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REVIEW

The roles of polyamines in microorganisms

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Abstract Polyamines are small polycations that are well conserved in all the living organisms except Archae, Methanobacteriales and Halobacteriales. The most common polyamines are putrescine, spermidine and spermine, which exist in varying concentrations in different organisms. They are involved in a variety of cellular processes such as gene expression, cell growth, survival, stress response and proliferation. Therefore, diverse regulatory pathways are evolved to ensure strict regulation of polyamine concentration in the cells. Polyamine levels are kept under strict control by biosynthetic pathways as well as cellular uptake driven by specific transporters. Reverse genetic studies in microorganisms showed that deletion of the genes in polyamine metabolic pathways or depletion of polyamines have negative effects on cell survival and proliferation. The protein products of these genes are also used as drug targets against pathogenic protozoa. These altogether confirm the significant roles of polyamines in the cells. This mini-review focuses on the differential concentrations of polyamines and their cellular functions in different microorganisms. This will provide an insight about the diverse evolution of polyamine metabolism and function based on the physiology and the ecological context of the microorganisms.

Keywords Cell cycle · Microorganisms · Parasidic activity · Polyamines · Stress response · Translation

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Introduction

Since they were first observed in human semen in 1678 by Antonie van Leeuwenhoek in the form of spermine phosphate and identified by Schreiner in 1878, polyamines have been of great interest to many researchers. They were identified in every living organism except two orders of Archaea, Methanobacteriales and Halobacteriales (Hamana and Matsuzaki 1992). This ubiquitous nature of polyamines indicates the significance of these small molecules in the organisms ranging from unicellular microorganisms to higher eukaryotes. This review specifically focuses on the roles of polyamines in microorganisms. Biosynthetic and regulatory pathways are also briefly explained to provide better understanding of the polyamine diversity, differential functions, significance of their roles and polyamine-based therapeutic approaches. The review covers the physiologically significant intracellular functions of polyamines such as cell division, stabilization of nucleic acids or gene expression as well as their involvement in cell-environment interactions such as stress response and pathogenic activity, in reference to their molecular structure, prevalence and ecological context.

Polyamines are aliphatic hydrocarbon chains with one or more amine groups. They are positively charged at physiological pH, which enables their interaction with polyanionic molecules such as DNA, RNA, phospholipid head groups in cell membrane or cell wall components. Putrescine, spermidine and spermine constitute the most common polyamines with some exceptions in bacteria and fungi. In bacteria such as *Escherichia coli*, putrescine is the most prominent polyamine, while in eukaryotes spermine and spermidine exist in higher concentrations. Yet in fungi *Saccharomycotina subphylum*, spermine is not detected at all. The other forms of polyamines involve long-chain and branched-chain polyamines, which are predominantly synthesized in thermophiles.

Polyamines, which vary in terms of molecular structure, valence and prevalence undertake different roles in the cells such as survival, growth, gene expression, stress response, cell division and parasidic activity (Miller-Fleming et al. 2015; Michael 2016). Thus, polyamine levels should be kept under strict control in the cells to enable the efficiency of these cellular functions. Polyamine biosynthesis and cellular transport are the major routes to regulate polyamine levels in the cells. Hence these mechanisms have been crucial targets for the experimental approaches to understand the roles of polyamines.

