Microbiol Monogr (1)
J.M. Shively: Inclusions in Prokaryotes
DOI 10.1007/7171_003/Published online: 8 April 2006

Acidocalcisomes and Polyphosphate Granules

Roberto Docampo

Department of Cellular Biology and Center for Tropical and Emerging Global Diseases, University of Georgia, 120 Cedar Street, Athens, GA 30602, USA
rdocampo@uga.edu

Abstract Polyphosphate (poly P) granules were one of the first subcellular structures described in bacteria and are characterized by their high content of phosphorus in the form of poly P. Recent work has shown that poly P granules have a limiting membrane and possess an enzymatic mechanism for their acidification. Their electron density and enrichment in pyrophosphate (PP_i), poly P, and cations such as calcium and magnesium are characteristics in common with those of the organelles described as acidocalcisomes in a number of eukaryotic cells, including human platelets, indicating that they have been conserved during evolution from prokaryotes to eukaryotes. Acidocalcisomes have multiple functions that are related to the functions of their main constituents, PP_i, poly P, and cations.

1 Introduction

The metachromatic or volutin granule was one of the first subcellular structures recognized in bacteria (Babes 1895; Meyer 1905). Its name was derived from its property to stain red when treated with toluidine blue and for its presence in the bacterium *Spirillum volutans*, respectively. Volutin granules were later shown to be present in algae, yeasts, and protists, and were named

polyphosphate (poly P) granules when it was found that their numbers in yeasts increased as the amount of this polymer increased (Wiame 1947). In recent years poly P granules were demonstrated to be similar to organelles first described in trypanosomatids and named acidocalcisomes (Vercesi et al. 1994; Docampo et al. 1995). Acidocalcisomes have been detected in a number of protist parasites, in green algae, in slime molds, and in human platelets (dense granules), which indicates that these organelles have been conserved from bacteria to man (Docampo et al. 2005).

Although early reports suggested the presence of a limiting membrane surrounding the granules (Jensen 1968; Friedberg and Avigad 1968), for many years they were assumed to lack an internal structure or limiting membrane (Shively 1974; Shively et al. 1988). More recent work has revealed a surrounding membrane (Seufferheld et al. 2003, 2004). Evidence for the presence of this limiting membrane was (1) its detection by electron microscopy of intact bacteria and subcellular fractions, (2) staining of the granules by dyes that accumulate in closed acidic compartments, and (3) detection by immunofluorescence and immunoelectron microscopy of a membrane-bound vacuolar pyrophosphatase (V-H^+-PPase) in the membrane of these organelles (Seufferheld et al. 2003, 2004) (Fig. 1b).

Most of the functions of acidocalcisomes are related to the presence in them of poly P, a linear chain of inorganic phosphate moieties linked by high-energy phosphoanhydride bonds. Poly P can be a phosphate store or an

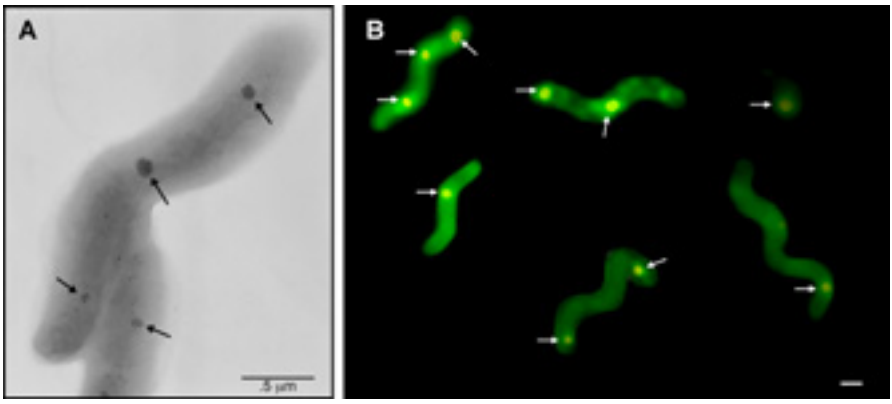

Fig. 1 Electron microscopy and immunofluorescence microscopy of *Rhodospirillum rubrum*. **a** Visualization of acidocalcisomes in whole unfixed cells allowed to adhere to a Formvar- and carbon-coated grid and then observed by transmission electron microscopy. Large granules appear located at bending sites and smaller granules of varying sizes appear distributed in the cytosol (*arrows*). *Bar*0.5 μm. **b** Confocal immunofluorescence analysis of the V-H^+-PPase of *R. rubrum* as detected using polyclonal antibodies against *Arabidopsis thaliana* V-H^+-PPase. *Bar* 0.5 μm. (Reproduced with permission from Seufferheld et al. 2004 *J. Biol. Chem.* 279:51193–51202)

