

Polyamine function in archaea and bacteria

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Most of the phylogenetic diversity of life is found in bacteria and archaea, and is reflected in the diverse metabolism and functions of bacterial and archaeal polyamines. The polyamine spermidine was probably present in the last universal common ancestor, and polyamines are known to be necessary for critical physiological functions in bacteria, such as growth, biofilm formation, and other surface behaviors, and production of natural products, such as siderophores. There is also phylogenetic diversity of function, indicated by the role of polyamines in planktonic growth of different species, ranging from absolutely essential to entirely dispensable. However, the cellular molecular mechanisms responsible for polyamine function in bacterial growth are almost entirely unknown. In contrast, the molecular mechanisms of essential polyamine functions in archaea are better understood: covalent modification by polyamines of translation factor aIF5A and the agmatine modification of tRNA^{Ile}. As with bacterial hyperthermophiles, archaeal thermophiles require long-chain and branched polyamines for growth at high temperatures. For bacterial species in which polyamines are essential for growth, it is still unknown whether the molecular mechanisms underpinning polyamine function involve covalent or noncovalent interactions. Understanding the cellular molecular mechanisms of polyamine function in bacterial growth and physiology remains one of the great challenges for future polyamine research.

Polyamines (Fig. 1) are a relatively overlooked component of the bacterial and archaeal metabolomes. This is due to several factors, including the patchwork phylogenetic distribution of any specific polyamine, the nontrivial problem of their detection and quantification, and the dearth of knowledge in bacteria about any molecular mechanisms that polyamines are involved in. Consequently, the terms “enigmatic” and “mysterious” occasionally decorate the titles of polyamine papers, yet the polyamine spermidine was almost certainly present in the last universal common ancestor of life (LUCA),² because LUCA likely encoded spermidine synthase (1). The extent to which polyamine functions are selected for by evolution can be inferred from the fact that two entirely independent biosynthetic path-

ways exist for spermidine production; similarly, two distinct, independent pathways exist for homospermidine biosynthesis, and polyamine biosynthetic enzyme arginine decarboxylase has convergently evolved from four different protein folds (2). This minireview will discuss polyamine function in archaea and bacteria but will not discuss production of agmatine, putrescine, or cadaverine by acid-inducible basic amino acid decarboxylases (2, 3) or the agmatine deiminase system that takes up exogenous agmatine and exports putrescine (4). It will not, for the most part, cover inferred functions of polyamines determined from observations of *in vitro* biochemical behaviors of polyamines in binding RNA and DNA and other macromolecules. To highlight the phylogenetically narrow scope of current published polyamine function studies in bacteria, the host phylum of the various bacterial species discussed is indicated.

Archaea

Archaea (Archaeobacteria) were formerly viewed as extremophile bacteria until they were unveiled as the third domain of life by Woese and Fox (5). They differ from bacteria in having isoprene lipids conjugated by ether bonds to glycerol-1-phosphate in their membranes, they lack peptidoglycan in their cell walls, and their informational proteins (e.g. those involved in transcription and translation) are more similar to eukaryotes than to bacteria (6). Recently, culture-independent sequencing approaches have greatly expanded the known phylogenetic diversity of archaea, with many new phylum-level lineages being discovered in diverse habitats (6–8). The lifestyles of archaea (e.g. hyperthermophiles, methanogens, and halophiles) do not necessarily reflect phylogeny but have been a useful descriptor before specific molecular phylogenetic attributions were possible.

The distribution of individual polyamines (Fig. 1) among archaeal groups is distinctive. Some of the earliest observations about polyamines in archaea were that they are absent in halophiles (9–11). It was also noted that the halophile *Halobacterium halobium* was unable to take up exogenous putrescine (9), but cell extracts of *H. halobium* and *Halococcus morrhuae* were able to produce agmatine from added arginine (11). Polyamines can be described by the number of methylene carbons between amine groups (e.g. putrescine is represented by [4] and spermidine by [34]). Analysis of diverse hyperthermophilic, acidophilic, and thermoacidophilic archaea found a variety of linear polyamines, including norspermidine [33], spermidine [34], homospermidine [44], norspermine [333], spermine [343], thermospermine [334], caldopentamine [3333], homocaldopentamine [3334], thermopentamine [3343], and caldohexamine [33333], and quarternary branched pentaamine *N*⁴-bis

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² The abbreviations used are: LUCA, last universal common ancestor of life; DHS, deoxyhypusine synthase; SAM, S-adenosylmethionine.

