

Physiological polyamines: simple primordial stress molecules

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Abstract

Physiological polyamines are ubiquitous polycations with pleiotropic biochemical activities, including regulation of gene expression, cell proliferation and modulation of cell signalling. Reports that the polyamines with cytoprotective activities were induced by diverse stresses raised the hypothesis that physiological polyamines may play a role in inducing stress response. In a wide range of organisms, physiological polyamines were not only induced by diverse stresses, such as reactive oxygen species (ROS), heat, ultra-violet (UV) and psychiatric stress but were able to confer beneficial effects for survival. Recent biochemical and genetic evidences show that polyamines can function as an ROS scavenger, acid tolerance factor and chemical chaperone, and positive regulators for expression of stress response genes which may explain their protective functions against diverse stresses. Taken together, these data suggest that physiological polyamines can function as primordial stress molecules in bacteria, plants and mammals, and may play an essential role in regulation of pathogen-host interactions.

Keywords: physiological polyamines • stress molecule • tolerance • pathogen-host interaction

Introduction

Physiological polyamines, such as putrescine, spermidine and spermine are ubiquitous aliphatic polycations synthesized endogenously in all organisms; their metabolic pathways are conserved, with minor variation, from bacteria to plants and animals. Polyamines can regulate the biochemical activity of various macromolecules, including DNA, RNA and

proteins by binding to them. Suggested pleiotropic biological functions for polyamines include regulation of transcription and translation [1–4], and cell proliferation [5–7]. In addition, complex regulatory mechanisms for the induction and the homeostasis of polyamines, such as metabolic regulation of polyamines and regulation of gene expression of the

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proteins responsible for polyamine metabolism were also reported [8]. However, still it remains to be largely unknown about how physiological polyamines exert their biochemical activities and what physiological function they serve. Increasing reports showing that the polyamines are induced in response to diverse physico-biochemical stresses, including osmolarity, heat, reactive oxygen species (ROS), ultraviolet (UV), and psychiatric stress in a wide range of organisms raise the question whether physiological polyamine can function as a conserved stress molecule. Little study has been done to address whether physiological polyamines are induced directly to give tolerance in response to stresses or how the polyamines can exert any direct protective activities against stress. This review is aimed to answer whether physiological polyamines can function as stress molecules and how polyamines mediate a stress response to diverse physiological and physical stresses. Finally, divergent physiological functions of the polyamines in terms of stress molecule in pathogen-host interactions will be discussed.

Metabolism and transport of physiological polyamines

Physiological polyamines conserved in both prokaryotes and eukaryotes, including putrescine, spermidine and spermine, are synthesized from two primary precursors, L-arginine and L-methionine as depicted in Figure 1. Ornithine and S-adenosylmethionine (AdoMet) are formed from these amino acids through action of arginase and methionine adenosyltransferase (MAT), respectively. Putrescine is synthesized from ornithine by ornithine decarboxylase (ODC); in plants and some bacteria it can be synthesized alternatively *via* agmatine formed by arginine decarboxylase (ADC). Gamma-aminobutyric acid (GABA), an important neurotransmitter, is formed through decarboxylation of glutamate by glutamate decarboxylation (GDC) or through catabolic degradation of putrescine by diamine oxidase and GABA dehydrogenase. Despite having only a single cationic charge, GABA seems also to play a polyamine-like role. Cadaverine is a bacterial polyamine formed from lysine by lysine decarboxylase (LDC). Decarboxylated AdoMet (DcAdoMet) is formed through decarboxylation of AdoMet catalysed by AdoMet decarboxylase (AdoMetDC). Transfer of an aminopropyl group from

DcAdoMet to putrescine and spermidine is catalysed by spermidine synthase and spermine synthase to yield spermidine and spermine, respectively.

The first step in the catabolic interconversion of polyamines is catalysed by spermidine/spermine N1-acetyltransferase (SSAT) to form N1-acetylspermidine and N1-acetylspermine that is back converted to spermidine and spermine by polyamine oxidase (PAO), respectively. The PAO reaction also yields stoichiometrically 3-acetamidopropanal and H₂O₂. Acetylated polyamines seem to be the major form of polyamines exported in eukaryotic cells. Spermine can be directly converted to spermidine by spermine oxidase (SMO) in animal cells because spermine is a preferential substrate for SMO over acetylated spermine [9, 10]. Spermidine also serves as a precursor for the synthesis of hypusine, an unusual amino acid residue in eukaryotic initiation factor 5A (eIF5A), which is essential for eukaryotic cell proliferation [5, 6].

In eukaryotes, regulation of polyamines levels is governed primarily by activity of ODC which catalyses the first step in the synthesis of canonical polyamines. Concentration levels of polyamines are tightly controlled by transcriptional regulation and rapid post-translational degradation, by antizyme (AZ) and antizyme inhibitor (AZI), and by exportation of the acetylated polyamines from the cells. ODC is known to be inactivated by proteasomal degradation through heterodimer formation with AZ which is synthesized through polyamine-mediated frame-shifting of the AZ gene [4]. On the other hand, in prokaryotes polyamines levels are regulated primarily by transcriptional regulation of enzymes catalysing the first step in polyamine synthesis, such as μ LDC, glutamate decarboxylase (GDC), ADC, and ODC, and by corresponding antiporters that are responsible for exporting polyamines as shown in Figure 1. Export of excess accumulated polyamines may be mediated in a membrane potential-dependent manner by polyamine antiporters (PAAXs), such as the Lys:cadaverine antiporter and the Glu: γ -aminobutyric acid antiporter, since they are induced by the polyamines and have a high K_m values (<100 μ M) for polyamines export. Uptake of the polyamines is carried out primarily by an ABC-type synporter with a low K_m value (few μ M) for polyamine uptake [11]. Although a mammalian polyamine transporter has not been identified, pharmacological studies showed that polyamines may be transported by diamine transporters (DAX) with broad specificity [12, 13].

Are physiological polyamines stress molecules?