energy source to replace ATP, and can have roles in cation sequestration and storage, cell membrane formation and function, transcriptional control, regulation of enzyme activities, response to stress and stationary phase, and in the structure of channels and pumps. The mobilization of poly P is mainly due to the action of enzymes that catalyze the synthesis and degradation of this polymer—the poly P kinases (PPKs) and the exopolyphosphatases (PPXs), respectively. Mutant strains of bacteria lacking these enzymes have helped in elucidating the structural and regulatory roles of this polymer.

2 Acidocalcisome Structure and Chemical Composition in Bacteria

Acidocalcisomes in bacteria are electron-dense, spherical, and from 15 to about 200 nm in diameter. Their number varies in different species. *Agmenellum quadruplicatum* contains an average of five granules per cell (Nierzwicki-Bauer et al. 1983), *Rhodospirillum rubrum* contains two to three granules per cell (Seufferheld et al. 2004), while *Agrobacterium tumefaciens* usually contains only one granule in one of the polar ends of the cell (Seufferheld et al. 2003) (Fig. 2). They usually occupy about 1% of the cell volume although in extreme cases, such as in metal-treated *Anabena variabilis*, this proportion can rise to 23% of the cell volume (Rachlin et al. 1985). Stress conditions increase the percentage of the cell volume occupied by these organelles. In *Helicobacter pylori* they accumulate under anaerobic conditions (Shirai et al. 2000).

The organelle contains an amorphous and electron-dense material, but the amount seen is dependent on the method of preparation of the sample for

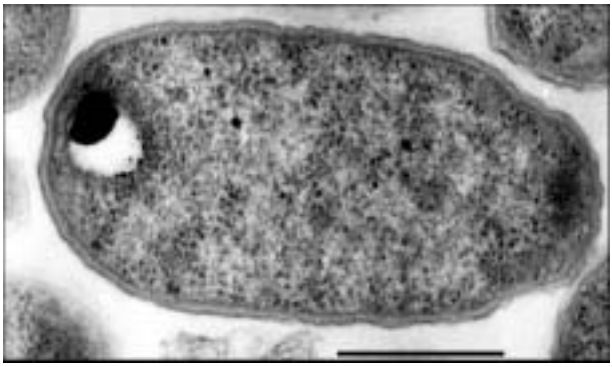

Fig. 2 Electron microscopy of *Agrobacterium tumefaciens*. Electron micrograph of a thin section of a whole bacterium showing an acidocalcisome containing electron-dense material in its periphery. *Bar* 0.5 μm. (Reproduced with permission from Seufferheld et al. 2002 *J. Biol. Chem.* 278:29971–29978)

electron microscopy. When standard staining methods for transmission electron microscopy are used, part of the dense material can be lost, either leaving an empty vacuole or a thin layer of dense material that sticks to the inner face of the membrane. In some bacteria the dense material adheres to one side of the membrane, as an inclusion (Fig. 2). Another useful method to observe acidocalcisomes is to allow whole cells to dry onto carbon- and Formvar-coated grids in the transmission electron microscope. When this technique is used, they appear as electron-dense spheres (Fig. 1a). The dense material appears to volatilize when submitted to the electron beam, giving them a spongelike appearance (Seufferheld et al. 2004).

At the optical microscope level, staining with 4′-6′-diamino-2-phenylindole (DAPI), which stains poly P (Fig. 3), and cycloprodigiosin or Lysosensor blue DND-167, which accumulate in acidic compartments, has also been shown to be effective (Seufferheld et al. 2002, 2004).

