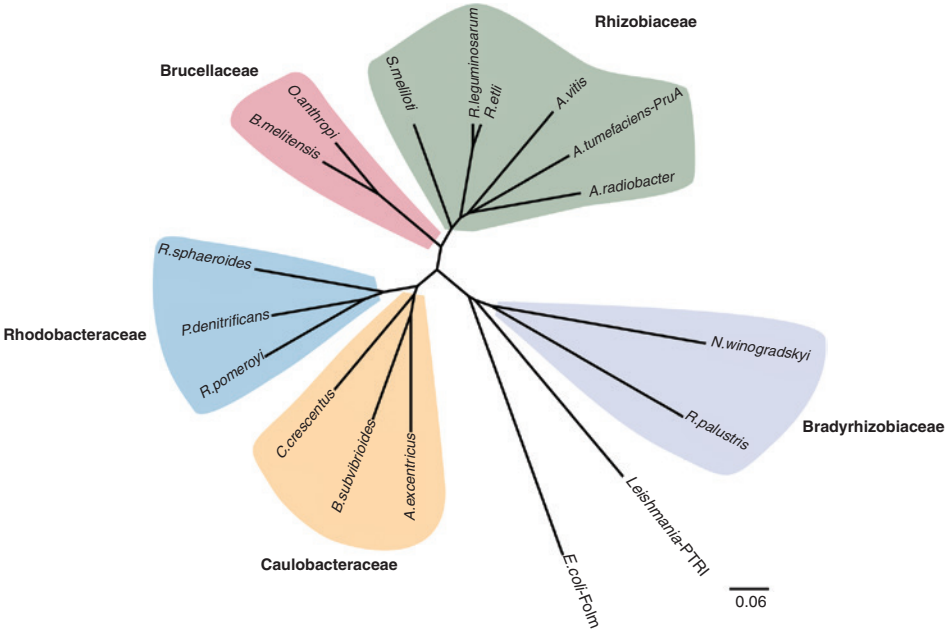


**Figure 3:** PruA/FolM phylogenetic tree. *Agrobacterium tumefaciens pruA* aligned to short chain-dehydrogenase orthologs from other *Rhizobiales* and Alphaproteobacterial species. Pteridine reductase homologs limited to protein sequences that contain characteristic YxxxK catalytic motif. Top protein BLAST hit for each species (querying with *A. tumefaciens pruA*) was used in alignment. Bacterial families highlighted by colored wedges. Alignments and phylogenetic tree construction performed using Clustal X 2.1 software [121]. Phylogenetic tree visualized using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Scale bar represents amino acid substitutions per site. Orthologous genes listed below: *Agrobacterium radiobacter* K84 Arad_1713, *Agrobacterium vitis* Avi_1559, *Rhizobium leguminosarum* Rleg_1233, *Rhizobium etli* RHE_RS07550., *Ruegeria pomeroyi* Spo3638 , *Sinorhizobium meliloti* SMc00603 , *Brucella melitensis biovar abortus BAB1_0721*, *Ochrobactrum anthropi* AYJ56_12940, *Caulobacter crescentus* CC0417, *Brevundimonas subvibrioides* Bresu_0918, *Asticcacaulis excentricus* Astex_1609, *Nitrobacter winogradskyi* Nwi_2393, *Rhodopseudomonas palustris* WP_044413957.1, *Paracoccus denitrificans* Pden_2311, and *Rhodobacter sphaeroides* Rsph17025_2858. *Escherichia coli FolM* and *Leishmania major* PTR1 used as outgroups.

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of photosynthetic vesicles (chlorophyll *a*, phycocyanin, and carotenoids) from UV exposure [113, 114]. BPt glucosides are synthesized by the specific activity of BPt glucosyltransferase (EC 2.4.1, BGluT) enzymes utilizing tetrahydrobiopterin ($H_4$BPt) and UDP-glucose precursors (Figure 2) [115].