Organisms respond continuously to environmental changes and evolutionary pressure selects those most prone to developing appropriate adaptive systems. The stringent response is a conserved adaptive regulatory system in prokaryotes. Under conditions of amino acid or carbon starvation, most prokaryotes and several plants rapidly turn off expression of unstringent genes for survival and up-regulate genes involved in protecting from starvation and maintenance of dormancy. These responses are mediated by induction of hyperphosphorylated guanosine nucleotides, ppGpp and pppGpp through an uncharged tRNA-sensing mechanism and sigmaS-dependent gene expression, respectively [14–16]. In eukaryotes, unoccupied tRNAs (utRNAs) and unfolded proteins in the endoplasmic reticulum (ER) increase during starvation or ER stress and suppress cap-dependent translation of many mRNAs; however, mRNAs involved in protective response to the stresses are activated *via* phosphorylation of eIF2 α by eIF2 α family kinases, including protein kinase R (PKR) and PKR-like ER kinase (PERK) [17, 18]. This conserved response to ER stress is called as the unfolded protein response (UPR) and constitutes an integral adaptive system in eukaryotes.

The basic principle underlying polyamine adaptive responses appears to be shared by the prokaryotic stringent response and the eukaryotic UPR. When subjected to starvation, ppGpp and adenosine triphosphate (ATP)-dependent proteases, Lon and Clp induced by utRNA or unfolded proteins stimulate induction of polyamine synthesis by liberating free amino acid substrates through the action of the proteases on ribosomal proteins. Increases in the levels of polyamines induce positive feedback control for the synthesis of polyamines [19, 22], and subsequently turn on multiple stress-related regulons and turn off general gene expression as depicted in Figure 2. Proposed mechanisms for polyamine modulated gene expression include chromatin remodelling *via* DNA methylation and histone acetylation [23, 24], induction of structural changes in DNA, tRNA or mRNA [25–27], and regulation of transcriptional regulators such as Myc, Oct-1, and enhancer binding protein (EBP) [28, 29]. Polyamines

in low concentration usually stimulate gene expression, but elevation of polyamines levels above certain concentrations could exert an inhibitory effect by differential interaction with target molecule. For example, binding of estrogen receptor or EBP to respective cognate DNA binding elements was stimulated by sub-millimolar concentrations of putrescine or spermine which induced DNA conformational change and polyamines in millimolar concentrations inhibited EBP binding to target DNA [28, 30–32]. Concentration-dependent differential effects of polyamines on the initiation and elongation of protein synthesis in a cell-free system was also reported [33]. The effective concentrations of polyamines (sub-mM to mM) in regulating gene expression in response to stresses seem to be physiologically relevant since a substantial fraction of putrescine and spermidine (< sub-mM) exist in free form even in unstressed cells, though most of spermine exists in RNA- and DNA-bound forms. It was reported that there remained 0.2 mM of free spermidine (15% of total spermidine) and 12.5 mM of putrescine (38% of total putrescine) in lymphocyte and *Escherichia coli*, respectively, based on calculations with binding constants of each polyamine to target macromolecules and the cellular concentration of the macromolecules under physiological concentrations of K⁺ and Mg²⁺ [34, 35]. In addition, weak ionic interaction of polyamines with RNA, DNA, proteins and anionic phospholipids ($K_s > 5 \times 10^{-4}$ M) can increase the local concentrations of polyamines in the vicinity of the interacting molecules; thus, direct protective functions of polyamines such as radical scavenging may be facilitated.

Based on observations that stress-responsive genes were turned on by stress-induced polyamines described in sections below and gene expression was suppressed by elevated polyamine levels, stress-inducible physiological polyamines may play a dual role by turning on stress-responsive genes and acting globally to shut down general genes. This may constitute a conserved basic adaptive stress response in living organisms. However, prolonged exposure to excess polyamines may cause direct or indirect detrimental effects on cell survival [36–39]; thus, strict regulation of cellular polyamine levels may be critical for balancing a coping response to acute stresses and reduction of the toxicity induced by excess polyamines.

Data supporting the hypothesis that physiological polyamines induce genes protective against stresses

have been reported, though the mechanism of how these simple polycations activate specific genes was not elucidated. Gene expression in response to heat shock was hampered in polyamine auxotrophic *E. coli*, and was restored to normal by addition of putrescine [40]. Polyamine depletion by α -difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC in hepatocarcinoma cell lines, impaired not only induction of HSP70 mRNA following heat shock [41], but also DNA binding activity of heat shock transcription factor (HSF), a transcriptional activator for heat shock proteins (HSPs) [42]. In addition, recent reports showed that physiological polyamines were involved in regulation of *rpoS*, an alternative sigma factor gene, and subsequent expression of its gene product sigmaS which effects expression of stationary phase genes and serves as a global stress response switch. SigmaS levels were up-regulated by polyamine-mediated transcriptional activation and post-translational stabilization which induced sigmaS-dependent gene expression of stress response genes in *E. coli* under starvation conditions and during transition to stationary phase [43]. Moreover, H₂O₂-mediated induction of OxyR, a ROS-responsive transcription factor responsible for the induction of a series of antioxidant genes, was impaired in polyamine-deficient *E. coli* generated by genetic deletion of *speABC*, putrescine biosynthetic genes. Tolerance to hydrogen peroxide and induction of OxyR and its target genes, such as *katG* and *ahpC*, were recovered by exogenous application of putrescine or spermidine [44]. Thus, physiological polyamines can regulate transcription and translation of two main stress responsive regulons, OxyR and RpoS, in response to stress, though the detailed mechanism is not yet known. SOS genes such as *recA* and *uvrA*, which are involved in DNA repair, were effectively induced by spermine and less effectively by spermidine in a concentration-dependent manner in *E. coli* [45, 46]. The polyamines were shown to be essential for synthesis of ColE7, an *E. coli* nuclease-type colicin, and the rate of polyamine synthesis was directly related to the SOS response [47]. SOS induction of *recA* by exposure to UV-, gamma-irradiation and mitomycin C was reduced in polyamine-deficient *E. coli*, 3.0-, 2.5- and 4-fold lower, respectively, as compared to wild type [48].

An array of data show that physiological polyamines are induced in response to diverse physio-biochemical stresses, including acid, osmolarity, UV, ROS and heat, in a variety of organisms

from bacteria to plants and mammals. In addition, reports have shown that induction of polyamines could exert protective effects on cells or organisms subjected to stresses, such as ROS [49–64], abnormal temperature [65, 66], pH [67, 68], heavy metals [69] and osmolarity [70–72]. Furthermore, down-regulation of polyamines by genetic interruption of polyamine synthesis caused a reduction in tolerance to stresses, while genetic elevation of polyamine synthetic enzymes such as ODC and ADC resulted in enhanced tolerance to stresses [71, 73–77].