Acidocalcisomes of *Agrobacterium tumefaciens* and *R. rubrum* have been shown to possess short-chain and long-chain poly P, as well as pyrophosphate (PP_i) and a number of cations such as calcium, magnesium, and potassium. In other species they have been shown to accumulate large amounts of other metals such as cadmium, cobalt, copper, mercury (Jensen et al. 1982), strontium, barium, manganese (Baxter and Jensen 1980), nickel (Gonzales and

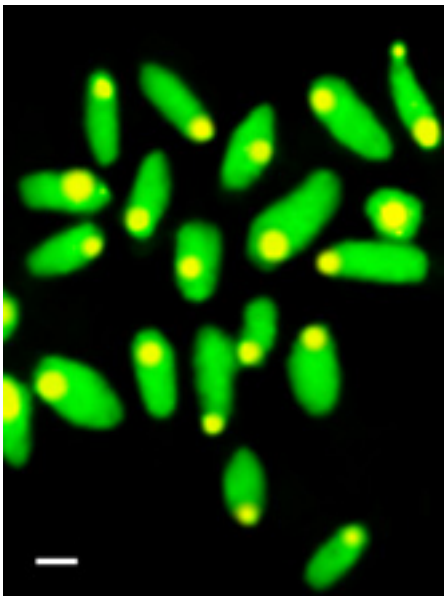

Fig. 3 Staining of acidocalcisomes from *Agrobacterium tumefaciens* with 4′-6′-diamino-2-phenylindole (*DAPI*). Cells were incubated with DAPI as described by Seufferheld et al. (2002), and were observed by fluorescence microscopy. Note the yellow staining at one pole of the cells. *Bar* 0.5 μm

Jensen 1998), lead (Jensen et al. 1982) and aluminum (Torres et al. 1998) when cultivated in their presence. The granules of *Micrococcus lysodeikticus* have been chemically analyzed and shown to contain 24% protein, 30% lipid, 27% poly P, as well as sodium, magnesium, calcium, potassium, manganese, iron, and copper. Zinc was found in the granules from *Plectonema boryanum* (Baxter and Jensen 1980) and is accumulated in *Anabena* spp. (Rachlin et al. 1985). In *Desulfibrio gigas* they were shown to contain a novel metabolite that was identified as α-glucose 1,2,3,4,6-pentakis(diphosphate) (Hensgens et al. 1996).

At least one proton pump has been identified in the membrane lining the acidocalcisomes of *Agrobacterium tumefaciens* and *R. rubrum*: a V-H^+-PPase. The acidocalcisomal V-H^+-PPase of *Agrobacterium tumefaciens* and *R. rubrum* is K^+-insensitive (type 2) and was used as a marker for acidocalcisome purification. The enzyme is also present in the chromatophore membranes of *R. rubrum* (Baltscheffsky et al. 1966). The low sulphur content detected by elemental analysis indicates that few proteins are present in bacterial acidocalcisomes.

3
Acidocalcisome Functions

Most of the functions of acidocalcisomes are those of their main constituents, cations and phosphorus compounds, which are stored in large quantities.

3.1
Phosphorus Storage

Acidocalcisomes are the major storage compartment for phosphorus compounds (orthophosphate, denoted hereafter as P_i, PP_i, and poly P) in several bacteria.

3.1.1
Pyrophosphate

PP_i is a by-product of biosynthetic reactions (e.g., synthesis of nucleic acids, coenzymes, and proteins, activation of fatty acids and isoprenoid synthesis) and is also synthesized in phototrophic bacteria by a reaction catalyzed by the V-H^+-PPase (H^+-pyrophosphate synthase) present in chromatophore membranes. The V-H^+-PPase of *R. rubrum* is capable of PP_i synthesis in the light (Baltscheffsky et al. 1999). Synthesis is driven by the energy derived from the electrochemical H^+ gradient generated across the membrane of the chromatophores during illumination (Baltscheffsky et al. 1999).

