Polyamines in cyanobacteria: biosynthesis, transport and abiotic stress response

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This review discusses recent advances on polyamines in cyanobacteria regarding their biosynthetic and transport aspects together with their responses to abiotic stress. Polyamines are ubiquitous and positively charged compounds which are essential constituents in all living organisms including cyanobacteria. There are three main naturally occurring polyamines namely putrescine, spermidine and spermine. The accumulation of these three classes of polyamines under various types of abiotic stress has been well documented in higher plants but not in cyanobacteria. Recent studies in a fresh-water cyanobacterium *Synechocystis* sp. PCC 6803 demonstrate the stimulatory effect by salt and osmotic stresses on the contents of the three polyamines as well as on the activity of arginine decarboxylase, the enzyme catalyzing the formation of putrescine. Other abiotic stresses such as high temperature and UV-irradiation also have an effect on polyamine metabolism. For instance, short-term irradiation with UV-A, B and C can drastically decrease the content of perchloric acid-insoluble (the bound form) spermidine. Polyamine transport system is also affected by abiotic stress. Both salt and osmotic stresses enhance the uptake of putrescine and spermidine. In the review, we will also discuss the involvement of a periplasmic polyamine-binding protein PotD. The role of PotD in the transport of polyamine has been confirmed using the *Synechocystis potD* knockout mutant.

Keywords polyamines; cyanobacteria**;** *Synechocystis*; biosynthesis; transport; abiotic stress

1. Introduction

Polyamines, the major polycationic compounds inside the cells, are present in all living organisms. These include kingdoms of “Plantae”; higher plants [1, 2], “Animalia”; animals, such as mouse [3] and human [4], “Fungi”; filamentous fungi [5] and yeast [6], “Protista”; green algae [7] and “Prokaryota”; bacteria, such as *Escherichia coli* [8], and cyanobacteria [9–11]. The most common polyamines in most living cells are triamine spermidine (1,8-diamino-4-azaoctane), tetramine spermine (1,12-diamino-4,9-diazadodecane) and their diamine precursor putrescine (1,4-diaminobutane). These three common polyamines are found in a cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), with spermine representing a minor content [9]. The polyamine localization inside the cells seems related to their positive charges at physiological pH. This allows polyamines to function in different forms such as free, conjugated or bound form *in vivo*.

The roles of polyamines are not only in the regulation of cell division and morphogenesis in plants, but they are also known to affect the folds of DNA by their binding [12], protein synthesis, membrane stability, and stress responses of plants and cyanobacteria [1]. Arginine decarboxylase (ADC) activity increases have been reported for cell growth and embryogenesis, DNA synthesis and stress responses. On the other hand, ornithine decarboxylase (ODC) has been reported to be associated with growth proliferation and fruit development [13]. Moreover, a role of hydroxycinnamic acid amide conjugates in defence mechanism against biotic and abiotic stress has been reported in higher plants [2]. In addition, there have been reports that the number of polyamine linkages occurring in the chloroplast is enhanced in the presence of light [14]. Spermine is thought to be bound intimately with chromatin [15]; consequently, spermine might have a direct role as a free radical scavenger in protecting DNA from free radical attack caused by oxidative stress [16]. Moreover, polyamines may contribute to the osmotic and excess ion adaptation by maintaining a proper cation-anion balance and by stabilizing membranes at high external salinity. Polyamine oxidation has been reported to be important in the oxidative burst which induces programmed cell death [17]. Although the known mechanisms and functions of polyamines have not been completely elucidated, they strongly act in a number of cellular processes including the responses toward environmental stresses. During the last decade very few studies on polyamines in cyanobacteria have been reported. Here we review the current knowledge on some aspects of the metabolism of polyamines in cyanobacteria with particular emphasis on *Synechocystis*.