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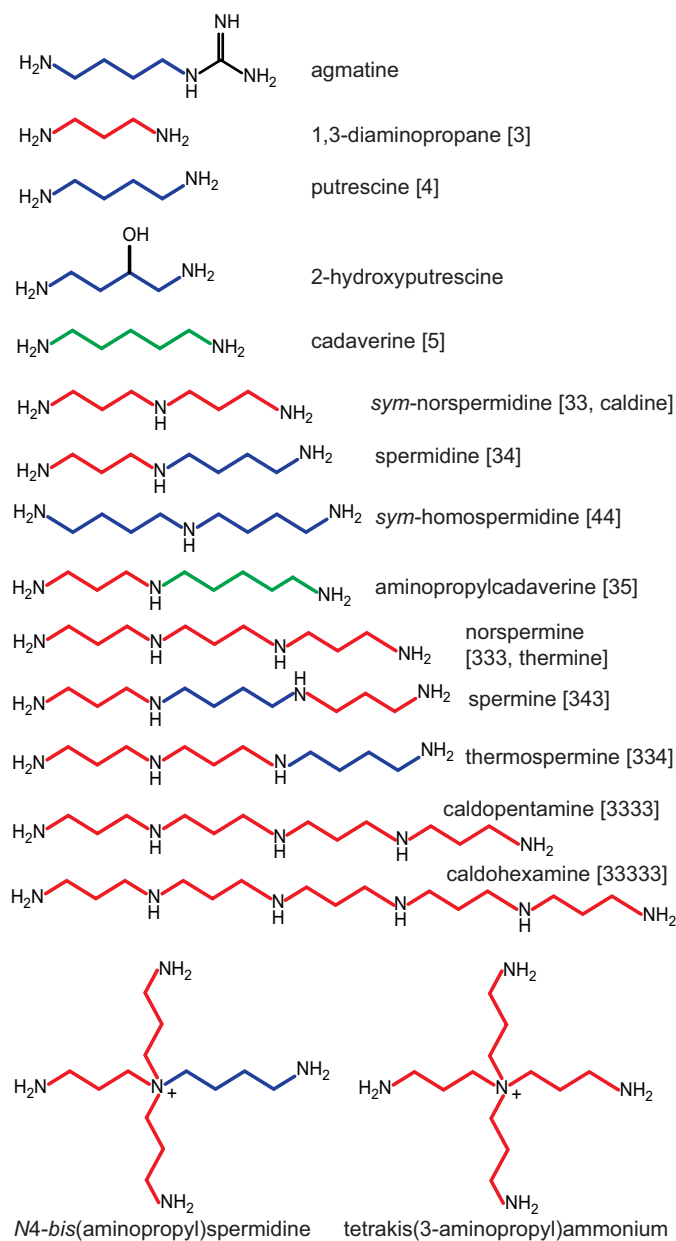


Figure 1. Some of the linear and branched polyamines found in bacteria and archaea. Blue, aminobutyl; red, aminopropyl; green, aminopentyl. Numbers in parentheses refer to the number of methylene groups between amine groups.

(aminopropyl)spermidine (12) (Fig. 1). Some methanogens contain only homospermidine and putrescine, the majority contain only spermidine, and some contain both homospermidine and spermidine (13). This diversity of polyamine structures and phylogenetic distribution suggests lifestyle-related functions for different polyamines. However, some core conserved functions of polyamines in archaea can be discerned.

Deoxyhypusine/hypusine modification of translation factor aIF5A

A common feature of archaea is the presence of the polyamine-derived deoxyhypusine/hypusine modification of translation elongation factor aIF5A (14). In eukaryotes, the aminobutyl moiety of spermidine is transferred by deoxyhypusine

