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MINIREVIEW

Role of Polyphosphates in Microbial Adaptation to Extreme Environments[▽]

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EXTREME ENVIRONMENTS AND EXTREMOPHILES

Anthropomorphically, an extreme environment is one in which physical conditions are not conducive for human life. In this review, extreme environments are defined as habitats that experience steady or fluctuating exposure to one or more environmental factors, such as salinity, osmolarity, desiccation, UV radiation, barometric pressure, pH, and temperature. Microorganisms that colonize extreme environments are called extremophiles, and they are categorized into subgroups according to the specific environmental characteristics of their habitats (for a review, see reference 78).

Prokaryotic and eukaryotic microorganisms provide sources for the discovery of novel biochemical pathways and biomolecules that allow microbes to adapt to extreme environmental conditions. Previous studies of microorganisms from extreme environments have led to the development of important industrial processes and the discovery of health-promoting biomolecules. Examples include biochemicals used for detergent formulations, leather and paper processing, biofuels, bioremediation, UV-blocking, and new antibiotics (1, 17, 27, 28, 57, 93). Potentially beneficial biomolecules still remain to be discovered from unexplored extreme environments. One such environment is the high-altitude Andean wetland (HAAW) ecosystems of the South American Andes. The HAAW ecosystems are systems of shallow lakes formed during the Tertiary geological period. These aquatic ecosystems are distributed in the geographical area called the Puna at altitudes from 3,000 to 6,000 m above sea level, where they are isolated from direct human activity, and they are almost unexplored (Fig. 1). The HAAW ecosystems are unique not only for their geographical characteristics and broad range of extreme environments but also for their abundant biodiversity. The microbial communities that have evolved within these high-altitude aquatic ecosystems must tolerate chemical and physical stresses such as wide fluctuations in daily temperatures, hypersalinity, and variable pH and be adapted to high levels of UV radiation, a low level of nutrient availability, and high concentrations of heavy metals, especially arsenic (13, 14, 18, 19, 99).

Polyphosphates (polyP), linear polymers of tens or hundreds of orthophosphate (P_i) residues linked by high-energy phosphoanhydride bonds, were apparently present on Earth before life appeared and were generated through a variety of abiotic processes (97). polyP have been associated with the capacity of microbes to resist both physical and chemical stresses (6, 39). The structural and physicochemical characteristics of polyP seem to have been criteria for the selection of these molecules as components of cellular processes during evolution (40). The early living organisms may have used polyP as long-term sources of P_i and energy. This may also have allowed microorganisms to survive Earth's primitive environment, which resembled some of Earth's current extreme environments. polyP granules or bodies (volutin granules, which stain red when treated with toluidine blue) are widely distributed among microbial species. Granules of polyP are homologous to the acidocalcisome, an organelle involved specifically in the storage and metabolism of cellular polyP (15). The association of acidocalcisomes with phosphorous metabolism and evidence that links polyP with the capacity of bacteria to overcome stress are of increasing scientific interest. polyP are some of the first examples of membrane-bound organelles with important functional roles found in both prokaryotic and eukaryotic kingdoms.

This minireview will focus on the ways that polyP, biologically versatile molecules, could be involved in the adaptation to extreme environments by modulating microbial stress responses in pristine HAAW ecosystems. These aquatic ecosystems are natural laboratories for exploring and monitoring in situ interactions between the environment and the dynamics of biodiversity.

polyP AS MODULATORS OF MICROBIAL STRESS RESPONSE

Microorganisms have developed diverse mechanisms of resistance that allow them to colonize and thrive in extreme environments such as those observed in the HAAW ecosystems. Within these habitats, microbial communities must withstand steady-state and fluctuating extreme environmental factors (13, 14, 18). Advances in biotechnology have provided the