The universal polyamine biosynthetic pathway in eukaryotes including Protozoa and Fungi, initiates by conversion of L-ornithine into putrescine by ornithine decarboxylase (ODC). Putrescine is converted into spermidine and then spermine by the successive transfer of aminopropyl groups, which are donated by decarboxylated AdoMet (dcAdoMet). The key enzymes in this pathway are (1) *S*-adenosylmethionine decarboxylase (AdoMetDC), which catalyzes the formation of dcAdoMet, (2) spermidine synthase (SpdSyn), which catalyzes conversion of putrescine into spermidine, and (3) spermine synthase (SpmSyn), which catalyzes conversion of spermidine into spermine. ODC and AdoMetDC catalyze the rate limiting steps of the biosynthetic pathway and deletion of one of these enzymes are frequently used in experiments to deplete polyamines in the cells. Although ODC, AdoMetDC and SpdSyn forms a universal polyamine biosynthetic pathway (Michael 2016), one interesting exception is the parasidic Protozoan *Trypanosoma brucei*, which doesn't possess ODC gene and therefore needs to uptake putrescine from its host. It, however, has AdoMetDC and SpdSyn to convert putrescine to spermidine. Spermine biosynthesis is thought to have evolved independently from putrescine and spermidine. Although it exists in Saccharomycotina yeasts, spermine doesn't exist in the rest of the fungi or Protozoa parasites *T. brucei, Trypanosoma cruzi, Leishmania* and *P. falciparum*. Also in bacteria the most common polyamines are putrescine, spermidine and cadaverine, leaving spermine out. Spermine is only found in the pathogenic bacteria if it is already present in the medium. In *E.coli*, for instance, spermine is not synthesized de novo, but exogenous spermine can be used in the cells (Dubin and Rosenthal 1960). These data indicate a transport system for the spermine without de novo synthesis in bacteria. An alternative biosynthetic pathway that can be observed in Bacteria and also in Archaea is the arginine decarboxylase (ADC) pathway. ADC converts arginine into agmatine, which is subsequently converted into putrescine by agmantinase. Putrescine is then converted into spermidine via AdoMetDC/SpdSyn route. Mutations of biosynthetic pathway components is a widely used experimental approach to deplete polyamines and understand their roles. For instance polyamine depletion via biosynthetic pathway mutants lead to reduced growth rate or even growth arrest in many different organisms such as *Yersinia pestis, Vibrio cholerae* and *Salmonella typhimurium* and *Thermococcus kodakaraensis* (Michael 2015).

Although less characterized than the biosynthetic pathway, polyamine transport is also involved in the regulation of intracellular polyamine levels. Polyamine transporters can be found in the plasma membrane or in organelle membranes, and they can provide influx or efflux of polyamines contributing to the polyamine homeostasis. For instance, deletion of yeast polyamine export protein Tpo1 showed sensitivity to excessive polyamine levels, while its overexpression increases tolerance to excess polyamine (Albertsen et al. 2003). These transporters are especialy important for organisms like *T. cruzi*, who lack ODC and depend on environmental uptake of polyamines such as putrescine.

Stabilization of nucleic acids

Polyamines can readily bind to anions in the cells due to their polycationic nature. Intracellular polyamines are predominantly found as polyamine-RNA complex rather than binding to cytoplasmic proteins. For instance in case of *E. coli*, 90% of the spermidine and 48% of the putrescine are found in RNA complex in the cells. In addition to the RNA, polyamines were also reported to bind to and stabilize double stranded DNA. Polyamine interaction with DNA and RNA, however, have different consequences. It was shown that polyamines stabilized the RNA by keeping it in a particular conformation, in which RNA stays soluble and capable of interacting with other molecules. In case of DNA, polyamines bind externally to the DNA, enabling intermolecular interactions (Katz et al. 2017). Stabilization of nucleic acids by polyamines is especially important in thermophilic microorganisms, which grow optimal at 50–60 °C temperature and in hyperthermophiles, which grow better at 80 °C or higher. There is growing evidence that polyamines contribute to their heat resistance.