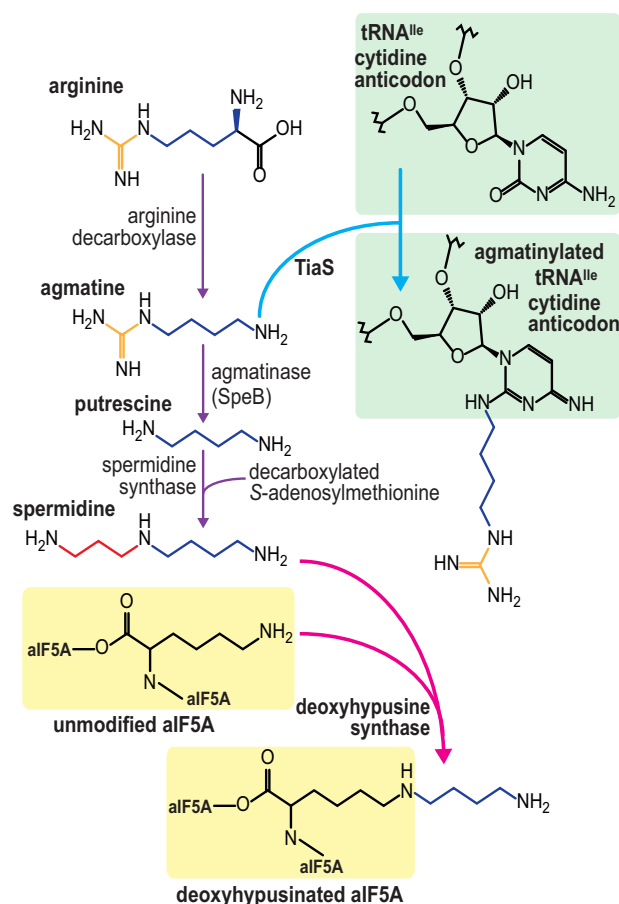


Figure 2. The pathways to tRNA^{Ile} and aIF5A modification by polyamines in archaea. TiaS, tRNA(isoleucine)-agmatidine(2)C synthetase.

synthase (DHS) to a single lysine residue in eIF5A to form deoxyhypusine, which, after hydroxylation by deoxyhypusine hydroxylase, forms the hypusine post-translational modification (15–17). Hypusine modification of eIF5A is required for translation of mRNAs encoding polyproline tracts that would otherwise cause ribosome stalling and translational arrest (18). DHS is essential for growth of eukaryotes as phylogenetically distant as budding yeast (19), mouse (20), and trypanosomatid parasites (21, 22). Diverse archaea have been shown to contain either deoxyhypusine- or hypusine-modified aIF5A (14, 23, 24). Analysis of archaeal genomes by BLASTP indicates that all archaea are likely to encode DHS. Inhibition of the thermoacidophilic crenarchaeote *Sulfolobus acidocaldarius* DHS by N¹-guanyl-1,7-diaminoheptane led to cell cycle arrest (25). Although deoxyhypusine formation in eukaryotes depends on spermidine as an aminobutyl group donor (15), and this is likely to apply to most archaea (Fig. 2), the mechanism of deoxyhypusine formation in halophiles does not depend on spermidine. Halophiles do not accumulate either spermidine or putrescine; however, they do accumulate agmatine. An agmatinase-like gene (agmatinase converts agmatine to putrescine) is necessary for deoxyhypusine formation in *Haloferax volcanii*, and only deoxyhypusine, and not hypusine, is detected in aIF5A (26). The *H. volcanii* agmatinase-like gene is essential for growth even though putrescine and spermidine are not accumulated. It was suggested that the aminobutyl moiety of deoxyhypusine in

H. volcanii might be derived from putrescine or from agmatine that is transferred to aIF5A and the guanidino group subsequently released to form deoxyhypusine by the action of the agmatinase-like enzyme. Currently, it is not certain whether putrescine is required for growth of halophiles, and, as mentioned above, the halophile *H. halobium* was unable to take up exogenous putrescine (9).

In contrast, the recombinant DHS of the hyperthermophilic euryarchaeote *Thermococcus kodakarensis* transfers the aminobutyl group of spermidine to *T. kodakarensis* aIF5A to form deoxyhypusinated aIF5A (26) (Fig. 2). Although spermidine is required for deoxyhypusine formation in *T. kodakarensis* (26), putrescine is not required for growth because spermidine is synthesized from agmatine via aminopropylagmatine rather than putrescine (27). Some methanogens, in particular the *Methanosarcinaceae*, accumulate only homospermidine rather than spermidine (13). Homospermidine contains two aminobutyl groups, and the human DHS can use homospermidine to donate an aminobutyl group to eIF5A (28). Intriguingly, homospermidine-accumulating members of the *Methanosarcinaceae* are capable of nitrogen fixation (29), and recently homospermidine biosynthesis was shown to be essential for normal diazotrophic (nitrogen-fixing) growth of the filamentous cyanobacterium *Anabaena* (30). Spermidine is therefore dispensable for growth in archaeal halophiles and some methanogens.

Agmatinylation of tRNA^{lle}

In most but not all archaea, agmatine is transferred to tRNA^{lle} to form the covalent modification known as agmatidine (2-agmatinylcytidine) on the cytidine of the anticodon CAT (31, 32) (Fig. 2). This modification, performed by the enzyme TiaS, is required for the discrimination of isoleucine and methionine codons and is essential for growth. The enzyme TiaS is also essential for growth (33). A few archaeal species, including *Candidatus Korarchaeum cryptofilum* OPF8 and *Nanoarchaeum equitans* do not encode a TiaS homologue and instead encode tRNA^{lle} genes with TAT anticodons (34). In the vast majority of archaea, it is likely that agmatine, specifically, will be necessary for growth due to the need for the agmatidine modification of tRNA^{lle}. The role of agmatine in agmatinylation explains why agmatine but not putrescine is essential for growth of *T. kodakarensis* (35) and why agmatine is the only polyamine accumulated in some extreme halophiles (36). An equivalent modification of tRNA^{lle} in bacteria is achieved by covalent attachment of lysine (37) by the enzyme TilS (38), a nonhomologous equivalent of TiaS that has arisen by convergent evolution (34). Due to the role of agmatine in agmatinylation of tRNA^{lle}, provision of agmatine by arginine decarboxylase is particularly important in archaea. A pyruvoyl-dependent arginine decarboxylase is present in Euryarchaeota (39), but this gene has been lost in Crenarchaeota, and instead a SAM decarboxylase paralogue has evolved to decarboxylate arginine (40). The covalent incorporation of agmatine into agmatidine in tRNA^{lle}, and the aminobutyl group of spermidine or homospermidine into deoxyhypusine in aIF5A means that agmatine and spermidine/homospermidine are independently essential for growth in most archaea.