2. Polyamine Biosynthesis

2.1 Current understanding of biosynthesis pathway of polyamines

In plants and bacteria, putrescine is formed either by direct decarboxylation of L-ornithine by the enzyme ornithine decarboxylase (ODC; EC 4.1.1.17), or by decarboxylation of arginine by arginine decarboxylase (ADC; EC 4.1.1.19) via agmatine and *N*-carbamoylputrescine intermediates (Fig. 1a). In mammals and fungi, only one pathway (ODC reaction) leads to putrescine formation [18]. The synthesis of spermidine and spermine is accomplished by the sequential addition of an aminopropyl group onto putrescine and spermidine, respectively, in reactions catalyzed by the enzyme spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22). The aminopropyl group is donated by decarboxylated *S*-adenosylmethionine (SAM), which is produced by *S*-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50). Until now, a large number of genes for polyamine biosynthesis have been isolated from *Arabidopsis* (http://www.arabidopsis.org/) in contrast to only a limited number of genes for cyanobacteria (http://genome.kazusa.or.jp/cyanobase/) (Table 1).

Although *Arabidopsis* genes are identified with strong homology to those in the photosynthetic bacterium *Synechocystis* [19], only four separate genes are found that correlate to polyamine biosynthesis in *Synechocystis*, as well as two related genes in *Anabaena* 7120 (Table 1). So far, the genetic information of *Synechocystis* reveals that the dominant pathway to produce putrescine is via the decarboxylation of arginine by ADC with agmatine as an intermediate (Fig. 1b). Recent studies on arginine catabolism in cyanobacteria have found the existence of ADC in all 24 strains of cyanobacteria including *Synechocystis* [20]. Even though ornithine can be detected by the action of arginase on arginine, the conversion of ornithine to putrescine is unlikely due to the lack of ODC in cyanobacteria. Polyamines and the activity of ADC are detectable in *Synechocystis*. SAMDC activity is also detectable in *Synechocystis*; however, its corresponding gene is not found in the genome sequence. Similarly, no gene sequences for spermidine and spermine synthases are found despite the detectable spermidine and spermine contents in *Synechocystis* [9]. This indicates that both spermidine and spermine are synthesized by some other enzymes or these two polyamines arise from the degradation of some related metabolites.