Induction of a stress response was first observed in 1962, when heat stressing lead to chromosomal puffs in the salivary glands of *Drosophila* lava. These anomalous puffs indicated that a group of proteins had been dramatically induced, which became known as HSPs. These proteins were found in all organisms and are more generally called stress proteins because they are induced in response to all known stresses. Stress proteins confer tolerance to stressors, and most act as molecular chaperones that assist refolding of damaged proteins or protect native proteins from damages. Here, physiological polyamines are presented as stress molecules, a general term describing both proteins and organic molecules induced in response to diverse stresses to provide protection against these stresses. In addition to direct protective activity such as ROS scavenging, acid tolerance and regulation of ion channels, polyamines in prokaryotes indirectly exerted protective functions against stresses by induction of stress responsive regulons by activation of RpoS, SoxR and SOS gene, but not RpoH, a well known HSF in prokaryotes. In eukaryotes, polyamines indirectly exert protective functions by activation of HSF. The polyamine-mediated induction of stress-responsive genes with concomitant repression of general gene expression may constitute a conserved stress-adaptive response. This hypothesis was further supported by the fact that polyamines are ubiquitous and abundant metabolites derived from amino acids, and can function as chemical chaperones. In addition, polyamine-mediated tolerance against stresses was demonstrated in a variety of genetically modified organisms overexpressing or lacking polyamine synthetic enzymes. Since ablations of polyamine synthesis impair cell survival under stressful conditions, induction of the polyamine-stress response seems to be essential in coping with the stresses. This along with induction of RpoH-mediated HSPs may constitute a main adaptive stress response in prokaryotes. In

eukaryotes, the polyamine-stress response may function in limited situations as described in sections below. Because prolonged induction of polyamines may not give beneficial effect anymore due to toxicity and/or may not be attained effectively due to homeostatic regulation of their levels, the polyamine-stress response may function with limited efficacy in coping with chronic or repeated stresses. Regulation of a broad range of target genes *via* polyamines-mediated transcriptional cascades may be unsuitable for tight regulation of expression of respective target genes. Due to these limitations, eukaryotes may utilize an alternative version of stress response, such as the UPR. Pathways for negative metabolic regulation of induced polyamines (Fig. 1) and positive feedback induction of physiological polyamines in response to stresses (Fig. 2) would facilitate polyamine function as a stress molecule. We posit that polyamines have been exploited by organisms from bacteria to plants and animals as a primordial form of stress molecules. They are ubiquitous and abundant metabolites capable of regulating differential gene expression *via* homeostatic regulation of their cellular concentration in response to environmental changes including starvation.

Role of physiological polyamines in oxidative stress response

Organisms utilizing aerobic respiration and oxygenic photosynthesis for energy encounter oxidative challenge by ROS, and have diverse adaptive defence systems for ROS and/or reactive nitrogen species (RNS), including expression of ROS- and/or RNS-scavenging enzymes, antioxidant metabolites and HSPs. Like starvation stress, oxidative stress is known to induce polyamines in a wide range of organisms. Cumulative data show that physiological polyamines can protect DNA from ROS damage *in vivo* and *in vitro* [49–51, 53, 78, 80–82]. Observation that DNA damage induced by ROS and 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO)-OH spin adduct formation of hydroxyl radical by DMPO were reduced by sub-millimolar concentrations of spermine *in vitro* suggested that polyamines could function as a direct radical scavengers [54]. Although excess polyamines can induce oxidative stress by ROS generation during catabolic oxidation of polyamines, a response

which may occur under special conditions such as host defence [55, 83], in most cases induction of polyamine synthesis protected cells from oxidative stress *in vivo* [50, 51, 53, 56, 57, 76, 82].

Under oxidative stress, a conserved protective mechanism is switched on in a manner similar to that of the response to starvation stress. Upon exposure to oxidative stress, polyamine synthesis was induced and followed by selective expression in a polyamine concentration-dependent manner of a series of genes involved in protecting against ROS stress and repairing damage. In *E. coli*, physiological polyamines were shown to be required for transcription of the genes responsible for oxidative stress defence such as *katG* and *katE* genes, whose expression was regulated by the expression of *oxyR* and *rpoS*, respectively [44]. Moreover, the expression of not only *rpoS*, a transcriptional regulon responsive to starvation conditions, but also *oxyR*, a transcriptional regulon responsive to ROS, was also induced by polyamines. These selective transcriptional activation cascades along with direct antioxidant effects by polyamines can confer tolerance to oxidative stress.

Protective functions of polyamines against ROS stress were further demonstrated by recent independent studies in prokaryotes. Rapid killing in cultures under 95% O₂/5% CO₂ or H₂O₂ was observed in polyamine deficient mutants lacking putrescine and spermidine due to deletion of *speABC* genes [77]. Addition of polyamines, including cadaverine, putrescine and spermine, or introduction into cells of a plasmid harbouring the gene for superoxide dismutase (SOD) abolished oxygen toxicity. Sensitivity to paraquat, a known superoxide anion-generating chemical, was abolished more effectively by spermidine than putrescine. A key determinant for RNS stress resistance in many uropathogenic strains of *E. coli* was identified as CadC, a transcriptional activator for the *cadB* operon, by screening for uropathogenic *E. coli* transposon mutants unable to grow in the presence of acidified nitrite. Mutation of *cadC*, or its transcriptional targets *cadB* and *cadA* which encode lysine: cadaverine antiporter and LDC, respectively, resulted in significant loss of cadaverine production and increased sensitivity to acidified nitrite [58]. CadB expression, which was up-regulated by CadC in response to acid stress, was also shown to be up-regulated by SoxR in response to superoxide stress through binding of SoxR to the promoter region of the *cadBA* operon [63]. The protective role of cadaverine against ROS stress was also