Long-chain and branched polyamines in hyperthermophilic archaea

The diverse roles of polyamines in archaea are best exemplified by the difference in polyamine content between hyperthermophiles and halophiles. Disruption of the gene encoding the agmatine aminopropyltransferase of the hyperthermophile *T. kodakarensis* (27) resulted in depletion of spermidine and branched-chain polyamines (*N*⁴-aminopropylspermidine and *N*⁴-bis(aminopropyl)spermidine, originally misassigned as spermine and *N*⁴-aminopropylspermidine) and severe growth defects at 85 °C, and even more so at 93 °C, that could be slightly reversed at 85 °C but not at 93 °C by supplying spermidine. If only the branched-chain polyamines are eliminated by deletion of the branched-chain aminopropyltransferase BspA, growth at 93 °C is abolished, but some growth can be restored by provision of 1 mM *N*⁴-bis(aminopropyl)spermidine (41). These findings establish the essential role of branched polyamines in high-temperature growth of *T. kodakarensis*. *In vitro* biochemical studies indicate that both linear long-chain polyamines and branched polyamines induce structural changes to DNA that are proposed to facilitate growth at extreme temperatures (42, 43).

Absence of polyamines in halophilic archaea

In notable contrast to the exotic polyamine content of hyperthermophiles that, in addition to branched polyamines, can contain long linear chain polyamines, such as caldohexamine featuring five aminopropyl group additions (12), halophilic archaea contain only agmatine. The genes encoding the spermidine biosynthetic enzymes SAM decarboxylase and spermidine synthase have been lost from halophiles. Archaeal halophiles grow in up to 3 M external NaCl by using a salt-in strategy, accumulating in their cytosol millimolar quantities of KCl (44). An interesting question then is whether polyamines have been lost from halophiles because they are not capable of performing their usual functions in high KCl or whether high KCl renders the noncovalent function of polyamines superfluous. Certainly, halophiles have evolved to supply an aminobutyl group for deoxyhypusine formation without the participation of spermidine or homospermidine (26).

In conclusion, agmatine and agmatidine formation are essential for growth in most but not all archaea, and agmatine is required for spermidine biosynthesis (Fig. 2). Agmatine or possibly putrescine is required for the essential deoxyhypusine modification of aIF5A in halophiles. Spermidine or homospermidine are essential in most archaea except halophiles for deoxyhypusine formation. Finally, long-chain and branched-chain polyamines are essential for growth at very high temperatures. Whether spermidine has a noncovalent role in archaeal physiology is not known, and due to its role in deoxyhypusine formation and long- and branched-polyamine biosynthesis, it will be technically and conceptually challenging to address this question. It also remains to be proven that the role of deoxyhypusinated aIF5A in archaea is the same as that of hypusinated eIF5A in eukaryotes.

Bacteria

Most of the known phylogenetic diversity of life is found in the bacterial domain, and that diversity has significantly expanded recently with the identification of multiple new candidate phyla using culture-independent metagenomic sequencing and single-cell sequencing (8). In addition to the 30 or so traditional phyla, such as Cyanobacteria, Proteobacteria, and Firmicutes, 134 new putative phyla have been identified (8). However, polyamine metabolism and potential function have been analyzed in only a few bacterial species, most extensively in *Escherichia coli* and *Thermus thermophilus*. The identification of polyamines has by definition been limited to culturable species, which represent a very small fraction of total bacteria (45). Much of the analysis of polyamine distribution in bacteria has been performed by the indefatigable Koei Hamana and co-workers (46, 47). Putrescine and spermidine are the most common polyamines in bacteria (48), but the presence of other polyamines has poor correlation with bacterial lifestyles, such as psychrophily, halophily, acidophily, or alkaliphily, except for thermophily, where a strong correlation exists with the presence of long-chain and branched polyamines (47). Some bacteria do not produce polyamines, and one of the earliest identified examples of polyamine auxotrophy was found in the firmicute opportunistic pathogen *Staphylococcus aureus* (49).