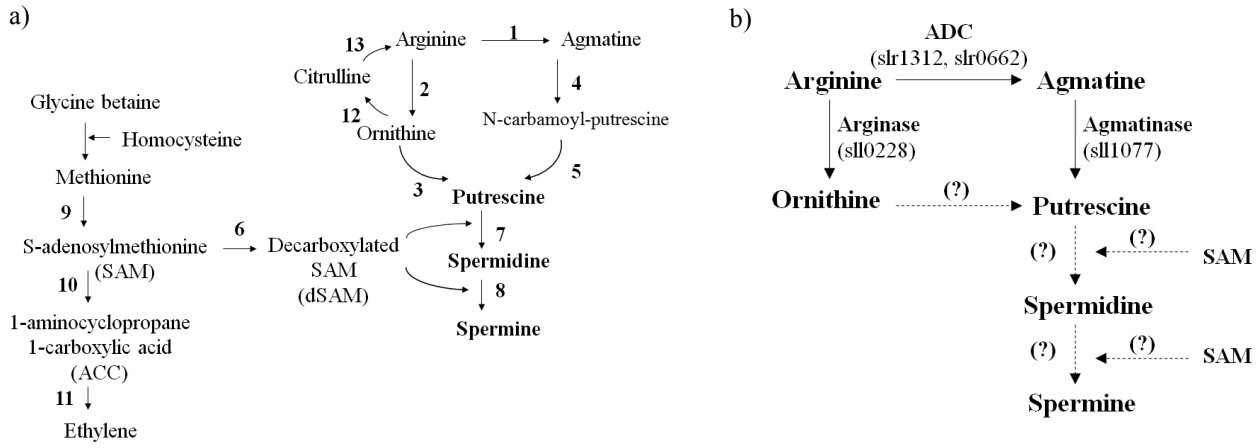

Fig. 1 (a) The pathway of polyamine synthesis in higher plants (modified from [1]). **1**, Arginine decarboxylase (ADC); **2**, Arginase; **3**, Ornithine decarboxylase (ODC); **4**, Agmatine iminohydrolase; **5**, *N*-carbamoyl putrescine amidohydrolase; **6**, *S*-adenosylmethionine decarboxylase (SAMDC); **7**, Spermidine synthase; **8**, Spermine synthase; **9**, SAM synthase; **10**, ACC synthase; **11**, ACC oxidase; **12**, Ornithine transcarbamylase; **13**, Arginine synthase. (b) The pathway of polyamine synthesis in cyanobacterium *Synechocystis* sp. PCC 6803.

From Table 1 and Fig. 1b together with all available data until now, ADC seems to be the most important enzyme for polyamine biosynthetic pathway in cyanobacteria. Based on structural modeling, *Synechocystis* ADCs have a putative extra domain, which might be involved in the posttranslational regulation of ADC activity in *Synechocystis*. Moreover, two symmetric inter-subunit disulfide bonds seem to stabilize the dimeric structure of ADCs [21].

Table 1 Genes related to polyamine biosynthesis in plant [75] and cyanobacteria based on Cyanobase

	Arabidopsis [75]	*Synechocystis* 6803	*Anabaena* 7120
Arginine decarboxylase	*adc1, adc2*	*adc1* (slr1312), *adc2* (slr0662)	all3401
Arginase	*aqrgah1, argah2*	*speB1* (sll0228)	-
Agmatinase	-	*speB2* (sll1077)	alr2310
Ornithine decarboxylase	-	-	-
Agmatine iminohydrolase	*aih*	-	-
N-carbamoyl putrescine amidohydrolase	*cpa*	-	-
S-adenosylmethionine decarboxylase	*samdc1, samdc2*	-	-
Spermidine synthase	*spds1, spds2*	-	-
Spermine synthase	*spms, acl5*	-	-
SAM synthase	-	-	-
Ornithine transcarbamylase	-	-	-
Arginine synthase	-	-	-

On the other hand, the mammalian ADC differs from ADC isoforms expressed in plants, bacteria, or *Caenorhabditis elegans* and is distinct from ODC [22]. The distribution of both ADC and ODC enzymes in different organism species is regulated in a developmental and tissue specific manner [23]. The location of ODC enzyme in animals and plants is observed in both cytoplasm and nucleus [24] whereas ADC is localized in chloroplasts associated with the thylakoid membrane [25]. In plants, SAM, aside from participating in numerous transmethylation reactions, as it does in other organisms, is also a precursor of the plant hormone ethylene. A variety of other related compounds have been found in plants, including cadaverine. Cadaverine diamine is synthesized predominantly as the result of lysine decarboxylase (LDC; EC 4.1.1.18) activity. This diamine is not as widely distributed as putrescine and is mainly found in Leguminoseae and in the flowers of Arum lilies [26]. However, recently we have found cadaverine in *Synechocystis* cells under either ionic or osmotic stress conditions. This is consistent with the presence of a gene encoding lysine decarboxylase (sll1683) in Cyanobase. Moreover, some uncommon polyamines including branched pentamines, hexamines and heptamines have been detected in the extreme thermophilic bacteria [2].

Specific inhibitors for each of these enzymes have been used in many tissues to manipulate cellular polyamine metabolism. DL-α-difluoromethylornithine (DFMO) is a highly effective inhibitor of all animal ornithine decarboxylases while its effectiveness for plant ornithine decarboxylases is quite variable. DMFO also exerts an inhibitory effect on the expression of ADC in *Synechocystis*. The *adc* transcripts decrease in cells grown in the presence of DMFO. Moreover, the increased *adc* transcripts observed in cells under osmotic stress is abolished upon addition of DMFO.