Thermophilic Archaea and Bacteria have unusual long-chain (e.g. homocaldopentamine, caldopentamine, caldohexamine and thermopentamine) and branched-chain (e.g. tris-(3-aminopropyl)amine, *N*4-aminopropylspermidine, tetrakis-(3-aminopropyl)ammonium and *N*4-bis(aminopropyl) spermidine) polyamines, in addition to the common polyamines putrecine and spermidine (Fukuda et al. 2015). For instance, *Pyrobaculum aerophilum* and *Hyperthermus butylicus* archaea have long-chain polyamines, while *T. kodakarensis* has no linear long polyamines but branched-chain polyamines, which was shown to stabilize compacted DNA and contribute to the regulation of gene expression (Fukuda et al. 2015). In vitro studies in aqueous solution showed that branched-chain polyamines forms bridges or

crosslinks within DNA, changing the higher order structure, while linear polyamines induces parallel alignment between DNA segments (Muramatsu et al. 2016). This could explain how these unusual polyamines in thermophiles contribute to the nucleic acid stability, which is an important part of thermostability. In addition to the branched-chain polyamines, aminobutryl-containing polyamines and (although less effectively) acetylated polyamines are known to stabilize DNA.

Translation

An important part of the requirement for polyamines in cellular growth and proliferation is believed to be due to their influence on gene expression through translation. This function of polyamines is not through the stabilization of nucleic acids and that is the reason aminopropyl and aminobutryl groups have similar effects on DNA stability but varying effects on translation efficiency (Wilson and Bloomfield 1979). The set of genes whose expression is upregulated by polyamines, at the level of translation is called 'polyamine modulon' (Igarashi and Kashiwagi 2006). Polyamine modulon in *E. coli* includes proteins involved in transcription, translation, nutrient transport, cell viability and signal transduction. Three different mechanisms were proposed to explain the effect of polyamines on translation: (1) polyamines exert structural changes and facilitate the formation of the initiation complex when Shine-Dalgarno (SD) sequence is far from the initiation codon (2) facilitate fMet-tRNA binding to inefficient UUG and GUG initiation codons (3) stimulate read-through or frameshifting. In thermophilic eubacteria *T. thermophilus*, polyamines are known to be indispensable for the efficient translation at temperatures above 65 °C, but not at 37 °C (Ono-Iwashita et al. 1975). Longer polyamines (e.g. homocaldopentamine, caldopentamine and thermopentamine) are shown to increase translation efficiency in the cellular extracts of *T. kodakarensis*, while putrescine and spermidine had no effect. Finally, studies on polyamine biosynthesis mutant *S. cerevisiae* showed increased Cox4 gene translation upon exogenous addition of spermidine, which is through ribosome shunting of the hairpin structures during scanning of the 5′UTR of the mRNA (Uemura et al. 2009). This first member of yeast modulon is a subunit of mitochondrial respiratory chain enzyme cytochrome *c* oxidase (Complex IV), which is responsible for the assembly and stabilization of this complex (Coyne et al. 2007).

The role of polyamines in the regulation of translation also contributes to their own feedback control in yeast. (Ivanov et al. 2000; Palanimurugan et al. 2004). Antienzyme (AZ) is an ODC inhibitor, which mediates its degradation. They have two open reading frames and functional AZ protein expression requires a+1 ribosomal frameshift, which is stimulated by polyamines. So, AZ expression increases with increasing cellular polyamine levels, which in turn induces the breakdown of the rate limiting biosynthetic enzyme ODC and constitutes a negative feedback loop for polyamine regulation. *S. pombe* SPA was the first antienzyme identified in a unicellular organism, whose deletion caused 40 times accumulation of putrescine in the cells and overexpression caused growth inhibition with cells accumulated in the G1 phase of the cell cycle (Ivanov et al. 2000). Another level of antienzyme-dependent feedback was identified in *S. cerevisiae*, in which polyamines also inhibited the ubiquitin dependent proteolysis of yeast antienzyme Oaz1 (Palanimurugan et al. 2004). Antienzyme-like proteins which are induced by purescine and inhibit ODC were also identified in bacteria *Selenomonas ruminantium* and *E. coli* (Ivanov et al. 1998; Yamaguchi et al. 2002).