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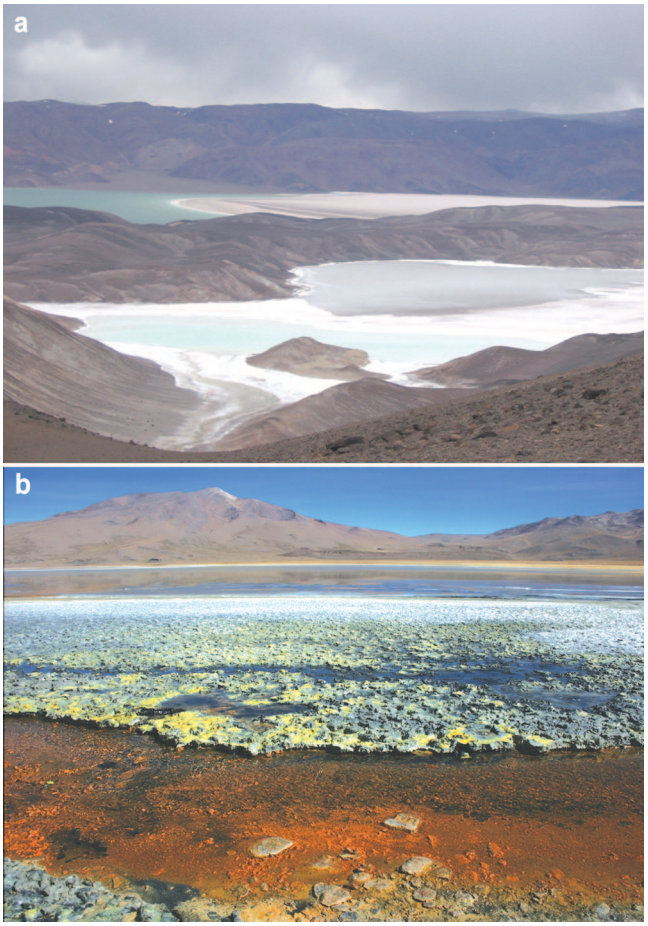

FIG. 1. HAAW ecosystem wetland in northwestern Argentinean Andes. (a) Salar de la Laguna Verde at 4,400 m above sea level; the HAAW ecosystem lakes are hypersaline and shallow, are separated by salar pads, and contain high levels of arsenic. (b) Laguna Vilama, at 4,600 m above sea level, has gradients of salinity along with microbial mats.

tools needed to generate a catalog of bacterial factors normally associated with stress resistance. However, the mechanisms of microbial regulation and coordination in response to stress remain poorly understood. Cellular polyP have been proposed to serve as reservoirs for phosphate and as chelators of metal ions (11, 37, 69) and in gene regulation (92). polyP have also been linked to a variety of microbial physiological processes, including motility, biofilm development, quorum sensing, and virulence (6, 70, 71, 72, 73, 82).

The key enzyme involved in the synthesis of polyP in bacteria is polyphosphate kinase (PPK). PPK synthesizes polyP by transferring the terminal phosphate of ATP to polyP. It also catalyzes the regeneration of ATP by the reverse reaction and the conversion of other nucleotides, such as GDP to GTP (38, 39). The involvement of polyP in microbial stress resistance is supported by the observation of phenotypic deficiencies in *ppk*-null mutants in which the levels of polyP are significantly reduced (11) (Table 1). *ppk* mutants of several bacterial species were unable to survive the stationary phase in culture, were highly susceptible to oxidative stress, displayed reduced adaptability to high concentrations of Ca^{2+}, were intolerant to acid and thermal shock, and showed reduced sporulation and virulence (5, 39, 60). PPK has also been associated with protein degradation during amino acid starvation stress (42). A lack of polyP resulted in decreased expression of the RNA polymerase sigma factor (RpoS), which is integral for the expression of stationary-phase-induced stress genes (48, 83) and impairs the SOS system of DNA repair (92). Mounting evidence indicates that polyP are key players in enabling microorganisms to respond to and resist stress.