In most plant tissues, DL-α-difluoromethylarginine (DFMA) and methylglyoxal bis-guanylhydrazone (MGBG) are generally quite effective in inhibiting the activities of arginine decarboxylase and S-adenosylmethionine decarboxylase, respectively. *S*-adenosylmethionine decarboxylase activity and transcript levels are known to increase in actively dividing tissues. On the other hand, due to its ability to inhibit cell division, MGBG has been widely used both in animal and plant cells for basic studies as well as for therapeutic applications in cancer treatment [27].

2.2 Polyamine forms existing inside the cells

Since the molecules of polyamines are basic with positive charges at physiological pH, they may not only occur as free molecules but also as conjugates to small molecules [2] like phenolic acids (conjugated forms) and also to various macromolecules like proteins (bound forms) [28]. Spermidine is the most abundant free forms of polyamines in most organisms including cyanobacteria [9] and it is present predominantly in the cytosolic fraction [29].

2.2.1 Conjugated forms

Conjugated polyamines are the most commonly found forms of polyamines in plants. Polyamines are conjugated by the formation of an amide linkage, utilizing esters of CoA for the provision of the activated carboxyl groups [30]. The most

common conjugated polyamines are those that are covalently linked to cinnamic acids. The levels of conjugated polyamines, such as hydroxycinnamic acid amides, are correlated with developmental phenomena. They accumulate in roots and shoots, upon floral initiation in tobacco. Polyamine content increases during all three organogenic programs, especially during meristemoid formation and up to the protrusion of the first organs [27]. Putrescine mainly forms monomers (perchloric acid-soluble fraction) with coumaric acid, caffeoyl acid or feruloyl acid. These conjugates are of particular importance both for the regulation of polyamine concentration inside the cell, and for their interaction. In fact hydroxycinnamic acid bridges, through ester-ester linkages, different cell wall polymers, essentially hemicellulose and lignin [2]. During all developmental programs, there is a possibility that the balance between the levels of free and conjugated polyamines may contribute to growth regulation and play a role during morphogenesis. Aliphatic amines (putrescine, spermidine and spermine) appear as water-soluble forms, whereas conjugated forms with aromatic and aliphatic amines that use each terminal amino group to bind cinnamic acid are water-insoluble [28]. It has been reported that only polyamines in the free form are translocated from one organ to another organ of plant and that conjugated polyamines have no effect on cell division process [2].

2.2.2 Bound forms

The delocalized positive charges of polyamines can provide the electrostatic linking to charged proteins and/or phospholipids and nucleic acids making their effect more complicated. Thus polyamines can bridge elements of membrane and cytoskeletal network and impart rigidity to biological membranes. Polyamine-binding proteins have been identified in a wide range of organisms including mammals, yeasts, and bacteria. The interaction between polyamines and membranes is suggested to be an intermediate in cellular membrane fusion [31]. The peptidoglycans, which are essential for both cell surface integrity and normal cell growth, form covalent linkage to polyamines in *Anaerovibrio lipolytica* [32]. The post-translational covalent linkage of polyamines to proteins is catalyzed by a class of enzymes known as transglutaminase (EC 2.3.2.13), which have been localized both intra- and extra-cellularly [33]. Transglutaminases are calcium-dependent enzymes capable of linking polyamines to glutamine residues and may thus cross-link proteins. Having an active site cysteine, transglutaminases change activity upon treatment with *N*-ethylmaleimide. The cross-linking of proteins through covalently attached polyamines makes tissues more stable and resistant to both proteolysis and physical degradation [34].