Covalent roles

Unlike the roles of agmatine and spermidine in covalently modifying translation factor aIF5A and tRNA^{lle} in archaea, the equivalent modification of the bacterial aIF5A orthologue EF-P uses distinct mechanisms, resulting in β -lysine, 5-aminopentanol, or L-rhamnose modifications to EF-P (50), and tRNA^{lle} is modified by lysine (34). A limited number of cases of polyamines being used covalently have been identified. The diamine cadaverine (Fig. 1) was found to be covalently attached to the cell wall peptidoglycan of the Negativicutes species *Selenomonas ruminantium* (51). Inhibition of the cadaverine-producing enzyme lysine decarboxylase by the specific inhibitor DL- α -difluoromethyllysine prevented cadaverine accumulation and incorporation into peptidoglycan, resulting in cell swelling and severe inhibition of cell growth (52). Cadaverine and spermidine were found to be covalently linked to the cell wall peptidoglycan in the related species *Anaerovibrio lipolytica* (53), and agmatine, putrescine, and cadaverine were found covalently linked to peptidoglycan in other *Selenomonas* species and related species (54). It was presumed that incorporation of polyamines into peptidoglycan increased the rigidity of cell walls, thereby contributing to their essential role in growth. However, the definitive proof of that role would require mutants that accumulate polyamines normally but that cannot covalently link the polyamines to peptidoglycan. There has been no systematic attempt to identify polyamines covalently linked to cellular proteins in bacteria where polyamines are known to be essential for growth.

Roles in cell growth

With growth being the most obvious readout for physiological importance, it was noted nearly 70 years ago that putrescine, spermidine, spermine, and agmatine, but not cadaverine

or ornithine, promoted the growth of opportunistic pathogen γ -proteobacterium *Haemophilus influenzae* (55). Norspermidine was subsequently shown to be more effective than spermidine or homospermidine, and the simple diamine 1,3-diaminopropane also allowed growth (56). Similarly, it was shown that the nonpathogenic β -proteobacterium *Neisseria flava* has an absolute growth requirement for exogenous putrescine (57). The most commonly used model bacterium for studying polyamine metabolism is γ -proteobacterium *E. coli*, and it has served as the primary template for understanding polyamine metabolism in other bacteria (2). It is generally thought that spermidine is the most important polyamine in archaea and eukaryotes due to its role in deoxyhypusine/hypusine formation and in bacteria due to its ubiquitous distribution (48). When Celia and Herb Tabor obtained a Mu transposon-disrupted SAM decarboxylase mutant strain of *E. coli* that was completely deficient in spermidine, the strain still grew at 75% of the WT rate (58). Disruption of all *E. coli* polyamine biosynthesis, including putrescine and cadaverine, reduced aerobic planktonic growth by 40–50% (59, 60). However, the polyamine-deficient strain exhibited an absolute requirement for spermidine for growth under strictly anaerobic conditions. The fact that a spermidine-deficient strain of *E. coli* could still grow at 75% of the WT rate under aerobic conditions suggests that there is not a specific requirement for spermidine *per se* in *E. coli* growth. Both homospermidine and aminopropylcadaverine could replace the role of spermidine in *E. coli* growth, albeit less efficiently (60). Recently, it was found that norspermidine is more efficient at replacing spermidine for growth at 37 °C but that homospermidine is the most efficient triamine at 42 °C (61). These findings indicate that the exact structure, number of methylene groups, and symmetry of the triamine are not critical for function during growth of *E. coli*, suggesting that the general physicochemical attributes (*i.e.* distributed positive charges over a flexible backbone) are more important.

In contrast to the important but not critical role of polyamines in planktonic growth of *E. coli*, polyamine biosynthesis is absolutely essential for growth of opportunistic pathogen γ -proteobacterium *Pseudomonas aeruginosa* PAO1 (62), food-borne pathogen ϵ -proteobacterium *Campylobacter jejuni* (63), plant pathogen α -proteobacterium *Agrobacterium tumefaciens* C58 (64), and plant pathogen β -proteobacterium *Ralstonia solanacearum* (65). Whereas *P. aeruginosa*, *C. jejuni*, and *A. tumefaciens* synthesize spermidine, *R. solanacearum* produces only putrescine and 2-hydroxyputrescine (66) (Fig. 1). The Lyme disease agent spirochete *Borrelia burgdorferi* has lost its polyamine biosynthetic pathway, but genetic abrogation of its PotABCD polyamine uptake transporter abolishes growth in polyamine-free medium (67). Nitrogen-fixing filamentous cyanobacterium *Anabaena* sp. strain 7120 normally accumulates only homospermidine, and genetic elimination of homospermidine biosynthesis abolished diazotrophic (nitrogen-fixing) growth in liquid culture (30). Extreme thermophiles, such as *T. thermophilus*, like archaeal thermophiles, accumulate a variety of long-chain and branched polyamines (reviewed by Oshima (68)). Depletion of long-chain and branched polyamines by deletion of the aminopropylagmatinase gene (*speB*) resulted in a severe growth defect at 78 °C but not at 75 °C (69).