The prokaryotic cell has been portrayed simplistically as a "sack" of plasma membranes and DNA adrift in cytoplasm and surrounded by a cell wall. Provocative findings have challenged the notion that the prokaryotic cell lacks a sophisticated cytoplasmic organization. For a long time, it was believed that bacterial shape was regulated by the organization of cellulose fibers within the cell wall. However, a protein homologous to actin, once thought to be an exclusive component of the eukaryotic cytoskeleton, has been discovered in bacteria (30, 53, 95). More recently, the idea that organelles similar to those present in eukaryotes were absent in bacteria was challenged by the discovery of an organelle similar to the acidocalcisome of unicellular eukaryotes within the bacterium *Agrobacterium tumefaciens* (81). The existence of this bacterial organelle has also been confirmed in the photosynthetic bacterium *Rhodospirillum rubrum* (80) (Fig. 2). The acidocalcisome is an acidic calcium and polyP storage organelle that was first observed in trypanosomes but is now known to be found in cells from diverse organisms ranging from bacteria to humans (blood platelets) (15). Acidocalcisomes are morphologically and chemically similar to the structures historically described as volutin or polyphosphate granules in a variety of microorganisms such as bacteria, algae, yeast, and protozoa (38). Meyer (52) described volutin granules in bacteria more than 100 years ago. In protists, the organelle has important roles in the regulation of intracellular Ca^{2+}, pH, and osmotic homeostasis (15). At this point, it is not known how this organelle was disseminated through the domains of the tree of life, but regardless of its origin, its conservation implies that it has an important functional role in cell biochemistry. The presence of acidocalcisomes in bacteria suggests that this organelle may play a fundamental role in microbial survival and could have effects on the dynamics of microbial biodiversity. In addition, acidocalcisomes may be important as virulence factors of many human pathogens and other microbial species.

Although many features of acidocalcisomes, especially of bacterial acidocalcisomes, are not yet understood, both eukaryotic and bacterial acidocalcisomes share the following properties. (i) Acidocalcisomes are membrane-bound structures. The origin of acidocalcisome membranes is not clear, but the inability to detect plasma membrane markers suggests that acidocalcisome membranes are not derived directly from the plasma membrane (81). When observed under a transmission electron microscope, acidocalcisomes are characterized by a high density of electrons, with their numbers fluctuating from more than 20 in eukaryotes (15) to 1 to 3 in bacteria (80, 81). The volume of this organelle represents approximately 1% to 2% of the volume of the cell. Under stress conditions, the organelle volume can significantly increase due to a massive accumulation of polyP (9, 55).

(ii) The characteristic feature of acidocalcisomes is their capacity to store polyP. Acidocalcisomes have been demonstrated to

TABLE 1. Functions of polyP in bacteria[a]

Organism	Phenotype observed	Reference
E. coli	Fails to survive stationary phase Shows loss of resistance to heat Susceptible to oxidative and osmotic stresses Deficient in adapting to nutritional deprivation	11, 42, 69
P. aeruginosa	Lacks biofilm and is deficient in quorum sensing Shows distortion of the cell envelope Lacks planktonic motility Susceptible to the beta-lactam antibiotic carbenicillin	22, 71, 72
Vibrio cholerae	Lacks motility; shows loss of virulence	72
Salmonella enterica	Lacks motility; shows loss of virulence Deficient in response to stress Deficient in growth in weak acids	37, 65, 72
Helicobacter pylori	Unable to colonize gastric mucosa Defective in motility	5
Porphyromonas gingivalis	Defective in biofilm formation	10
Shigella spp.	Show loss of resistance to acid and heat Deficient in response to starvation and stress Has decreased virulence	37
Bacillus cereus	Defective in motility, biofilm formation, and sporulation	82
Myxococcus xanthus	Defective in motility and has delayed fruiting body formation Has low level of spore production and delayed germination Has reduced level of predation	100
Mycobacterium tuberculosis	Lacks stringent response Shows impaired survival in macrophages	89
Bordetella pertussis	Shows loss of virulence	39
Yersinia pestis	Shows loss of virulence	39
S. lividans	Susceptible to oxidative stress Lacks regulation of antibiotic biosynthesis	24, 25
Campylobacter jejuni	Defective for survival during osmotic shock Shows low-nutrient stress tolerance and loss of virulence	8
Corynebacterium glutamicum	Shows growth disadvantages under low-P_i conditions	47

[a] Characteristics were inferred from phenotypic deficiencies of mutants lacking the gene encoding PPK, which is responsible for the synthesis of polyP.