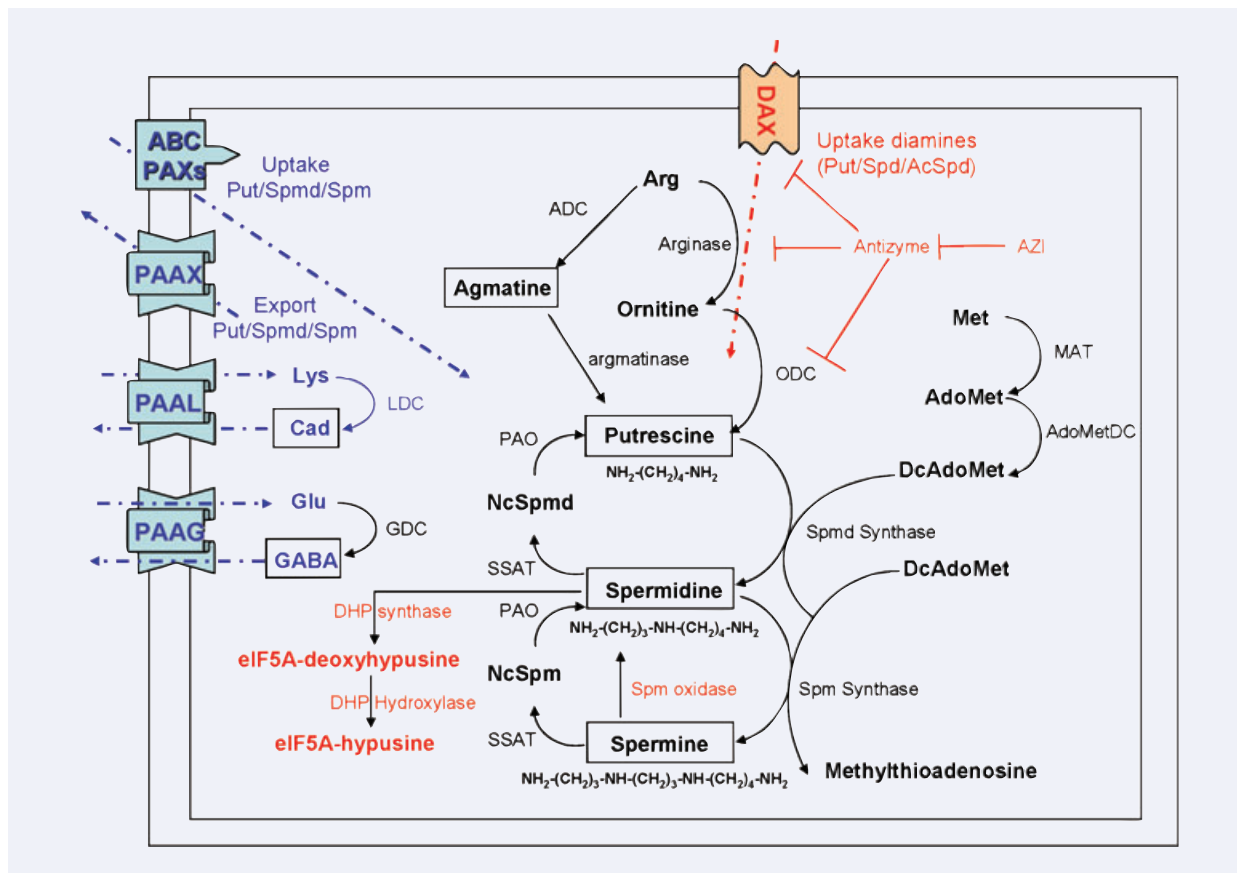


Fig. 1 Metabolic pathways of physiological polyamines in prokaryotes and eukaryotes. Eukaryote- and prokaryote-specific pathways are represented in red colour and blue one, respectively. ODC, ornithine decarboxylase; LDC, lysine decarboxylase; GDC, glutamine decarboxylase; ADC, arginine decarboxylase; AZI, antizyme inhibitor; AdoMET, S-adenosylmethionine; DcAdoMET, decarboxylated AdoMET; Spmd, spermidine; Spm, spermine; Cad, cadaverine; GABA, γ -aminobutyric acid; PAO, polyamine oxidase; SSAT, spermidine/spermine N1-acetyltransferase; NcSpmd, N1-acetylspermidine; NcSpm, N1-acetylspermine, eIF5A, eukaryotic initiation factor 5A; PAAXs, polyamine antiporters; PAAL, Lys:Cad antiporter; PAAG, Glu:GABA antiporter; DAX, diamine transporter.

demonstrated by the induction of Mn-containing SOD (Mn-SOD) under ROS stress that was reduced by elevating gene dosage of *cadBA*; sensitivity to paraquat-induced oxidative stress was higher in a *cadA* mutant than in wild type.

Protective effects of physiological polyamines on oxidative stress were also observed in mammals. Ethanol-induced hepatotoxicity was decreased by GABA in a dose-dependent manner in rat hepatocytes which are known to express GABA synthetic enzymes and its transporter. [64]. Addition of physiological polyamines also reversed ethanol-induced cytotoxicity that may be primarily a result of ethanol-induced ROS. Acute maternal hypoxia in rat was

shown to cause an increase in ODC activity and polyamines levels in foetal brains [84, 85]. In addition, physiological concentrations of spermine (0.1 mM) prevented genistein-, glycyrrhetic acid- and salicylate-induced mitochondrial swelling and membrane potential ($\Delta\Psi$) collapse caused by ROS stress in rat liver mitochondria, and protected rat liver from glutathione oxidation, lipid peroxidation and protein oxidation [86]. Spermine-mediated protective effects on mitochondria were further demonstrated by spermine suppression of ROS-induced glutathione efflux from liver mitochondria, probably by the protection against oxidation of critical thiol groups responsible for the mitochondrial pore opening [87]. A recent