3. Polyamine transport

3.1 Proteins involved in polyamine transport

Despite the fact that *de novo* synthesis is the major source of polyamines, transport in and out of the cell, also contributes to polyamine homeostasis. Organisms are equipped with a well-organized transport system for exogenous polyamines uptake. Indeed, there is only a single transporter or, individual transport systems for the various polyamines, capable of transporting all the polyamine molecules. In mammalian cells, two polyamine transport systems have been suggested [35]. In one model, polyamines are transported through unidentified membrane transporters driven by a membrane potential. The second model proposes a role for the heparin sulfate side chains of recycling glypican-1 (GPC-1). For higher plant cells a model for a polyamine transport system has not yet been available. However, some early studies on long-distant polyamine transport suggest the existence of a nonpolar translocation within the plant which occurs mainly via the xylem vessels [36]. In *E. coli* and yeast polyamine transport systems have been well investigated. In *E. coli*, six systems, (1) spermidine preferential uptake system (PotABCD), (2) putrescine specific uptake system (PotFGHI), (3) putrescine/ornithine exchanger (PotE), (4) cadaverine/lysine exchanger (CadB), (5) spermidine excretion system (MdtJI) and (6) putrescine transporter (PuuP), have been identified [37]. In yeast, nine proteins have been identified as polyamine transport proteins [38]. TPO1-5 are efflux-pumps for polyamines. UGA4 takes up putrescine along with γ-aminobutyric acid. GAP takes up polyamines into cytoplasm along with amino acids. DUR3 and SAM3 also carry polyamines into the cytoplasm.

There are a few scattered reports on putrescine transport in cyanobacteria. The earliest one was the study in *Anacystis nidulans* where the mechanism of putrescine transport was passive diffusion and ion trapping within the cells [39]. Polyamine transport in *Synechocystis* has been characterized for the first time by our research group. This polyamine uptake system is very rapid and energy-dependent driven by proton-gradient and membrane potential. The K_m values for putrescine and spermidine in the *Synechocystis* uptake system are 92 and 67 μM, respectively [40]. The exogenous spermidine was found to be metabolized rapidly to diaminopropane in *Anacystis nidulans* [41]. Most of the known details of polyamine transport in prokaryotes have been characterized in *E. coli*. The uptake systems which belong to ATP binding cassette (ABC) polyamine transporters are typically composed of the substrate-binding protein (PotD), two transmembrane proteins (PotB and PotC) and a membrane-associated ATPase (PotA); of these only PotD has been annotated in *Synechocystis* based on sequence similarity to *E. coli* PotD [42]. The homology model of *Synechocystis* PotD was verified based on the structural comparison with *E. coli* PotD and PotF, the three amino acids that are known to be crucial for polyamine binding [43, 44] were totally conserved in *Synechocystis* PotD (Glu209, Trp267 and

Asp295) indicating a polyamine binding capacity. The overall sequence identity between *E. coli* PotD and *Synechocystis* PotD is 24 %, but the residues within 8 Å radius from the active site are more conserved and share a sequence identity of 38 %. Based on this analysis, the active site of *Synechocystis* PotD is more similar to the active site of *E. coli* spermidine-preferential PotD than to putrescine-specific *E. coli* PotF. Binding studies using recombinant PotD overexpressed in *E. coli* showed that *Synechocystis* PotD binds specifically to polyamines but not to other amino acids and prefers spermidine over putrescine. This is in line with results from the uptake experiment using radioactively labeled spermidine, which revealed that the K_i value of putrescine is about 4-fold higher than the K_m of spermidine transport in *Synechocystis* suggesting spermidine preferential binding for the transport system [40]. Machius et al. [45] have recently solved the crystal structure of *Treponema pallidum* PotD (TpPotD, PDB: 2V84), which binds putrescine stronger than spermidine but does not bind spermine at all, and summarized some structural features that might be used to predict the specificity of polyamine binding. The *in vivo* transport assays with *potD* knockout mutant confirm that the PotD homolog in *Synechocystis* has a physiological role in polyamine transport [43]. The mutants showed a 50% lower spermidine uptake than the wild type. More experiments are needed to clarify the properties of transmembrane protein (PotB and PotC) and membrane-associated ATPase (PotA). For example, there are possibilities that polyamine transport system in *Synechocystis* might have its own transmembrane and ATPase subunits (Fig. 2a) or it might share the channel-forming proteins (PotB and PotC) and ATPase (PotA) with another ABC transport system (Fig. 2b). The outcome of such investigation would improve our understanding of the mechanism of polyamine transport in cyanobacteria.