A more recent report elucidated the role of cyanopterin in the phototactic response to blue and near-UV light [116]. Cyanopterin is a pterin glycoside (Figure 1) that can be synthesized from either a MPt or a NPt precursor (Figure 2) that was first discovered in the freshwater cyanobacteria *Synechocystis* sp. PCC 6803 [109]. *Synechocystis* normally exhibits phototactic behavior towards blue light and no net movement in response to UV light. Deletion of *pgtA*, a gene shown previously to be required for cyanopterin synthesis [117], diminished movement towards blue light and induced movement away from UV light [116]. These results led to the conclusion that cyanopterin may be one of the main cyanobacteiral chromophores involved in the photoreception of light in the 280–460 nm range. This role is perhaps not surprising, as pterins have previously been implicated in plant photoreception [118].

## Pterin signaling

Despite evidence in eukaryotic systems, up until recently, pterins had not been reported to play regulatory roles in bacteria. We have changed this view with a recent report on the influence of pterin metabolism in the regulation of biofilm formation and polysaccharide production in the plant pathogen *Agrobacterium tumefaciens* [119]. In *A. tumefaciens* and many other species, surface-associated phenotypes are controlled by the intracellular concentrations of the second messenger cyclic-diguanylate-monophosphate (c-di-GMP) [120]. We found that the *A. tumefaciens* protein diguanylate cyclase-phosphodiesterase A (DcpA), via discrete diguanylate cyclase (EC 2.7.7.65, DGC) and phosphodiesterase (EC 3.1.4.52, PDE) domains, is able to both synthesize and degrade c-di-GMP, respectively. The catalytic activity of DcpA is controlled by the activity of the pteridine reductase (EC 1.5.1.33) PruA, which is widely conserved in a variety of diverse α-proteobacteria (Figure 3). Ectopic expression of *pruA* homologs, such as *E.* coli *folM*, in *A. tumefaciens* is sufficient for the regulation of DcpA. We found that PruA reduced dihydromonapterin to tetrahydromonapterin and plays a role in the synthesis of the novel pterin 2′-O-methylmonapterin (Figures 1 and 2) [119].

It is hypothesized that a putative pterin receptor PruR, through association with pterins, links PruA pterin synthesis to the control of DcpA enzymatic activity (Figure 4). Preliminary data support the hypothesis that PruR resides in the periplasm, where it may interact with excreted pterins. Pterin excretion has been observed in *E. coli* species [24], and it is possible that a subset of PruA-synthesized pterins transit to the *A. tumefaciens* periplasmic space, where they could interact with PruR, and perhaps mediate a response to extracellular stimuli.

Another intriguing aspect of the PruA-PruR-DcpA regulatory cascade is the possible role of osmotic stress on this pathway. Folate molecules have been shown to bind to osmolytes in solution, with the pterin ring mediating this interaction [122]. In addition, the activity of *E. coli*