One major mechanism polyamines exert their effects on translation is through a unique post translational modification of the tranlation factor eIF5A, which involves conversion of its lysine residue into an unusual amino acid hypusine. Hypusine is synthesized by a two step process: (1) cleavage and transfer of the aminobutyl group of the spermidine to a specific lysine residue of the precursor eIF5A by deoxyhypusine synthase (DHS), which yields deoxyhypusine intermediate (2) conversion of the deoxyhypusine intermediate into hypusine containing active eIF5A by deoxyhypusine hydroxylase. This modification is known to convert inactive eIF5A into its active form and directs it to the cytoplasm enabling its association with ribosomes (Lee et al. 2009). The active form of eIF5A is shown to stimulate ribosome peptidyltransferase activity and especially essential for the translation of proline rich regions on the mRNA (Gutierrez et al. 2013). Disruption of eIF5A or DHS genes in *S. cerevisiae* are known to cause growth arrest and loss of viability. Additionally, *S. cerevisiae* polyamine biosynthesis mutant can grow at normal rate upon addition of polyamines at 0.2% of the physiological level, 54% of which was used for the hypusination, indicating that polyamine requirement in cells is mostly due to its hypusination function (Chattopadhyay et al. 2008). *DHS* gene is also known to be essential for protozoa such as *Leishmania donovani* (Chawla et al. 2010) and *T. brucei* (Nguyen et al. 2013).

Stress response

One of the major roles of polyamines in the cells is to provide resistance to intracellular and environmental stress, which could be in the form of reactive oxygen species, temperature changes, osmotic pressure or other toxic compunds. A growing number of evidence showed that intracellular polyamine levels change in response to stress and depletion of polyamines (by chemical or genetic methods) renders cells

more sensitive to stress. Engineered *S. cerevisiae* strains with high spermidine levels were resistant to chemicals such as acetic acid and furfural, which would otherwise inhibit microbial growth, metabolism and ethanol fermentation (Kim et al. 2015). Polyamines can exert their effects as part of the stress response either directly (e.g. acting as reactive oxygen species/ROS scavengers) or indirectly (e.g. regulating the expression of stress response genes). Also in fungi such as *Glomus mosseae, Rhizopus stolonifer, Botryodiplodia theobromae, Gigaspora rosea* and *Glomus etunicatum*, polyamines were shown to be involved in spore germination, which forms a more stress resistant form of cell compared to the vegetative cells (Valdes-Santiago and Ruiz-Herrera 2013). A similar situation could be observed in *E. coli*, in which putrescine was shown to promote persister cell formation by upregulating rpoS expression. Persister cells are known to survive lethal antibiotics such as netilmicin and stresses (Tkachenko et al. 2017).

Oxidative stress

Oxidative stress is caused by ROS such as hydrogen peroxide (H_2O_2), which can be harmful for intracellular macromolecules. ROS can be a byproduct of metabolism and increases with cellular metabolic rate, which is high in rapidly proliferating cells or infective bacteria. Cells can also be exposed to ROS from the environment. Either way, cells respond to stress by increasing antioxidant proteins, arresting cell cycle and adjusting metabolism. Polyamines are known to help fight against the oxidative stress by directly interacting with free radicals or by altering gene expression. In fact, polyamines are known to be very strong ROS scavengers due to their positive charge. Putrescine, spermidine, spermine and cadaverine are shown to be very efficient against alkyl, hydroxyl and peroxyl radicals, while spermidine and spermine can work as scanvengers against superoxides (Valdes-Santiago and Ruiz-Herrera 2013). In *E. coli*, putrescine level was shown to increase upon oxidative stress (Tkachenko et al. 2001). Also putrescine and spermidine were shown to increase the transcription of OxyR and SoxRS, which are the transcription factors associated with the subsequent expression of stress response genes *ahpC, katG* and *katE* (Tkachenko and Nesterova 2003; Jung and Kim 2003). In fungi *Ustilago maydis*, polyamine mutants were shown to be more sensitive to environmental H_2O_2 compared to wild type cells (Valdes-Santiago et al. 2010), while *S. cerevisiae* polyamine mutants lost viability under oxygen atmosphere (Balasundaram et al. 1993). Additionally, in H_2O_2 exposed *S. cerevisiae*, Tpo1 polyamine transporter is shown to export spermidine and spermine, which in turn induces stress response proteins such as Hsp70, Hsp90, Hsp104 and Sod1, and prolongs cell cycle arrest (Krüger et al. 2013). Tpo1 is also shown to be involved in the resistance against benzoic acid (Godinho et al. 2017) and its deletion was shown to render the yeast cells more sensitive to environmental H_2O_2, which could be rescued to some extent by exogenous supply of spermidine and spermine in the growth media.