possess high concentrations of short- and long-chain polyP, phosphate, and pyrophosphate (PP_i) (15, 80, 81). PP_i can be found in cells of both eukaryotic and prokaryotic microorganisms as a by-product of cellular activity (15). In bacteria, it intervenes in numerous enzymatic reactions (12, 54, 75). *R. rubrum* stores PP_i within acidocalcisomes (80) and can synthesizes PP_i during photosynthesis to create a H^+ gradient across the membranes of chromatophores. Both prokaryotes and eukaryotes synthesize polyP (15, 39). In prokaryotes, the major synthetic enzymes of polyP are PPK (38) and exopolyphosphatase (PPX), which degrades polyP by splitting P_i from the end of the long chain of polyP. PPK and PPX are membrane-bound enzymes (2). In response to needs within the cell, the polyP are hydrolyzed to generate phosphate (15). The capacity to store polyP originally suggested that the function of acidocalcisomes was to sequester and store phosphorous. Now emerging evidence has began to elucidate polyP's roles in a wide variety of dynamic physiological processes. Although the exact relationship between bacterial acidocalcisome-mediated storage and the degradation of polyP and the capacity of cells to survive under environmental stress conditions is still poorly understood, it can be argued that bacterial acidocalcisomes could potentially have functions similar to those demonstrated for eukaryotic acidocalcisomes (15).

(iii) The lumen of the acidocalcisome in both prokaryotes and eukaryotes is acidic, as demonstrated by staining with LysoSensor blue DND-167 and acridine orange (15, 80, 81). Acidification is important in the maintenance of high levels of polyP in this organelle.

(iv) Both eukaryotic and bacterial acidocalcisomes are resources of calcium (15) and other cations. An elevation in calcium content in response to high extracellular calcium concentrations suggests that acidocalcisomes have calcium-accumulating activity (81). Within acidocalcisomes, polyP can chelate Mn, Zn, Fe, K, and other cations, including heavy metals present in the environment. In contrast, depletion of cellular stores of polyP under phosphate-limiting conditions correlates with the resistance of microorganisms to heavy metals (4, 34).

(v) Vacuolar pyrophosphatase (H^+-V-PPase), which serves as a proton pump in the acidocalcisomes of eukaryotes, is present in the acidocalcisomes of *Agrobacterium tumefaciens* and *R. rubrum*. H^+-V-PPase hydrolyzes PP_i to generate a proton gradient across the membrane of the acidocalcisome. The mechanisms of PP_i transport and storage in the acidocalcisome are poorly understood. H^+-V-PPase has been used as a marker for acidocalcisome purification (80, 81).

SURVIVAL SYSTEMS FOR ENVIRONMENTAL STRESS: THE RELATIONSHIP BETWEEN polyP AND BACTERIAL STRESS RESPONSES

Oxidative stress: UV radiation and nutrient deficiency. When bacteria are exposed to the hazards of environmental stresses such as UV radiation, a series of signals are responsible for triggering physiological responses. UV radiation enhances the generation of reactive oxygen species (ROS), with two different outcomes. First, ROS act as cell signals that allow