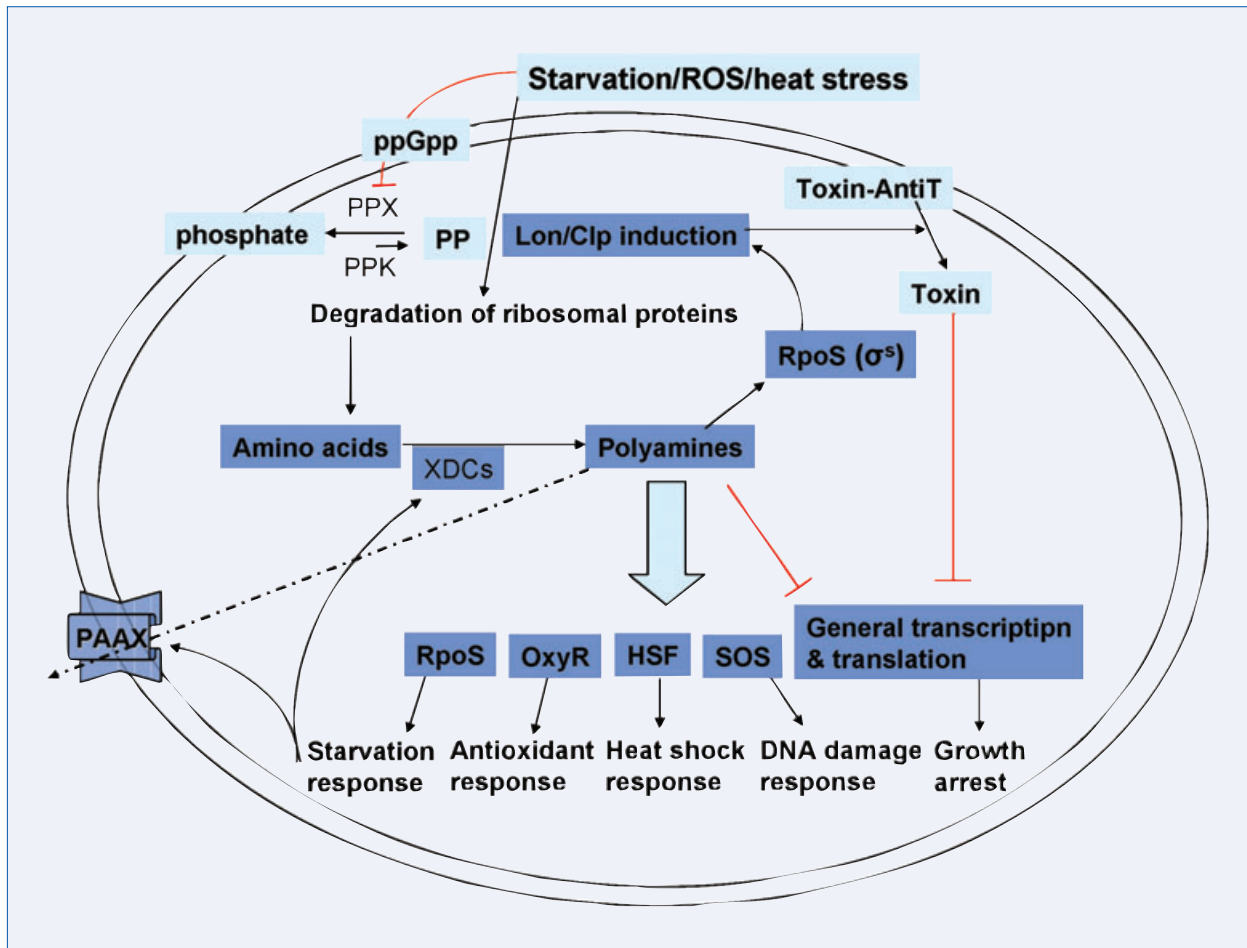


Fig. 2 Schematic model for the stress response mediated by physiological polyamines. In response to diverse stresses, levels of physiological polyamines are up regulated by positive feedback regulation, which activates stress-related regulons represses general gene expression. AntiT, antitoxin; PP, polyphosphate; PPX, exopolyphosphatase; PPK, polyphosphate kinase; HSF, heat shock transcription factor; PAAXs, polyamine antiporters; XDCs, amino acid decarboxylases.

report showed that exogenous spermine and induction of endogenous spermine played roles in protecting *Trypanosome cruzi* insect stage epimastigotes, the causative parasite of Chagas'disease, from membrane lipid peroxidation by ROS. However, putrescine or cadaverine in *T. cruzi* had no effect on protection [60].

Physiological polyamine-mediated acid stress response

Upon exposure to acid pH, organisms turn on pH homeostasis systems to cope with intracellular acid-

ification that can cause enzyme inactivation and protein denaturation. Response to acid stress was studied most extensively in enteric bacteria, including *E. coli*, *Shigella*, *Salmonella* and *Vibrio*, bacteria that survive an acidic environment en route to intestinal colonization. When *Salmonella typhimurium* was pre-incubated at pH 6, survival of adapted bacteria exposed to severe acid conditions (pH 3.3) increased 100-fold over that of unadapted bacteria. This acid tolerance response (ATR) required proteins induced during pre-adaption and during the acid response, including several HSPs [88]. ATR appears to be comprised of two components: an acid-inducible pH homeostasis system, and sigmaS-dependent induction of a series of acid-shock proteins. In addition,

acid induction of the *cadBA* operon, which encodes a lysine:cadaverine antiporter and LDC was responsible for acid tolerance, as well as pH homeostasis under acidic pH in *S. typhimurium* [67]. *E. coli* uses two types of acid response systems to survive acidic environments. One is an oxidative or glucose-repressed system that is acid-induced during stationary phase in an RpoS- and cyclic AMP receptor protein (CRP)-dependent manner. The other is composed of three inducible polyamine synthesis and polyamine antiporter systems. These systems operate to consume protons with concomitant generation and export of polyamines, while co-importing the precursors of polyamines including CadB and CadA, GadA/GadB (GDC) and GadC (glutamate: γ -aminobutyric acid antiporter), and AdiA (ADC) and AdiC antiporters.

ATR by induction of polyamine synthetic enzymes and polyamine transporters appears to be conserved in a wide range of enteric bacteria, despite species-dependent variable combinations of acid-inducible polyamine transporter systems. Mutational analysis of each acid-inducible polyamine transporter gene in *Vibrio cholerae* revealed that CadA, but not SpeF (ODC) or RpoS, plays a major role in ATR, although *Vibrio cholerae* had almost the same acid-inducible polyamine transporter systems as those in *E. coli*. *In vivo* competition assay using CadA- and SpeF-mutant strains revealed that both CadA and SpeF were required for colonization of the intestine of suckling or adult mice [89]. Our recent report showed that *Vibrio vulnificus* expression of *cadBA* was regulated not only by acidic pH via CadC, a known pH-dependent transcriptional activator [90], but also by SoxR, a ROS-induced transcription factor [63]. Thus, increases in the synthesis of cadaverine can impart tolerance to acidic pH, as well as ROS stress. The GadABC system was also shown to play a role in acid tolerance in *E. coli* O157 [91], *Shigella flexneri* [92,93], and *Listeria monocytogenes* [94]. Moreover, exogenous addition of polyamines, such as putrescine and spermidine restored GadA and GadB expression and acid tolerance in polyamine deficient *E. coli* mutants [19]. Thus, for acid tolerance expression of genes responsible for GABA synthesis is subjected to positive feedback regulation by end products, GadA or GadB. AdiA and AdiC also play a role in ATR of *S. typhimurium* [67] and *E. coli* [95, 97]. *S. enterica* serovar Typhimurium can display arginine-dependent acid tolerance, provided the cells are

grown under anoxic conditions. Loss of acid tolerance was observed in mutants lacking *adiA*, *adiC* or *adiY* which encode an AraC-like regulator [98].