Spermidine essential for growth
<i>Pseudomonas aeruginosa</i> (γ -Proteobacteria) (62)
<i>Campylobacter jejuni</i> (ϵ -Proteobacteria) (63)
<i>Agrobacterium tumefaciens</i> (α -Proteobacteria) (64)
<i>Borrelia burgdorferi</i> (Spirochaetes) (67)
Putrescine essential for growth
<i>Ralstonia solanacearum</i> (β -Proteobacteria) (65)
Homospermidine essential for nitrogen-fixing growth
<i>Anabaena</i> sp. strain 7120 (Cyanobacteria) (30)
Long-chain and branched polyamines essential for growth above 78°C
<i>Thermus thermophilus</i> (Deinococcus-Thermus) (69)
Spermidine essential for anaerobic growth
<i>Escherichia coli</i> (γ -Proteobacteria) (59)
Spermidine required for normal aerobic growth
<i>Escherichia coli</i> (γ -Proteobacteria) (58,59)
<i>Salmonella enterica</i> sv. Typhimurium (γ -Proteobacteria) (72)
<i>Yersinia pestis</i> (γ -Proteobacteria) (71)
<i>Sinorhizobium meliloti</i> (α -Proteobacteria) (74)
<i>Bacteroides dorei</i> (Bacteroidetes) (75)
<i>Bacteroides thetaiotaomicron</i> (Bacteroidetes) (76)
Norspermidine required for normal aerobic growth
<i>Vibrio cholerae</i> (γ -Proteobacteria) (73)
Homospermidine required for normal aerobic growth
<i>Anabaena</i> sp. strain 7120 (Cyanobacteria) (30)
Spermidine required for normal autolysis
<i>Streptococcus pneumoniae</i> (Firmicutes) (78)
Polyamines dispensable for growth
<i>Bacillus subtilis</i> (Firmicutes) (77)
<i>Streptococcus pneumoniae</i> (Firmicutes) (78)
Norspermidine required for/stimulates biofilm formation
<i>Vibrio cholerae</i> (γ -Proteobacteria) (73,81)
Spermidine required for biofilm formation
<i>Bacillus subtilis</i> (Firmicutes) (77,85)
<i>Yersinia pestis</i> (γ -Proteobacteria) (71)
Spermidine biosynthesis inhibits biofilm formation
<i>Agrobacterium tumefaciens</i> (α -Proteobacteria) (88)
<i>Synechocystis</i> sp. strain PCC 6803 (Cyanobacteria) (89)
Acid-inducible-type ornithine decarboxylase inhibits biofilm formation
<i>Shewanella oneidensis</i> (γ -Proteobacteria) (87)
Putrescine required for swarming
<i>Proteus mirabilis</i> (γ -Proteobacteria) (90)
Spermidine required for swarming
<i>Escherichia coli</i> (γ -Proteobacteria) (91)
1,3-diaminopropane required for surface-associated mobility
<i>Acinetobacter baumannii</i> (γ -Proteobacteria) (92)

Figure 3. Polyamine-dependent growth and surfaces behaviors among different bacterial species. Only cases where genetic mutations have been used to confirm polyamine dependence are listed. The host phylum is indicated in parentheses followed by the relevant reference.

The long-chain and branched polyamines of *T. thermophilus* were found to be necessary for the maintenance of the ribosome, tRNA^{His}, and tRNA^{Tyr} structural integrity during growth at high temperatures (70).

In other species investigated, polyamine depletion reduces growth, but nevertheless, polyamines are not essential. A polyamine auxotrophic strain of the bubonic plague agent γ -proteobacterium *Yersinia pestis*, lacking both putrescine and spermidine, grew at 65% of the WT rate (71). Similarly, a sper-

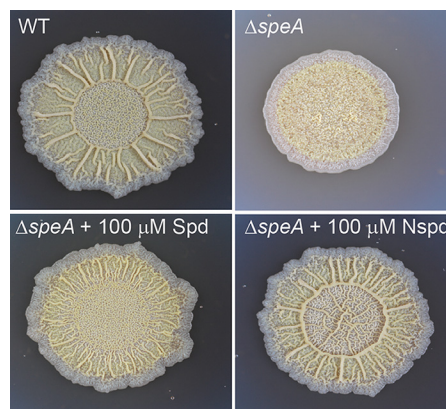


Figure 4. Polyamine dependence of biofilm formation in *B. subtilis*. WT, *B. subtilis* NCIB3610; Δ speA, deletion mutant of arginine decarboxylase; Spd, spermidine; Nspd, norspermidine. Complex colony biofilms were grown on solid MSgg medium. Adapted from Hobley et al. (86).