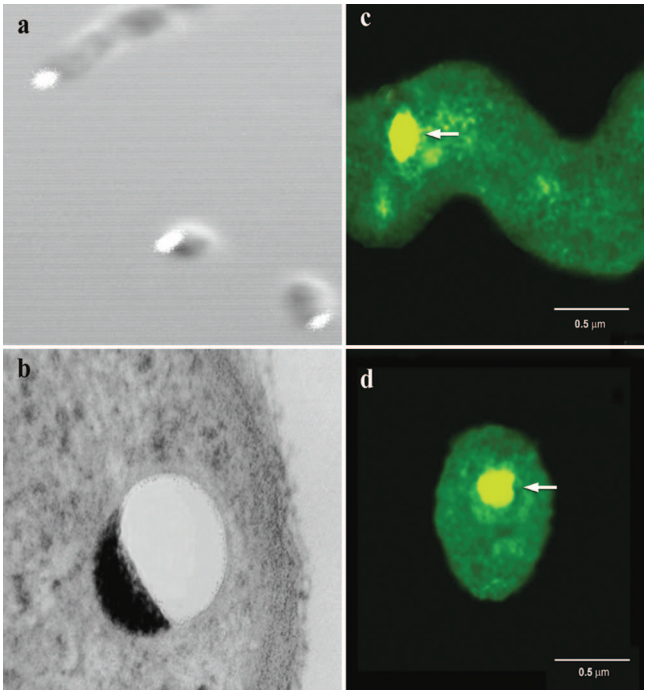

FIG. 2. Confocal immunofluorescence and ultrastructural analysis of the acidocalcisomes of *A. tumefaciens* and *R. rubrum.* (a) Analysis of the H^+-V- PPase of *A. tumefaciens* using polyclonal antibodies against the H^+-PPase of *Trypanosoma cruzi*. Labeling is at one pole of the cells, where acidocalcisomes are located. (b) Thin-section micrograph of an acidocalcisome volutin granule of *A. tumefaciens* showing a partially empty vacuole that corresponds to an acidocalcisome containing electron-dense material. (c) Confocal immunofluorescence image of the H^+-V- PPase of *R. rubrum*. Arrows show labeling of the acidocalcisomes. (d) Confocal thin section of *R. rubrum*. Arrows show the internal label of the acidocalcisome. Bars, 0.5 μm. (Panels c and d are reprinted from reference 80.)

cells to arm themselves against these stressors (7), and second, when the level of ROS overwhelms the defense mechanisms of the cell, extensive cell damage and apoptosis can occur. The amount of UV radiation (A and B) that the organism is exposed to depends on several factors, such as solar zenith, latitude, altitude, water depth and transparency, and cloud cover and thickness. In HAAW ecosystems, all these factors promote exposure of the microbes to very high levels of UV radiation, which is viewed as one of the most-limiting factors in bacterioplankton community survival (13, 14, 18). One of the strategies that microorganisms have evolved to colonize environments with extreme levels of UV radiation, such as HAAW, is the formation of biofilms. Biofilms are layers of planktonic bacteria attached to one another at their surfaces, forming an elaborate, growing, three-dimensional structure. In order to form this three-dimensional colony, bacteria need to communicate with one another (quorum sensing), and polyP have been proposed as the modulators of quorum sensing and biofilm development. Although *Escherichia coli* and several other bacterial species mentioned in this minireview are not extremophiles, they have been used as models for biochemical insights to infer the biological function of polyP. PPK mutants of *Pseudomonas aeruginosa*, which are incapable of synthesizing polyP, are also defective in quorum sensing and biofilm formation and are susceptible to UV-A exposure (22, 66, 73). While the involvement of polyP in biofilm formation has been demonstrated experimentally, the functional aspects of polyP in biofilm formation are not yet understood. Recently, it was demonstrated that human platelet cells also contain acidocalcisomes (79) and that polyP act as modulators for blood clotting (84). Blood clotting is a complex cascade mechanism in which polyP secreted from the acidocalcisome act as "sticky molecules" by activating the contact pathway. Without polyP, clot formation is impaired (84). As with platelet activation in homeostasis, bacterial biofilm formation requires intercellular signaling (29, 63), and polyP, which could be secreted from the bacterial acidocalcisome as a quorum-sensing factor, may act as the necessary sticky molecule to modulate biofilm formation.

The accumulation of polyP also regulates the activation of the RNA polymerase RpoS (83) and the synthesis of the guanosine nucleotides, guanidine 5′-triphosphate 3′-diphosphate, and guanidine 5′-diphosphate 3′-diphosphate (ppGpp). ppGpp (a major signaling component of the stringent responses) accumulates when cells cannot meet the demands of protein synthesis, as in stress situations such as oxidation, starvation, heat, desiccation, and hyperosmolarity/hypersalinity (6). Elevated levels of ppGpp induce the accumulation of polyP by inhibiting polyphosphate exophosphatase PPX activity (41). Mutants of *E. coli* and *Salmonella* that do not produce stringent factors are deficient in the accumulation of polyP (43). polyP represent inorganic adaptors of the ATP-dependent Lon proteases. Interaction of the Lon proteases with ribosomal proteins is strictly dependent on the presence of polyP as adaptors (39). It has been suggested that Lon protease is released from its DNA binding site by first binding to polyP. This mechanism stimulates the proteolytic activity of Lon (42, 59). The degradation of ribosomal proteins would release amino acids for synthesis of the key enzymes required for adaptation to the extreme stresses prevalent in HAAW ecosystems.