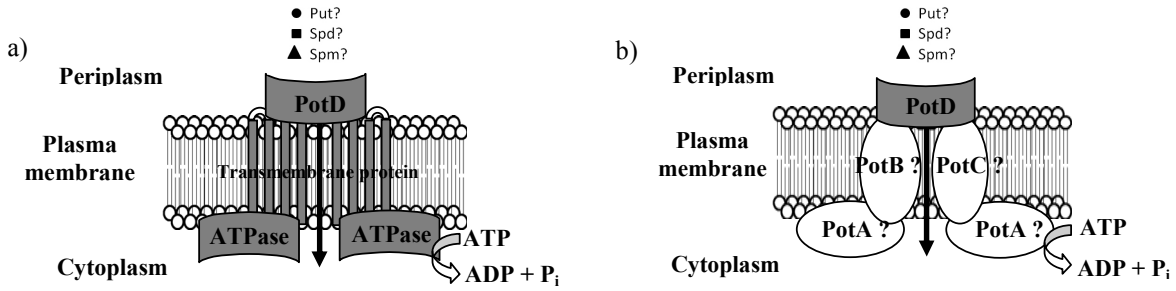

Fig. 2 The proposed polyamine transport system in *Synechocystis* sp. PCC 6803. (a) PotD is substrate-binding protein which might have its own transmembrane and ATPase subunits, (b) PotD is substrate-binding protein which might share the channel-forming proteins (PotB and PotC) and ATPase (PotA) with another ABC transport system.

3.2 Regulation of polyamine uptake

Intracellular polyamine levels are actively maintained by their synthesis, degradation, and transport [46, 47]. In mouse embryonic fibroblast cells the uptake rate is negatively regulated by an antizyme, a small protein of 227 amino acid residues, which is known to be induced by polyamines [48]. In *E. coli*, transcription of the spermidine-preferential transporter genes is down-regulated by the PotD protein and the inhibition is enhanced by spermidine [49]. In *Saccharomyces cerevisiae*, Tpo1 transporters are regulated at the post-transcriptional level by activation of serine/threonine protein kinases [46]. None of these mechanisms have been yet described in cyanobacteria. However, we have initially characterized the basic function of polyamine transport in *Synechocystis*. We found that *Synechocystis* can regulate the polyamine uptake according to the environmental conditions including extracellular pH, salinity and osmotic fluctuation [11, 40]. The putrescine transport operates optimally at pH 7.0, whereas the spermidine transport is at pH 8.0. The different pattern of uptake of putrescine and spermidine, especially at alkaline pH, suggests that these two polyamines might bind at distinct sites on the transporter. This is substantiated by the results showing noncompetitive inhibition of spermidine transport by putrescine [40]. The dependence of polyamine uptake on the extracellular pH has also been reported in *Anacystis nidulans* [50] and a sea water red alga *Ulva rigida* [51]. Upshift of the external osmolality generated by either NaCl or sorbitol causes an increase in putrescine and spermidine transport in *Synechosystis* with about 1.5-2.0 fold increase at 10-20 mosmol/kg upshift [11, 40]. This suggests that cells require polyamines to better thrive against osmotic upshift. Furthermore, it is concluded that the increase in polyamine uptake due to NaCl or sorbitol is caused by an osmotic rather than an ionic effect. This conclusion is drawn from the results showing that sorbitol with no ionic effect has a similar pattern of stimulated uptake seen for NaCl. The polyamine uptake appears to be dependent on *de novo* synthesis of a transport protein or protein(s) regulating the activity of preexisting transport protein(s) because it can be inhibited by chloramphenicol. In fact PotD is an important subunit for polyamine transport in bacteria and parasites [37, 52]. Moreover, PotD has been shown to contribute to virulence in both a murine sepsis model and a pneumonia model with capsular type 3 pneumococci [53].