Osmotic and salt stress

Polyamines can influence the stability and permeability of cellular membranes by binding to the negatively charged phospholipid head groups or other anionic sites (including membrane bound proteins) on these membranes (Marton and Morris 1987), which is especially important in providing defense against osmotic or acidic stress. In *E. coli*, for instance, polyamines were shown to inhibit the activity of porins OmpF and OmpC, which results in decreased membrane permeability and consequently contributes to the resistance against acidic or osmotic stress (Vega and Delcour 1996). Also in *S. cerevisiae* osmotic stress induced by NaCl, KCL or sorbitol downregulated a major high effinity permease AGP2, which is responsible for polyamine import (Lee et al. 2002; Aouida et al. 2005). Serine/threonine protein kinases Ptk1p and Ptk2 in yeast were also shown to regulate polyamine uptake, and disruption of ptk2 resulted in salt tolerance while over expression of serine/threonine kinase genes Ptk2 and Sky1p caused salt sensitivity (Erez and Kahana 2002). In cyanobacterium *Synechocystis* sp., spermine level was shown to increase upon osmotic stress, while spermidine level increased upon salt stress, which was provided by both increased expression of adc and increase in the uptake of putrescine and spermidine (Jantaro et al. 2003). Additionally, in *U. maydis*, spdsyn and odc mutants were shown to decrease growth rate only in the presence of salt stress induced by KCl and SDS, but not at optimum conditions without stress (Valdes-Santiago et al. 2009).

Temperature stress

In thermophilic archaea and bacteria, long-chain and branched-chain polyamines are thought to contribute significantly to thermostability. These specific polyamines contribute to the heat resistance of thermophiles. Consistent with their role, branched-chain polyamines are more abundant in thermophiles growing at high temperatures compared to the cells growing at lower temperatures (Fujiwara et al. 2015). The gene disruptions that result in loss of long-chain and branched-chain polyamine production also decrease the viability of thermophiles at high temperatures (Ohnuma et al. 2005; Morimoto et al. 2010). In thermophilic eubacterium *Thermus thermophilus*, for instance, long and branched chain synthesis mutants showed that these polyamines were required for viability at high temperatures and for the maintenance of $tRNA^{Tyr}$, $tRNA^{His}$, rRNAs and 70S ribosomes (Nakashima et al. 2017). In addition to their essential role

as nucleic acid stabilizers at extreme temperatures in thermophilic microorganisms, polyamines provide resistance to high temperatures in other organisms as well. In fungi such as *Tapesia yallundae, U. maydis* and *S. cerevisiae*, polyamine biosynthesis mutants showed sensitivity to elevated temperatures (Valdes-Santiago and Ruiz-Herrera 2013).

Cell cycle

Polyamines have been shown to be involved in the progression of cell cycle. Studies in *S. cerevisiae* showed that ODC enzymatic activity is highest in the exponentially growing cells and its activity decreases as cells approach stationary phase. The nongrowing cells, which are arrested at the G1 phase also have decreased ODC activity (Kay et al. 1980). Also in *S. pombe*, spermidine depletion at the early stages slowed down the cell cycle with cells accumulating at the G1. Prolonged polyamine depletion leads to more cells accumulating at G1 with morphological abnormalities such as disruption of the actin network, absence of septum and disintegration of nucleus. Notably, even a very small amount of polyamines could restore normal growth with cells mostly at the G2/M stage similar to the wild type cells (Chattopadhyay et al. 2002). Due to the sensitivity of cell proliferation to intracellular metabolic activities as well as outer environment, polyamines also indirectly effect the cell division. For instance, in archae *Sulfolobus acidocaldarius*, inhibition of eIF5A hypusination, which is dependent on spermidine, leads to cell cycle arrest (Jansson et al. 2000). Or in *S. cerevisiae*, environmental H_2O_2 exposion induces polyamine transport, which contributes to the induction of stress response proteins and consequently the timing of H_2O_2-dependent cell cycle arrest at G2 (Krüger et al. 2013).