The mechanism by which physiological polyamines protect cells from acid stress is poorly understood, but polyamine-induced tolerance could be explained by several biochemical properties of the polyamines. Induction of polyamine antiporters and corresponding polyamine synthetic enzymes at acidic pH increases proton consumption in the cytoplasm. Protons are consumed during anabolic decarboxylation of amino acids in polyamine synthesis which protects the cell from acidification; subsequent antiporter-excretion of the polyamines protects against toxic polyamine accumulation [99, 100]. However, in conditions of very low pH, sustaining pH homeostasis by continuous proton consumption and membrane potential-coupled excretion of polyamines in the presence of their precursors is not feasible. For example, in stationary-phase *E. coli* cellular pH dropped to 4.5 under severe acid stress conditions (pH 2.5), and negative transmembrane potential changed to positive. Additional levels of protective actions may operate to maintain cellular pH above a given lower limit; for example, at pH 4 critical deformation in protein structures and loss of function may lead to cell death. In addition, secreted polyamines have been shown to directly inhibit porin through specific binding, which regulated outer membrane permeability and may contribute to acid tolerance [101–110]. Finally, chaperone activity of s and physiological polyamines may also contribute to acid stress protection, but not pH homeostasis, by facilitating folding of proteins denatured by acidic pH.

Physiological polyamines in osmotic stress response

Maintenance of cell turgor in response to fluctuations in external osmolarity is prerequisite for cell division, cell expansion and survival in living organisms. Upon exposure to hypoosmotic stress, cells use mechanosensitive channels in response to swelling to remove excess cytoplasmic solutes. Upon a shift to hyperosmotic stress, K⁺ uptake and glutamate synthesis in response to dehydration, followed by accumulation of compatible solutes, including proline, betaine, ectoine and trehalose are induced in order to stabilize cell volume and protein folding.

Although mechanosensitive channels, potassium channels, and accumulation of compatible solutes by synthesis and uptake are primarily responsible for maintenance of cell turgor, physiological polyamines, small polycationic molecules, porins and aquaporins may also contribute. The question is raised whether polyamines in millimolar concentrations in the cytoplasm under conditions of osmotic stress may function as a compatible solute or a stabilizer for proteins and anionic phospholipids, in addition to regulating porin- and aquaporin-gating. In *E. coli*, hyperosmotic shift was shown to increase putrescine synthesis and subsequent secretion of the polyamines [111]. Induction of spermidine, spermine, ADC and AdoMET synthases by hyperosmotic stress was also observed in *Synechocystis* sp. PCC 6803 [112, 113]. Many authors have reported that in mammalian cells, hypoosmotic stress induced increases in polyamines such as putrescine [114–116]. Osmotonicity-dependent increases in polyamine levels in rat hepatoma were shown to be mediated by induction of ODC *via* rapid reduction in both level and stability of antizyme, a negative ODC regulator, in response to osmotic stress [117]. Moreover, physiological polyamines are known to directly regulate porin gating, which may facilitate maintenance of osmotic pressure [102, 106, 108]. Direct regulation of inward rectifier K⁺ channels by polyamines and specific regulation of aquaporins by diverse divalent cationic metals were shown [118, 119]. However, there have been few direct evidences supporting the hypothesis that polyamines can function in osmoregulation by regulating the channels involved in osmoregulation, such as mechanosensitive channels, K⁺-channels, and aquaporin.

In plants complex, osmoregulatory systems may exist as higher cell turgor in plants compared with bacteria or mammalian cells is maintained due to the presence of the cell wall. A number of recent reports demonstrated that physiological polyamines were commonly adopted in plants for protection against osmotic stress. While ODC is subject to extensive regulation *via* antizyme, and polyamines are commonly involved in the osmotic response in animals, ADCs seems to be used widely in plants and play a central role in exerting polyamine-mediated osmotic tolerance [120]. Higher putrescine levels established by overexpression of ADC in transgenic rice conferred drought tolerance. In addition, impairment of ADC in *Arabidopsis* subjected to osmotic shock lead to more serious cell injury than that in wild type.

[121]. Genetic evidence suggested that ADC2, but not ADC1, was induced by osmotic stress and may also play a role in development [122, 123].

Physiological polyamines in neuronal stress response

In adult brain, early transient increase in polyamine metabolism, particularly putrescine synthesis, along with induction of universal stress proteins, is a common response to acute traumatic and emotional stresses [124]. Stress responses by physiological polyamines, a primordial form of stress molecule, were most widely observed in highly differentiated brain tissues in which cellular stress defence systems are not fully developed as a result of the functional complexity of neuronal cells. Severe traumatic stresses induced sustained activation of ODC in rodent brain, while transient activation of ODC was induced by acute, mild stresses. Putrescine levels were elevated (130–157% of the control) in all parts of the brain, with little or no reduction in spermidine and spermine levels, 24 hrs after subjecting mice to water-immersion or restraint stress [125, 126].