PP_i can be used in a number of reactions in bacteria, such as the PP_i-dependent phosphoenolpyruvate carboxykinase (Wood et al. 1977), the pyru-

vate phosphate dikinase (Evans and Wood 1968; Reeves 1968), the PP_i-dependent 6-phosphofructokinase (O'Brien et al. 1975), and in the direct phosphorylation of serine to *O*-phospho-L-serine (Cagen and Friedmann 1972). Hydrolysis of PP_i is catalyzed by soluble pyrophosphatases (sPPases) and vacuolar pyrophosphatases (V-PPases). sPPases are divided into different groups (family I and family II) according to their molecular properties and phylogeny. Family I sPPases are the most widespread, require Mg^{2+}, and are present in the cytosol of archaea, bacteria, fungi and metazoa, as well as in organelles (mitochondria, plastids) (Serrano 2004). The distribution of family II sPPases, first discovered in *Bacillus subtilis* (Young et al. 1998; Shintani et al. 1998), is restricted to archaea and bacteria, and they require Mn^{2+} or Co^{2+} for activity. V-PPases are also divided into two groups, type I, which is K^+-sensitive, and type II, which is K^+-insensitive. Both types are present in archaea, bacteria, protists, and plants and their K^+ sensitivity depends on a single amino acid substitution (Belogurov and Lahti 2002).

Little is know about how PP_i is transported into and out of acidocalcisomes and the reasons for its storage.

3.1.2 Polyphosphate

Poly P is ubiquitous from bacteria to mammals (Kornberg et al. 1999; Kulaev and Kulakovskaya 2000). Poly P has several functions in bacteria.

As a *phosphate store* poly P reduces the osmotic effect of large pools of this important compound. The amount of poly P in bacteria depends on the phosphate content in the medium. Bacteria from wastewaters, such as *Acinetobacter johnsonii* (Deinema et al. 1985), *Microlunatus phosphovorus* (Nakamura et al. 1995), and *Microthrix parvicella* (Erhart et al. 1997) can accumulate large amounts of poly P that can account for to up 30% of their dry biomass (Deinema et al. 1985). There is great interest in the role of poly P accumulation in biological P_i removal from wastewaters (Ohtake et al. 1998). Algal blooms reduce water quality by producing an offensive odor and taste, render boating and fishing difficult, and discourage swimming. When aerobic bacteria consume algae in decomposition there is an increase in dissolved oxygen consumption, causing mass mortality of fish and other aquatic organisms. Algal toxins are also a problem for water supplies. Since P_i is a limiting factor for algal growth in nature, its removal by microorganisms that accumulate it as poly P becomes very important (Ohtake et al. 1998) and efforts have been made to genetically improve bacteria to remove P_i from wastewaters (Kato et al. 1993; Morohoshi et al. 2002). The ability of *Escherichia coli* to accumulate poly P has been enhanced by manipulating the genes involved in the transport and metabolism of P_i and those encoding the enzymes involved in poly P metabolism (Kulaev et al. 1999). A decrease in the level of the PPX and an increase in the level of PPK could lead to an increase in the amount of poly P in

bacteria (Ohtake et al. 1994). When *E. coli* recombinants accumulate high levels of poly P, they release poly P into the medium concomitantly with P_i uptake (Hardoyo et al. 1994), but the mechanism for poly P release is still unclear.

On the other hand, phosphate starvation drastically reduces the amount of poly P in bacteria (Nesmeyanova et al. 1974). When P_i is added to some bacteria, such as *Klebsiella aerogenes*, previously subjected to P_i starvation, they rapidly accumulate poly P, and this is called the poly P overplus (Harold 1966). The mechanism underlying this poly P accumulation is not clearly understood. In *E. coli*, poly P accumulation is not induced by P_i starvation stress alone but takes place as a result of the stringent response (Kuroda and Kornberg 1997). The stringent response is a physiological response caused by a failure in the capacity for transfer RNA aminoacylation to keep up with the demands of protein synthesis (Kuroda and Ohtake 2000). Guanosine 5′-triphosphate 3′-diphosphate (pppGpp) and guanosine 5′-diphosphate 3′-diphosphate (ppGpp) accumulate during the stringent response. ppGpp, which is the major regulatory signal for the stringent response, inhibits the PPX activity without affecting the PPK activity. This leads to slow accumulation of poly P in response to amino acid starvation (Kuroda and Kornberg 1997). Mutants that fail to produce stringent factors are deficient in poly P accumulation in response to amino acid and P_i starvation. As a result of its accumulation, poly P can form a complex with the ATP-dependent protease Lon, and can promote ribosomal protein degradation, thereby supplying the amino acids needed to respond to starvation (Kuroda et al. 2001). It has been shown that poly P first binds to Lon, which stimulates Lon-mediated degradation of ribosomal proteins. Poly P can also compete with DNA for binding Lon, suggesting that poly P may control the cellular activity of Lon not only as a protease but also as a DNA-binding protein. Poly P binding is hypothesized to activate Lon by freeing it from DNA (Nomura et al. 2004). If poly P competes with DNA or RNA for binding sites to other proteins, this may explain how poly P levels influence many different cellular processes, including DNA replication (Stumpf and Foster 2005).