4. Effect of abiotic stress on cellular polyamines

Polyamine metabolism is responsive to wide arrays of environmental stress conditions [1]. The levels of polyamines are an integral part of the response mechanisms of living organisms to various stresses such as osmotic [54, 55], salinity [9, 20, 56-59], heat, drought, and light [60, 61], as well as chilling temperature [62] and UV radiation [63, 64]. One possible mechanism of salt resistance in cyanobacteria is due to the highly increased contents of spermidine and spermine against the small increase in putrescine content [9]. Alternatively, the salt sensitivity in plants is due to a large increase of putrescine and incapacity to maintain high levels of spermidine and spermine [65]. In general, putrescine accumulation is very often characteristic of a stress response. Although this accumulation could play a protective role in the cell, it has also been reported that putrescine excess may have some negative effects [66]. The endogenous levels of polyamines may serve as markers for different phases of the growth response under NaCl concentrations [65]. However, no clear relationship is observed between the mean levels of salinity resistance and the endogenous concentrations of spermidine or spermine [67]. Table 2 shows the effect of various abiotic stresses on ADC and PotD in terms of transcript, activity and protein levels. The transcriptional levels of *adc1* (slr1312) in *Synechocystis* is highly induced under ionic stress whereas another *adc* gene, namely *adc2* (slr0662) is not affected, showing constant levels after ionic-stress treatment (Pothipongsa *et al*., unpublished). The uncharacterized environmental regulation of the functionally active *Synechocystis potD* gene at the transcript and protein levels has been studied [43]. The steady-state transcript amounts of the *potD* gene are under regulation by a wide spectrum of environmental factors including light, osmolarity, temperature and nutrient availability and the PotD protein amount also shows regulation but its direction cannot be predicted from the transcript levels.

The levels of perchloric acid-soluble and -insoluble polyamines show a dynamic change in response to ionic, osmotic and UV stresses. UV radiations (UV-A, UV-B and UV-C) decrease the perchloric acid-insoluble polyamine contents, under short term stress of one hour [64]. Putrescine and spermine are increased under UV-B and UV-C. UV-A increases the free form of spermidine as opposed to the decrease in the bound form of spermidine. Osmotic stress highly regulates the perchloric acid-insoluble levels whereas ionic stress has no effect on the bound form of polyamines [68]. In a green alga *Scenedesmus obliquus*, high CO_2 concentrations cause an increase in the levels of thylakoid-bound putrescine which leads to an increase of the active reaction center density with a decrease in the light harvesting complex II size [7].

The osmotica with widely different assimilation routes, such as sorbitol [69], mannitol, sucrose, glycerol [71], polyethylene glycol [67, 70], all induce a rise in putrescine. These changes are associated with measurable signs of stress, such as wilting and protein loss [1]. On the basis of osmotic strength, NaCl, KCl, sucrose, or glycerol can induce similar decreases in cellular homospermidine content in the soybean rhizobia *Rhizobium fredii* P220. Homospermidine, an analog of spermidine, is an organic polycation detected ubiquitously in the soil environment and its occurrence has been demonstrated in a wide variety of microorganisms. Subsequently, the cellular levels of homospermidine in strain P220 may be regulated by mechanisms related to their pH and osmotic tolerance [71]. In a highly salt-tolerant strain, Mg^{2+} and homospermidine, a major polyamine in *Rhizobium*, might be closely associated with osmoregulation, since the cellular levels of Mg^{2+} and homospermidine are also regulated critically in response to the external medium osmolarity [71]. The decline of spermidine and spermine levels beyond 30 min of stress in tolerant rice callus, indicates a shift towards the production and accumulation of the higher molecular mass rare polyamines, norspermidine and norspermine. The pattern of accumulation of uncommon polyamines under heat stress in the tolerant cultivar's callus is consistent with that observed in heat-tolerant cotton [72]. Recent studies in a bacterium *Yersinia pestis* have shown that polyamines are required for the formation of biofilm. When the genes for ADC and ODC were mutated; both putrescine and spermidine levels were hardly detectable and this led to a loss of biofilm formation. This situation was reversed by the addition of putrescine [73].The biofilm formation renders a valuable protective mechanism against various detrimental conditions such as pH, UV stress as well as the host defence mechanism [74]. These indicate that the changes of polyamine forms and contents might be the adaptation mechanism for the survival under insulting environments in all organisms.