midine-deficient mutant strain of the food-borne pathogen γ -proteobacterium *Salmonella enterica* serovar Typhimurium grew at 60% of the WT rate (72). Cholera agent γ -proteobacterium *Vibrio cholerae* accumulates norspermidine rather than spermidine, and a norspermidine auxotrophic mutant strain grew at 40–50% of the WT rate (73). The nitrogen-fixing α -proteobacterium *Sinorhizobium meliloti* accumulates putrescine, spermidine, and homospermidine, and a polyamine auxotrophic mutant strain grew at 60% of the WT rate (74). Outside of the Proteobacteria, a polyamine auxotrophic mutant strain of the commensal gut bacterium *Bacteroides dorei* exhibited a severe growth defect (75), and growth was ~50% of WT in a polyamine auxotrophic mutant strain of the key gut microbe *Bacteroides thetaiotaomicron* (76). In stark contrast to the examples discussed above, polyamine-deficient mutant strains of the firmicute species *Bacillus subtilis* and *Streptococcus pneumoniae*, which normally produce spermidine, exhibit no planktonic growth defect in the absence of spermidine (77, 78). Polyamine-dependent growth among different species is listed in Fig. 3.

Roles in biofilm development and other surface behaviors

Biofilms (Fig. 4) are communities of bacterial cells sheathed in a protective matrix of exopolysaccharides, proteins, and DNA, and the biofilm matrix protects the encapsulated cells against environmental insults (79, 80). The first indication that polyamines affected biofilm development was the observation that exogenous norspermidine stimulates biofilm formation of *V. cholerae* via a norspermidine sensor (NspS) that is a homologue of the spermidine transporter substrate-binding protein PotD (81). It was subsequently shown that norspermidine biosynthesis is required for normal biofilm formation in *V. cholerae* (73). Exogenous spermidine was found to inhibit biofilm formation, an effect dependent on the PotD protein (82), whereas inhibition of *V. cholerae* biofilm formation by the tetraamine spermine is achieved via NspS (83). Biofilm formation in *Y. pestis* is also dependent on polyamine biosynthesis, and polyamines are required for expression of the matrix exopolysaccharide biosynthetic genes, although it is not clear whether it is putrescine or spermidine that is the necessary polyamine

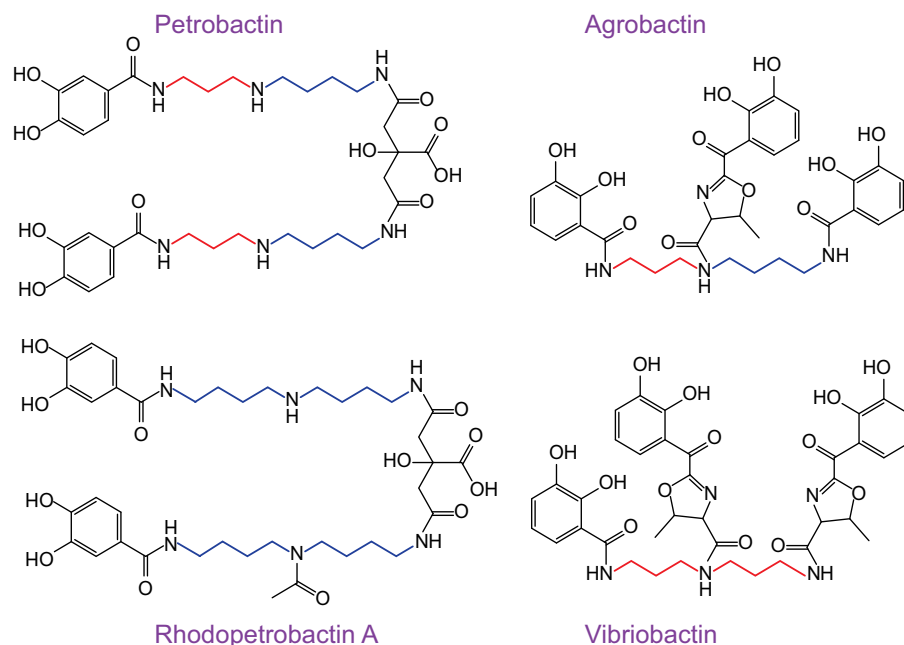


Figure 5. Structures of polyamine-based catecholate siderophores. *Petrobactin*, spermidine; *Rhodopetrobactin A*, homospermidine; *Agrobactin*, spermidine; *Vibriobactin*, norspermidine.