Poly P in bacterial acidocalcisomes could potentially have similar roles to those demonstrated in eukaryotic acidocalcisomes, such as a buffer against alkaline stress or in recovery of cell volume after hypo-osmotic stress (Docampo et al. 2005), but these functions have not been investigated in bacteria.

In addition to its functions within acidocalcisomes, bacterial poly P is a component of the cell capsule in *Neisseria* species (Tinsley et al. 1993). Poly P also forms a complex with Ca^{2+} and poly(β-hydroxybutyrate) in the membrane of *E. coli* that is believed to act as a channel allowing DNA passage through the cells during transformation (Huang and Reusch 1995) or to act as an ion channel (Reusch and Sadoff 1988; Zakharian and Reusch 2004). It has been postulated that the complex is a double helix, in which the outer chain is formed by poly(β-hydroxybutyrate) and the inner chain by poly P molecules linked to each other by Ca^{2+} ions (Reusch 1999).

Poly P can also be used as an energy donor in a number of reactions in bacteria. The PPK can convert poly P to ATP using ADP (Kornberg 1995) (Eq. 1):

$$\text{Poly P}_n + n\text{ADP} \rightarrow n\text{ATP}\,. \quad (1)$$

An AMP phosphotransferase could use AMP and poly P to generate ADP (Eq. 2), which is then converted to ATP by coupling with PPK (Eq. 3) or with adenylate kinase (Eq. 4) (Ishige and Noguchi 2000, 2001):

$$\text{Poly P}_n + \text{AMP} \rightarrow \text{Poly P}_{n-1} + \text{ADP}\,, \quad (2)$$

$$\text{Poly P}_n + \text{ADP} \rightarrow \text{Poly P}_{n-1} + \text{ATP}\,, \quad (3)$$

$$2\text{ADP} \longleftrightarrow \text{AMP} + \text{ATP}\,, \quad (4)$$

$$\text{Poly P}_n + \text{glucose} \rightarrow \text{Poly P}_{n-1} + \text{Glucose 6-P}\,. \quad (5)$$

AMP phosphotransferase has been identified in *Acinetobacter* (Bonting et al. 1991), *E. coli*, and *Myxococcus xanthus* (Kornberg 1995), while adenylate kinase is a ubiquitous enzyme (Kornberg 1995). Poly P can also replace ATP in the phosphorylation of glucose catalyzed by glucokinases (Eq. 5) (Hsieh et al. 1993), and can also phosphorylate proteins in *Sulfolobus acidocaldarius* (Skorko 1989). Enzymes with both poly P- and ATP-dependent NAD kinase activities were isolated from *Micrococcus flavus* and *Mycobacterium tuberculosis* (Kawai et al. 2000), The gene for a poly P- and ATP-dependent NAD kinase from *B. subtilis* has been cloned, expressed, and characterized (Garavaglia et al. 2003). A novel poly P- and ATP-dependent glucomannokinase was isolated from the bacterium *Arthrobacter* sp., which also has several poly P- and ATP-dependent kinases, including glucokinase, NAD kinase, mannokinase, and fructokinase (Mukai et al. 2003). The gene encoding this enzyme was found to be homologous to glucokinases of other bacteria and to proteins of unknown function, and the crystal structure of its protein product has been resolved recently (Mukai et al. 2004).

Since the metabolic turnover of ATP is considerably higher than that of poly P (Chapman and Atkinson 1977), it has been suggested (Rao and Kornberg 1996) that poly P is not an efficient supply of energy and that it has a mainly a regulatory role.