Table 2 Changes in ADC and PotD in *Synechocystis* sp. PCC 6803 under different abiotic stresses

Stress conditions	ADC		PotD	
	Transcript	Activity	Transcript	Protein
Short-term treatment[a]				
Darkness	± [21]	± [21]	± [43]	ND
High light intensity	+ [21]	+ [21]	ND	ND
UV B irradiation	- [64]	+ [64]	ND	ND
Low temperature	+ [21]	- [21]	± [43]	ND
High temperature	+ [21]	+ [21]	± [43]	ND
Salt stress	+ [21]	+ [21]	+ [43]	ND
Salt stress + High light intensity	+ [21]	+ [21]	ND	ND
Long-term treatment[b]				
Darkness	± [21]	± [21]	± [43]	- [43]
Low temperature	ND	ND	+ [43]	± [43]
High temperature	ND	ND	- [43]	- [43]
Salt stress	+ [9]	± [9]	+ [43]	- [43]
Osmotic stress	+ [9]	+ [9]	+ [43]	± [43]

[a]: incubation time from 15 min to 18 h
[b]: incubation time from 3 d to 10 d
The symbols +, - and ± indicate an increased, a decreased and an unchanged level, respectively, based on data from references shown in the brackets. ND: not determined.

5. Concluding remarks and future perspectives

The overall picture of polyamine metabolism in cyanobacteria is still far from clear, encompassing the biosynthesis, transport and catabolism. This should encourage more researchers to unveil the mechanism governing the regulation and function of polyamines *in vivo*. Although ADC has been shown to play a role in the adaptation of cells under various stress conditions [2, 9, 20]; the localization of the enzyme still remains obscure. SAMDC activity is found in *Synechocystis* cells [9] although its gene has not been found. Two other enzymes, spermidine synthase and spermine synthase, which are involved in the addition of the propylamine group onto putrescine to produce spermidine and onto spermidine to produce spermine, still remain elusive in cyanobacteria. New biochemical approaches such as gene walking technique, bioinformatic tools and appropriate mutant construction are recommended as a research tool for further study on polyamine metabolism in cyanobacteria. Importantly, the forms of polyamines which can influence the function and regulation of polyamines *in vivo* are still mysterious. Polyamines bound with proteins or enzymes have impacts on the intracellular processes and actions. Membranes are also one target of polyamine binding inside the cell based on the positive charges of polyamine molecules. On the other hand, the actual mechanism of polyamine in response to environmental stress is also another important topic. The aspect regarding polyamine catabolism is also worth studying. There are many findings confirming that polyamine oxidation is involved in the induction of programmed cell death. The study on polyamine catabolism in cyanobacteria, may help us to better understand how programmed cell death occurs in living organism.

The properties of the polyamine transport systems in *Synechocystis* sp. PCC 6803 have been presently studied in detail. However, many characteristics still remain unknown or poorly understood. This is especially true for the membrane-associated proteins such as ATPase and transmembrane proteins. The polyamine uptake and its binding study defines the characteristics of the polyamine binding sites on PotD proteins. It is known that a sequence comparison with *Synechocystis* PotD and *E. coli* PotD revealed several amino acid residues crucial for polyamine binding. We expect that the polyamine transporter in *Synechocystis* is structurally similar to the polyamine transporter in *E. coli*. Analyses of knockout strain of *Synechocystis* lacking PotD and other subunits are vital for the insight into the mechanism of polyamine transport in cyanobacteria. Last but not least, the possibility that PotD might also be involved in the excretion of polyamines to overcome the toxicity problems inside the cells cannot be overlooked.

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