Physiologically, polyP are also correlated with protection against oxidative stress generated by stressing conditions. In superoxide dismutase (SOD)-deficient *E. coli* mutants, transient accumulation of polyP is correlated with increased resistance to H_2O_2. Protection against oxidative damage to DNA, which appears to be correlated with the induction of catalase and other DNA repair enzymes, is regulated by the induction of the RpoS sigma factor (3). polyP have been reported to be required for the transcriptional induction of *E. coli* RecA following DNA damage (92). The RecA protein is essential for the repair and maintenance of DNA and has structural and functional homology in many species. Recently, it has been demonstrated that the accumulation of polyP modulates the DNA repair system by regulating the activity and fidelity of DNA polymerases (86). polyP could mimic DNA by binding to DNA binding proteins that regulate DNA reparatory enzymatic machinery, which was demonstrated in vitro, where polyP inhibited restriction enzymes, ligases, *Taq* polymerase (76), and the Lon protein (59).

Under oxidative stress, proteins are subject to conformational changes leading to protein unfolding and aggregation. DnaK and GroEL chaperones are the main drivers of protein folding and, together with other cochaperones, stabilize pro-

teins by promoting appropriate folding and preventing their self-association. polyP can regenerate ATP, which can then be used to synthesize chaperones or to activate efflux ATPases to eliminate toxic metals. In addition, it has been suggested that polyP can also act as a "chemical chaperone" that promotes protein stabilization and the proteolysis of denatured protein and its aggregates. The chaperone activity of polyP is useful in providing amino acids and avoiding toxicity (65). Many effects of polyP can be pleiotropic rather than specific, and their direct roles in most extremophiles have yet to be demonstrated.

In high-altitude extreme environments, UV radiation promotes mutagenesis. Mutations can have lethal consequences but also play an evolutionary role in microbial adaptation to these extreme environments. As discussed above, polyP can regulate DNA protective mechanisms. polyP have also been found to increase the rate of mutagenesis under stress conditions. Recently, Stumpf and coworkers (86) reported that polyP regulate the activity of the *E. coli* Y-family polymerases. These enzymes have reduced fidelity on undamaged templates and have the capacity to replicate through damaged DNA (91). When cells are subjected to environmental stresses, the Pol6 polymerases (members of the Y-family polymerases), which have mutagenic and error bypass activities, are activated, resulting in the induction of Pol Lac^+ and UV- dependent mutations (86). However, the mutagenic and lesion bypass activities of these polymerases are lost if the cells are unable to synthesize polyP (21, 87).

Microorganisms that flourish in HAAW ecosystems are under extreme UV radiation and nutritional stress (14, 99), and many of them have acidocalcisomelike volutin granules (M. J. Seufferheld, unpublished results). It can be argued that under these circumstances, the accumulation of polyP increases the activity of RpoS, thus increasing the cellular levels of Pol4, which would then lead to the generation of adaptive mutations (21) such as the generation of the antibiotic resistances that have been detected in microbial communities of HAAW ecosystems (14, 18). Remarkably, the antibiotic resistances in the microbial consortium of the HAAW ecosystems appear to be in response to UV radiation stress (14, 99). These findings, the first reported in ecosystems isolated from direct human influence, have important epidemiological implications in antibiotic resistance and its relationship with environmental and landscape variables. This ecosystem could serve as a model to study how polyP modulate physiological and molecular responses that impact antibiotic resistance in other locations subjected to elevated UV radiation due to reduced atmospheric ozone.