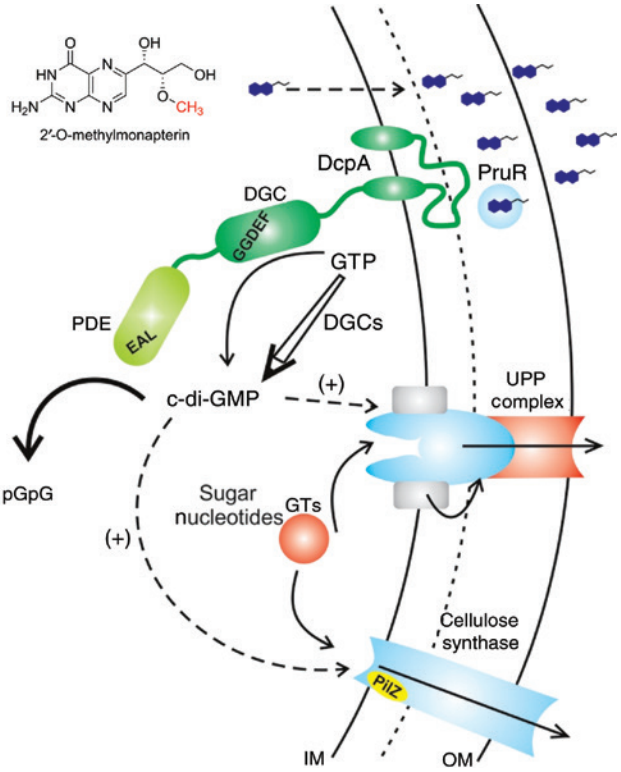


**Figure 4:** Model of *Agrobacterium tumefaciens* pterin-mediated control of c-di-GMP metabolism. DcpA DGC-PDE activity in *A. tumefaciens* is controlled by the combined action of PruA pterin synthesis and the putative pterin-binding capacity of PruR. A subset of intracellular pterins may be exported into the periplasm where they are hypothesized to interact with PruR, possibly working in concert to sense and transduce an environmental signal. This signaling cascade eventually controls the switch between DcpA c-di-GMP synthesizing and degrading activities. The c-di-GMP signal has been shown to control the levels of the *A. tumefaciens* UPP by an unidentified mechanism. Figure modified from Feirer et al. [119]. DGC, diguanylate cyclase; PDE, phosphodiesterase; UPP, unipolar polysaccharide adhesin.

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pteridine reductase FolM is diminished in the presence of a variety of osmoloytes [123]. It is possible that osmotic stress, manifesting in the alteration of the level of certain osmolytes, feeds into the regulation of the pterin-mediated DcpA regulatory circuit either at the level of PruA activity or pterin availability.

Few, if any, examples exist of pterin derivatives modifying enzymatic activity in an allosteric fashion. A recent report [124] has demonstrated that the activity of the *Vibrio cholerae* dinucleotide cyclase DncV is modulated *in vitro* by the pterin derivative 5-methyltetrahydrofolate diglutamate. This folate derivative binds in a pocket opposite the active site and inhibits the synthesis of c-GMP-AMP (cGAMP) by DncV. DncV and cGAMP syntheses have been shown to play a role in *V. cholerae* virulence [125].

## Conclusions

Pterins play diverse roles in a wide range of different bacteria. Perhaps the best described is the activity of pteridine molecules as enzymatic cofactors. Pterin-associated redox enzymes play an important part in global nutrient cycles, atmospheric composition, and several forms of autotrophic and heterotrophic metabolism. The diversity of pterin structure in bacteria is notable, with unconjugated and conjugated pteridine derivatives common throughout bacterial phyla. Pterins also exhibit diverse roles as cofactors for nonredox enzymes. These enzymes, such as cyanide oxygenase, phenylalanine hydroxylase, and tryptophan hydroxylase, allow bacteria to utilize alternate carbon and nitrogen sources and produce various pigments, both processes that can greatly affect bacterial fitness. The prospect of future pterin research is exciting, as these discoveries could have considerable implications on the understanding of bacterial pathogenesis, metabolism, bioremediation, and signaling.

The conservation of pterin molecules throughout the tree of life leads to the strong possibility of novel roles being described for these ubiquitous molecules. This is especially true in the prokaryotes, where the number of different pterin species currently characterized vastly outnumber their ascribed biological roles. An excellent example is in the cyanobacteria, home to perhaps the greatest variety of pterin structures with only a few reported molecular or cellular functions. Future work should aim to correlate specific pterin species with distinct phenotypic outputs. Combining phenotypic analysis with comprehensive pterin profiling will allow for a more complete understanding of the role of pterins in bacterial physiology, metabolism, and environmental interactions.

The biological function of extracellular or excreted pterins is another outstanding question. Pterin molecules are often found in the extracellular space, but their roles in this environment are unknown. The mechanism of pterin excretion, the pterin chemistry outside the cell, and the regulation of these processes are entirely unstudied. Further insights into the roles of extracellular pterins in nutrient acquisition, osmoprotection, antibacterial competition, or mediation of host-pathogen interactions await future research.

Finally, the concept of pterins functioning in a signaling capacity is intriguing. As alluded to above, pterin molecules may be able to act as cellular sensors of environmental pertubations such as osmotic or oxidative stress. These pterin-transduced signals are likely to involve pterin-binding proteins that can then interact with downstream signaling cascades. Thus, pterin-mediated signaling pathways may be important in bacterial lifestyle transitions such as biofilm formation or the transition from an environmental reservoir to a mammalian host, both of which are known to involve osmotic and oxidative challenges. It is also possible that pterins may be able to exert a signaling capacity by binding directly to enzymes and influencing their activity. We look forward to these and other exciting discoveries into new roles of pterins in bacteria.

**Acknowledgments:** Research on bacterial pterins was supported by the National Institutes of Health (NIH) grant no. GM080546 (C.F.) and the Indiana University Genetics, Molecular and Cellular Sciences NIH Training grant no. T32-GM007757 (N.F.).

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