Studies with transgenic animals overexpressing ODC or SSAT have supported the idea that induction of putrescine, in response to these diverse neuronal insults is a neuroprotective adaptive response rather than a result of neuronal damage. Overexpression of ODC in transgenic mice showed elevation of putrescine in brain and significantly protected the transgenic animals from physical and chemical induced-seizure activity [127]; there was no sign of neuronal degeneration at 2 years of age in transgenic animals with life-long overexpressing ODC [128]. The elevated seizure threshold was neither due to changes in the major neurotransmitter amino acids, glutamate and GABA [127], nor due to elevation of free Mg²⁺-mediated N-methyl-D-aspartate (NMDA) blocking [129]. Substantial expansion of brain putrescine pools in transgenic rat or mouse overexpressing SSAT protected these animals from kainite-induced toxicity and pentylentetrazol-induced seizure activity involving both tonic and clonic convulsions [130, 131]. Although the molecular mechanism of induced putrescine protection against neuronal damage is obscure, polyamine interaction with cationic channels, especially with

NMDA receptor, may provide a clue. Prolonged activation of NMDA can lead to neuronal damage. Putrescine is believed to act as a weak NMDA receptor antagonist, while spermidine and spermine are agonists. Antagonistic activity of putrescine [132, 133] may block agonist activation of NMDA receptor and contribute to putrescine-mediated neuroprotection. This explanation was consistent with the observation that spatial learning and memory test performance was impaired in transgenic mice overexpressing *ODC*, as activation of NMDA is essential in the learning and memory process [127].

ODC was induced by amyloid beta ($A\beta$) and imparted protection against $A\beta$ -induced toxicity which is presumed to be an important neurotoxic factor in Alzheimer's disease [134, 135]. Neurodegenerative disorders, such as Alzheimer's, Parkinson's and Huntington's diseases are characterized by the accumulation of intracellular toxic protein aggregates, $A\beta$, α -synuclein, and huntingtin, respectively. These protein aggregates are generated from misfolded proteins, which should have normally removed or refolded by stress proteins [136] or removed by stress-inducible autophagy, a cellular self-eating [137, 138]. Neuroprotective functions of physiological polyamines may facilitate protection of neuronal cells from cell damage along with stress proteins induced by UPR; thus, the polyamines may constitute integral components of a neuronal stress-response system. This hypothesis was supported by findings that aliphatic diamines could function as chemical chaperones [139]. Further experiments to address whether accumulation of putrescine can function in protection from neurodegenerative diseases through chemical chaperone activity would be intriguing.

Both agmatine and spermine were shown to protect against ischaemic damages *in vivo* by diverse ways, including reducing the size of ischaemic infarcts and the loss of cerebellar neurons, and decreasing both nitric oxide synthase (NOS) and arginase activity [140–142]. In addition, agmatine was reported to show diverse neuroprotective activities, such as attenuation of neuronal death following excitotoxic spinal cord injury [143], prevention of neurotoxicity produced *via* NMDA or glutamate [144, 145], suppression of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity [146], inhibition of inducible nitric oxide synthase (iNOS) [147]

and reduction of hippocampus neuronal damage caused by glucocorticoids [75]. However, contradictory findings that the polyamines were implicated in the pathogenesis of diverse neurodegenerative diseases including ischaemia were also reported [148–150]. These conflicting results may be explained by the fact that oxidation of polyamines induced by traumatic stress can generate ROS, which can cause neuronal death, particularly in microglia that are sensitive to sub-micromolar concentrations of polyamines [59]. This explanation was supported by observations that MDL 72527, a specific inhibitor of PAO, reduced ischaemia-induced neurotoxicity, putrescine levels, and N1-acetylspermidine levels [149, 150]. However, more compelling evidence is needed to elucidate the mechanism and roles of physiological polyamines and their metabolites in neuronal stress responses, neuroprotection or neurotoxicity.

Moreover, the stress-inducibility of polyamines in brain is developmentally regulated, and its increase coincides with maturation of the hypothalamic–pituitary–adrenocortical (HPA) axis, a central component of the stress-response system [124]. Thus, induction of polyamines may be involved in the developmental maturation of HPA.

Physiological polyamines in other stress responses

Metabolic profiling analyses of the induction of acquired thermotolerance and freezing tolerance in response to heat shock and cold shock, respectively, revealed that the majority of heat-shock responses were shared with cold-shock responses in *Arabidopsis*. A co-ordinated increases in polyamine precursors in addition to an increase in the pool sizes of amino acids derived from pyruvate and oxaloacetate and compatible solutes during both heat and cold shock were observed [65].

Although few studies have been conducted on the temperature stress-induced polyamine response, recent findings showed that polyamines can function as chemical chaperones and shed light on the mechanism by which small polycations play a role in protection from cytotoxic stress. Essential structural determinants of diamines for suppressing thermal

aggregation of lysozymes were determined. Small diamines including 1,5-diaminopentane were shown to be active, though 1,3-diaminopropane was most effective, whereas diamine derivatives with diols or monoamines had no protective effect [139]. Chemical chaperone activities of the polyamines can directly contribute to the protective function of these stress molecules by facilitating folding of denatured proteins generated by various stresses.

Solar ultraviolet B (UV-B) radiation induced cutaneous ODC in *Ptch1*^{+/-} mice, in which patched (*PTCH*) was known to be a tumour-suppressor gene [151]. In addition, physiological polyamine response to UV exposure was studied in detail in tobacco cultivar [152]. An increase in polyamines, especially in putrescine levels, in thylakoid membranes upon elevated UVB exposure comprised one of the primary protective mechanisms in the photosynthetic apparatus of tobacco Bel B. The UVB sensitivity of tobacco cultivar Bel W3 was attributable to its inability to enhance putrescine levels in thylakoid membranes. After full adaptation to UVB including induction of carotenoids and additional flavonoids, the polyamine levels were reduced.

Upon exposure to heavy metals, such as Cadmium, Copper, Nickel and Zinc, an array of amino acid derived metabolites, proline, betaine and polyamines accumulated to high millimolar concentrations in plants. The response molecules may have three major functions, namely metal binding, antioxidant defence and signalling [153]. In the leaves of *Nymphoides peltatum*, copper induced increases in putrescine levels and lowered spermidine and spermine levels. Exogenous application of spermidine or spermine markedly reversed the Copper-induced effects, including enhanced levels of proline, superoxide anion generation and Copper-induced lipid peroxidation [69].

Thus, physiological polyamines have been reported to be induced by a variety of stresses including radiation, heavy metal and emotional stress in addition to common stresses such as ROS, osmolarity, low pH and heat, which have been shown to confer tolerance against them. Herein, it was proposed that in response to physiological and physical stresses, physiological polyamines could function as a conserved primordial stress molecule in a wide range of organisms from bacteria to plants and animals.