HAAW ecosystems are nutritionally limited with regard to the availability of inorganic P_i, which is present at only trace levels (14). To overcome P_i starvation, microbes express the two-component system that is composed of two proteins, PhoR and PhoP (24, 77, 85). This system is suggested to regulate the transcription of several genes and operons (collectively known as the Pho regulon) involved in phosphate-scavenging mechanisms, nitrogen metabolism, morphological differentiation, and the production of secondary metabolites. Under conditions of limited P_i, cells are more sensitive to oxidative stress and react by increasing catalase activity (98), which is indirectly activated by PhoP (77). It was recently reported (25) that under P_i starvation and oxidative stress, *Streptomyces lividans* is able to use internal sources of polyP that use PPK to regenerate ATP from ADP and polyP (25). The same study suggested that under these circumstances, both cell growth and the energy charge are low. The ATP generated can be used to activate metabolic pathways that enhance the carbon and phosphate uptake, which is translated to antibiotic synthesis and an increase in oxidative stress (24). Superoxide radicals are responsible for oxidative cell damage through combination with hydrogen peroxide, by which they produce hydroxyl radicals that are capable of strong reactions with the majority of biomolecules (23). ROS can react with any cellular compound at unsaturated bonds and with thiols. The iron-sulfur centers of proteins are particularly sensitive to attack by superoxide radicals. Thus, oxidative stress induced under P_i starvation can increase the levels of cellular iron, which in turn catalyzes the production of more ROS by Fenton reactions, and as a balance response, the Pho regulon responds, inducing iron-scavenging-enzyme genes that prevent the progression of oxidative damage (77).

As Martin and Russell discussed (50), there are many energy sources used by organisms. Proton gradients are a strategy broadly used to generate potential energy for work in a cell. Acidocalcisomes, which are rich in PP_i, (two orthophosphate molecules linked together), are able to generate a proton gradient across their membranes by the action of the H^+-translocating pyrophosphatase. This protein uses pyrophosphate as a substrate to pump H^+ inside the acidocalcisome. Proton gradients are as universal as proteins and nucleic acids, and as an energy source, the acidocalcisome may have played a role in the survival of organisms in both primitive and contemporary extreme environments.

Salinity. Available free water is essential for all cellular biochemistry. The measurement of water availability is expressed as water activity, which depends on the amount of solutes present in the solution. High concentrations of sodium chloride, ranging from 2 to 260 ppm, are present in HAAW ecosystems (13, 14). polyP could have a regulatory function in the responses of microorganisms to stress from high salinity. polyP are directly involved in the regulation of gene expression (36, 44, 68). They also play a role in the promoter selectivity control of RNA polymerase in *E. coli* grown in high concentrations of salt during the stationary phase (45). At high osmolarity, polyP can assist RNA polymerase sigma factors in discriminating between vegetative and stationary-phase promoters (45). Within the HAAW microbial ecosystem, *Archaea* comprise an important component of the community (13, 18). *Methanosarcina mazei* Gö adapts to concentrations of NaCl up to 1 M by inducing genes that encode a phosphate transport system, which results in the increase of transport and synthesis of polyP (64).

Salinity stress is also associated with desiccation, an even more critical environmental condition that microorganisms that colonize hypersaline environments such as HAAW ecosystems encounter (13). Cellular water loss imposes a series of structural, physiologic, and biochemical challenges that microbes must overcome in order to survive. Several studies have suggested that for microorganisms found in extreme environments exposed to hypersalinity, desiccation and UV radiation are capable of producing large amounts of extracellular exopolymers (62, 67). Extracellular polymers enhance biofilm formation and may be major components of the water loss

prevention mechanism that seals the cell (61). As discussed previously, polyP are important players in biofilm formation. It was recently demonstrated that in the absence of polyP, *Pseudomonas aeruginosa* (an environmental and opportunistic human pathogen) was impaired in the production of extracellular polymers, making cells susceptible to desiccation and UV radiation damage and impairing biofilm formation (22).

It has been found that microorganisms exposed to osmotic stress, an unavoidable companion of salinity, develop multiantibiotic resistance (51). In contrast, starvation, which is also associated with salinity stress, can stimulate antibiotic synthesis (24). HAAW ecosystems have been found to be a source for microbial multiantibiotic resistance (18) and of new antibiotics (M. E. Farias, unpublished results).

Heavy metal (arsenic) toxicity. The aquatic ecosystems of the HAAW have high concentrations of arsenic, ranging from 3 mg/liter to 59 mg/liter (13, 14, 49). In these environments, arsenic is the most abundant metal, and the microbial communities from these ecosystems must be capable of coping with elevated concentrations of arsenic (20).