Pathogenic activity

The significance of polyamines for the survival through vital mechanisms such as gene expression and cell division, makes polyamine metabolism a proper target for drug design against pathogenes. This idea has been successfully applied to Protozoa parasites such as *T. brucei, T. cruzi, Leishmania* and *Plasmodium*, which cause HAT (Human African Trypanosomiasis/sleeping sickness), American trypanosomiasis (Chagas disease), leishmaniasis and malaria, respectively. Biosynthetic enzymes such as ODC, AdoMetDC and SpdSyn have proved to be promising targets against these parasites. ODC inhibitor DFMO and AdoMetDC inhibitors (such as MDL 73811 analogues, aryl and heteroaryl bis-guanylhydrazones) were shown to efficiently cure *T. brucei* infections (Bitonti et al. 1990; Bacchi et al. 1992; Li et al. 1998; Barker et al. 2009), reduce Leishmania (Gradoni et al. 1989) and *Plasmodium* infection (vonBrummelen et al. 2009). In case of *T. cruzi*, which lacks ODC and depends on the uptake of the putrescine from the host, the inhibition of the only polyamine transporter TcPAT12 by isotretinoin turned out to be a promising treatment (Reigada et al. 2017).

A similar polyamine dependent pathogenic activity can be observed in fungi *Penicillium marneffei* (a pathogen for people with immune deficiency), whose pathogenesis is disrupted by *spe* (yeast ODC) mutation and *Botrytis cinerea*, whose virulence is enhanced by the expression of *spe* gene and increased polyamine levels and returned to normal upon polyamine inhibitor treatment (Marina et al. 2008). Polyamine biosynthesis and transport system was also shown to be proper drug targets in bacteria such as *Strestococcus pneumoniae* and *Salmonella enterica* serovar Typhimurium (Shah et al. 2011; Jelsbak et al. 2012).

Emerging roles of polyamines and concluding remarks

Microorganisms constitute very efficient models for genetic manipulations (e.g. knock-out mutants of polyamine biosynthesis proteins or transporters), which widened our general understanding of polyamines, both functional and structural. Understanding the functions of polyamines in microorganisms serve important functions in drug design against pathogens or render microorganism more resistant to stress in case they are used for biofuel, etc. production. Polyamines are gathering more and more attention in biotechnology due to their cationic nature and high catalytic activity. In nanotechnology, for instance, polyamines are used in the construction of carbon fiber surfaces (Baumgärtner et al. 2017) or in protein based films (Sabbah et al. 2017). Their high proton affinity also enables them to be used as proton sponges in mass spectrometry (Wirth et al. 2017) and also chemical chaperones to suppress protein aggregation in biochemical methods (Kara et al. 2017). The use of polyamines in biotechnology also serves important functions in fight against diseases. Polyamines were recently shown to successfully target amyloid aggregation in Alzheimer's Disease (Simoni et al. 2016) and spermidine was used to produce super-cationic carbon quantum eye drops for the treatment of bacterial keratitis (Jian et al. 2017) as well as provide cardioprotection (Eisenberg et al. 2016). Finally polyamines also contribute to therapeutic applications in regenerative medicine as spermine coating enhanced adenoviral transduction of mesenchymal stem cells (Wan et al. 2016). All these examples point to the fact that polyamines will stay as hot topics for the following years.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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