Divergent roles of physiological polyamines in adapting to pathogen-host interaction

Once pathogens or parasites infect their host, both pathogen and host are mutually stimulated to produce a set of stress proteins including chaperones, a group of proteins with activities facilitating assembly and disassembly of macromolecular structures. Induction and/or secretion of stress proteins can modulate several levels of pathogen-host interaction, such as pathogen-host adhesion signalling, pathogen virulence and host pathogen defence systems [154].

Many pathogenic enteric bacteria pass through and survive acid environments in the process of colonization. Acid tolerance factors including polyamine synthetic enzymes may contribute to the virulence of these bacteria, particularly in acid sensitive *V. cholera*. In concerted action with *speF*-encoding inducible ODC, CadA was shown to be essential for colonization of the intestine of suckling and adult mice [89]. The role of CadA as a colonization factor may reflect its inducibility by acid and its protective function against the acid environments encountered during infection. In addition, RpoS, whose transcription is known to be induced by polyamines and low pH, was also shown to be an essential virulence factor, as demonstrated by *rpoS* null *Salmonella* attenuated for infection of mice through oral or intraperitoneal routes.

Physiological polyamines can modulate modified tRNA-mediated translational fidelity, maintenance of reading frame and efficiency for modified nucleosides such as queuosine or epoxy queuosine (Q) in the 3', or wobble, position of an anticodon. VirF is an upstream regulator of virulence cascades, such as VirG and VirB in the *Shigella*. Putrescine or a combination of methionine and arginine restored VirF expression through modified tRNA-mediated translational control in *Shigella flexneri*, while expression of virulence genes including VirF was 10-fold reduced in tRNA modification-deficient mutant *Shigella* due to the lack of epoxy Q in a subset of tRNA, when the bacteria were grown in minimal media [155].

PotD, a constitutive putrescine or spermidine transporter, was implicated in the pathogenicity of *Streptococcus pneumoniae*, a leading agent of

bacterial pneumonia and meningitis [156]. Immunization with *S. pneumoniae* PotD abolished infection in a systemic mouse model [157]. There is no clear understanding of how PotD functions as an essential virulence factor, but recent findings [47] show that levels of PotD are increased by polyamine-mediated induction of ColE7. Polyamine-mediated induction of PotD, an ABC-type synporter responsible for preferential uptake of putrescine in low concentrations (few μM), can rapidly increase cellular polyamine concentrations sufficient to turn on virulence genes by importing extracellular putrescine and spermidine abundant in the host intestine and stomach.

On the other hand, results showing that polyamines can function as anti-virulent factors have also been reported. Pathoadaptive mutations in *cadA* were observed in pathogenic *E. coli* O111 producing *Shiga* toxin, and might be involved in the regulation of pathogenicity through adhesion to host cells [158]. The negative role of *cadA* in cell adhesion could reflect the fact that physiological polyamines regulate cytoskeletal structure adhesion by modulating F-actin stress fiber formation and the expression or function of RhoA [159, 160].

As described above, a number of bacteria including pathogens have inducible transport systems for excretion of accumulated excess polyamines that may be harmful to cells. Polyamines excreted during infection may affect infectivity of pathogens in several ways. *Helicobacter pylori* which induces an innate mucosal immune response and selectively colonizes the human stomach was shown to induce macrophage apoptosis by activation of inducible arginase followed by induction of ODC. Apoptosis of macrophage was also induced by exogenous polyamines [161]. Another virulence factor of secreted polyamines could be mediated by induction of polyamine oxidase 1 (PAO1) in *Helicobacter pylori*, which was demonstrated to cause H_2O_2 release and mitochondrial membrane depolarization [162]. The second pathological consequence of polyamine metabolism in *H. pylori* is gastric carcinogenesis which may be initiated through ROS stress induced by catabolic oxidation of spermine [163]. Polyamines excreted by a pathogen can also play a negative role in pathogenicity. Pathoadaptive mutation in *cadA* was observed in *Shigella* species evolved from non-pathogenic *E. coli* with intact *cadA*. Introduction of *cadA* into *Shigella*-inhibited *Shigella* induced transmigration of polymorphonuclear leucocytes (PMN)

and exogenous cadaverine also blocked PMN transmigration and enterotoxicity induced by enterotoxins [164, 165].

The finding that blood forms of ODC null *Trypanosoma brucei* mutants could not proliferate without exogenous polyamines suggested that physiological polyamines may be essential for proliferation of the parasites, which render ODC and AdoMETDC important anti-parasitic drug targets. Inhibition of ADC by irreversible inhibitors was shown to reduce infection capacity of blood forms of *Trypanosoma cruzi* in mouse peritoneal macrophage and heart myoblasts [166]. ADC-generated argmatine could protect protozoan parasites in multiple ways. As iNOS was inhibited indirectly by agmatine aldehyde [147], agmatine-induced suppression of NOS would aid in survival of the parasite. Moreover, reactivation of ODC through denitroylation of cys360 [167] due to nitric oxide (NO) reduction could increase polyamine levels to support cell proliferation.

A number of reports suggested that physiological polyamines or their catabolic enzymes were exploited as a defence mechanism in plants with unique catabolic enzymes, particularly PAOs. The function of polyamine catabolism in plant defence systems is extensively reviewed elsewhere [168]. H_2O_2 generated by PAOs in plants is involved not only in induction of pathogen apoptosis, but also in cell wall strengthening during pathogen infection [169, 170]. In addition, induction of putrescine and ADC2, but not ADC1, by mechanical damage, methyl jasmonate and abscisic acid in *Arabidopsis* suggested that physiological polyamines may be involved in wound response in plants that lack special mobilizing cells [171].

Conclusions

Physiological polyamines, ubiquitous polycations, were induced by diverse stresses, and induction of polyamines exerted multiple cytoprotective actions from ROS scavenging to polyamine-mediated induction of stress-responsive genes, though polyamines are simple aliphatic metabolites. This polyamine-stress response may constitute an integral part of the stress response to a variety of stresses. As they may have evolved to play a role in maintaining cell function and viability, detailed knowledge of the molecular control mechanism for the cytoprotective functions of

physiological polyamines may provide better ways for regulating pathophysiological conditions, including infection of pathogens and parasites, cancer and inflammation, and for generating environmentally adaptable plants to enhance crop production.

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