An important function of polyP is the detoxification of heavy metal cations. The sequestration of heavy metals using polyP in bacteria is well documented (26, 33, 35). The hydrolysis of polyP under heavy metal stress has been observed among eukaryotes and prokaryotes alike (32, 58). This mechanism may be involved in the detoxification of heavy metals and the excretion of metal-phosphate complexes out of cells (32, 33, 74). In arsenic-rich aquatic environments, the novel bacterium *Herminiimonas arsenicoxydans* expresses genes encoding inorganic-phosphate transport and phosphate-specific transport systems while accumulating polyP in acidocalcisomelike granules (56). This study showed that the locus of the phosphate transport system is located near the alternative oxidase gene (*aox*) cluster that encodes arsenic resistance genes. Taken together, these data suggest that arsenate, phosphate, and the phosphate transport systems are tightly associated. Arsenic-tolerant bacteria may mobilize polyP as a signal to modulate gene expression and enzyme activity, fulfilling metabolic requirements while transporting phosphate/arsenic in and out of acidocalcisomelike granules and the cell as a detoxification mechanism. It is also important to note that many bacterial species, including the arsenic-tolerant *Acidithiobacillus ferrooxidans* (16), have a Pho regulon that contains the *ppk* and *ppx* genes involved in the synthesis and degradation, respectively, of polyP (96). In an environment with high levels of arsenic and low levels of phosphate, such as the HAAW ecosystems, it is plausible that the Pho regulon system is induced to capture P_i and that the arsenate (structural analog of P_i) present in the medium is incorporated instead. Then, as acidocalcisomes sequester arsenic and its cellular concentration increases, PPX activity is stimulated, mimicking P_i starvation, upon which P_i is released from polyP and the metal-phosphate complexes are transported out of the cells (33).

Exposure to high concentrations of arsenic can also result in the generation of ROS and nitrogen-reactive species (nitrile oxide) that damage DNA and membranes and induce abnormalities in calcium and sulfhydryl homeostasis in the cell (46, 88, 94). Defense and adaptive mechanisms used by *H. arsenicoxydans* include the activation of catalases, DNA repair protein, and DNA polymerase Pol4 (56), each of which has expression regulated by polyP (3, 21, 76, 86, 87). Another strategy used by bacteria is sequestration of heavy metals such as Cr and Pb by biofilm adsorption (31, 90). Recently, it was demonstrated that biofilms from *H. arsenicoxydans* are able to bind large amounts of arsenic by producing extracellular polymers (56) whose synthesis was also proposed to be modulated by polyP (22).

CONCLUDING REMARKS

The multifunctionality of polyP places these molecules at a crossroads between the living and the inorganic world. While polyP have maintained chemical continuity through the course of evolution, their functional roles have diverged and become more specialized. polyP have been found to play fundamental roles in stress resistance in both prokaryotic and eukaryotic organisms and in organisms from bacteria to mammals (blood platelets).

Microorganisms are very dependent on the conditions of their habitats, especially in extreme environments where it is important to conserve P_i and energy. Bioenergetically, polyP is a flexible molecule that can serve as a source of short-term energy released during its hydrolysis. polyP can also generate ATP and proton gradients through the action of PPK and V-H^+-PPase for a more-sustainable supply of energy.

The versatility of polyP is also manifested by their ion exchange properties, which can be used to regulate the homoeostasis of heavy metals and other cations. Since polyP can mimic DNA and complex with RNA, they can influence gene expression under stress conditions. By regulating specific enzymatic activity during stressful conditions, polyP can promote mutagenesis. Therefore, polyP not only modulates adaptive mechanisms that protect organisms from environmental stress but may also be involved in the adaptive evolution of microorganisms in extreme environments.

There is limited information concerning the dynamics of polyP in microbial communities in extreme environments with restricted phosphorous availability. In addition, the range of acidocalcisome distribution among the microbial species of these ecosystems and the relationship between the function of this organelle and stress responses are not known. Examination of microbial communities in extreme ecosystems such as those found in the HAAW ecosystems can provide the basis for functional studies to determine the relationship of phosphorus acquisition and storage and the function of acidocalcisomes among molecular mechanisms and how microorganisms survive in extreme environments.

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