(71, 84). Spermidine biosynthesis is required for robust colony and pellicle biofilm formation in *B. subtilis* (77) (Fig. 4). Exogenous norspermidine is twice as effective as spermidine in promoting *B. subtilis* biofilm formation, but homospermidine is completely ineffective (85). The effect of spermidine on biofilm formation is mediated by activation of the matrix regulator SlrR, although it is likely that the actual target of spermidine is upstream of SlrR (86). In contrast to the stimulatory role of polyamines in biofilm development in some species, in others, depletion of polyamines stimulates biofilm formation. A mutant of the acid-inducible-type ornithine decarboxylase in γ -proteobacterium *Shewanella oneidensis* increased biofilm formation, although it is not known in this case whether endogenous polyamine levels were decreased (87). A partially spermidine-depleted biosynthetic ornithine decarboxylase mutant strain of *A. tumefaciens* exhibited increased cellulose production and biofilm formation, even though planktonic growth was inhibited (88). However, complete spermidine depletion resulted in growth arrest (64). A spermidine-depleted mutant strain of cyanobacterium *Synechocystis* sp. strain PCC 6803 also exhibited increased biofilm formation (89). Polyamine-dependent biofilm development among different species is listed in Fig. 3.

Another surface behavior of bacteria is the coordinated multicellular migration known as swarming, by which a population of cells can move across surfaces. Putrescine was found to be a self-produced extracellular signal for swarming and was necessary for effective migration across agar surfaces, in the γ -proteobacterial common urinary tract pathogen *Proteus mirabilis* (90). Spermidine rather than putrescine was found to be important for swarming behavior of *E. coli* (91). Surface-associated motility of the nosocomial γ -proteobacterial pathogen *Acinetobacter baumannii* was found to depend on biosynthesis of 1,3-diaminopropane, a relatively rare polyamine (92). It is perhaps

salient that *A. baumannii* also uses 1,3-diaminopropane and its biosynthetic precursor 2,4-diaminobutyrate in the synthesis of the iron-scavenging siderophores baumannoferrin A and B (93). Polyamine-dependent surface behaviors among different species are listed in Fig. 3.

Polyamine-based siderophores

Due to their distributed positive charges and flexible backbone, polyamines are often incorporated into natural products, especially iron-scavenging siderophores. They are usually employed as a structural chassis onto which are incorporated hydroxamate groups (94) or independently functional groups, such as catecholate ligands and polycarboxylate groups (2). The spermidine-based dicatecholate siderophore petrobactin (Fig. 5) produced by the anthrax agent *Bacillus anthracis* is a stealth siderophore that avoids sequestration by the human innate immunity protein siderocalin (95). A homospermidine-based petrobactin-related siderophore rhodopetrobactin (Fig. 5) is produced by the metabolically flexible environmental α -proteobacterium *Rhodopseudomonas palustris* (96). The tricatecholate siderophore agrobactin (Fig. 5) uses a spermidine scaffold and is synthesized by the plant pathogen *A. tumefaciens* (97), whereas a norspermidine-based tricatecholate siderophore vibriobactin (Fig. 5) is produced by *V. cholerae* (98). An extensive review of polyamine-containing siderophores and other natural products is not possible in this minireview, but it is worth mentioning the analytical chemical and biochemical tour de force by Codd *et al.* (99, 100) that revealed the biosynthetic sequence of the linear and cyclic diamine-based desferrioxamine-type siderophores.

Conclusions

The polyamine spermidine is probably as old as cellular life, but we know very little about its molecular function in bacteria.

What we do know about the role of spermidine in bacterial physiology comes from a very limited part of the bacterial tree of life, and even fewer studies of the role of homospermidine have been made. Although it is assumed that homospermidine is simply a replacement for spermidine, some bacteria produce both, indicating different functions in those species. Diamines are likely to have functions independent of their roles as precursors of triamines, because many bacteria produce only diamines. Within the archaea, with the exception of the role of long-chain and branched polyamines in growth of hyperthermophiles, the function of polyamines was not discovered intentionally. The role of spermidine in deoxyhypusine modification of aIF5A was inferred from the similarity of aIF5A and eIF5A, and the role of agmatine in modification of tRNA^{lle} was discovered tangentially when it was realized that, unlike bacteria, lysine was not the archaeal modification of tRNA^{lle}. There remains a considerable challenge to understand polyamine function in archaea and bacteria, and especially in bacteria requiring a greater sampling of phylogenetic diversity because published studies have focused mainly on Proteobacteria and Firmicutes and mainly on pathogens rather than environmental bacteria. If it is determined that polyamines are not involved in covalent modifications in bacteria, larger-scale approaches are required, combining omics, genetics, and fresh thinking.

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