3.2 Cation Storage

Bacterial acidocalcisomes are the main storage compartment for calcium, magnesium, sodium, potassium, and other cations, which are combined with poly P. Poly P in acidocalcisomes can also accumulate heavy-metal cations when they are present in the environment (Kulaev et al. 1999). As indicated earlier poly P within acidocalcisomes can sequester nickel, cadmium, lead, and other heavy metals. Their accumulation into the cells also stimulates the

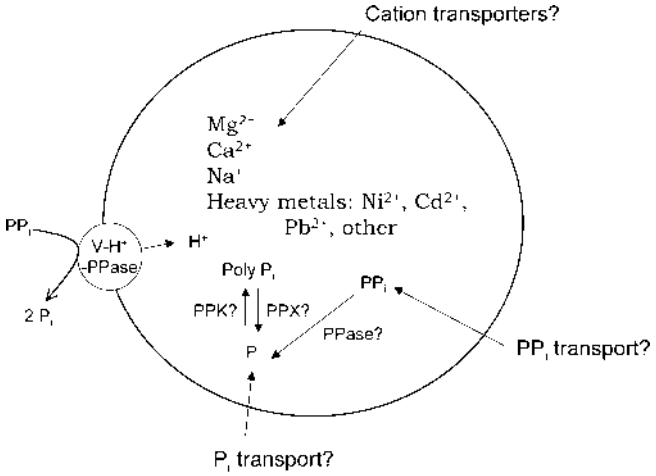

Fig. 4 Representation of a bacterial acidocalcisome. An aminomethylene diphosphonate sensitive vacuolar H^+-PPase is responsible for proton uptake. Other transporters (e.g., cation, P_i, and PP_i transporters) are probably present. The acidocalcisome is rich in PP_i, poly P, magnesium, calcium, and sodium and can accumulate heavy metals. Enzymes involved in PP_i and poly P metabolism (PPX, PPK, PPase) could also be present. See text for an explanation of the abbreviations

PPX activity, releasing P_i from poly P and the metal–phosphate complexes can be transported out of the cells (Keasling and Hupf 1996; Keasling 1997). It has been shown that poly P generated in recombinant bacteria confers mercury resistance (Pan-Hou et al. 2002).

In some cases the chelating properties of poly P could play a significant role in cell metabolism. For example, *Lactobacillus plantarum*, which is devoid of a superoxide dismutase, an important enzyme for the detoxification of superoxide anion, contains very high concentrations of Mn^{2+} (30 mM) that is chelated to 60 mM poly P and effectively replaces the function of a superoxide dismutase (Archibald and Fridovich 1982). Figure 4 shows a schematic drawing of the structure of a bacterial acidocalcisome.

4
Enzymes Involved in Poly P Synthesis and Degradation

In bacteria the mobilization of poly P is performed primarily by the action of enzymes that catalyze the synthesis and degradation of this polymer. The most important enzymes involved in the synthesis of poly P in bacteria are the PPKs. Two PPKs have been described: PPK1, which catalyzes the reversible transfer of phosphate residues from ATP to poly P and from poly P to ADP (Eq. 1); and PPK2, which catalyzes the synthesis of poly P from GTP

or ATP (Zhang et al. 2002; Ishige et al. 2002). The gene encoding PPK1 was first cloned from *E. coli* (Akiyama et al. 1992), and was later found in several bacteria. The gene encoding PPK2 was first found in certain mutants of *Pseudomonas aeruginosa* that lacked PPK1 and still had persistent levels of poly P (Zhang et al. 2002). BLAST searches have detected homologs to PPK2 in 32 species that contained both PPK1 and PPK2, only three species contained only PPK2, and 43 species contained only PPK1 (Zhang et al. 2002). Interestingly, mutants deficient in PPK1 in one of these species, *E. coli*, have been shown to still possess poly(β-hydroxybutyrate)–calcium–poly P membrane complexes (Castuma et al. 1995). This indicates that another PPK with ability to synthesize poly P chains of about 60 P_i residues (those present in the complexes) must be present in *E. coli*.

PPK1 is membrane-bound and has been studied in more detail. The first step of PPK1-catalyzed poly P synthesis involves the autophosphorylation of its histidine residues, and PPK is therefore a histidine kinase. The crystal structure of *E. coli* PPK1 has been resolved recently (Zhu et al. 2005). PPK forms an interlocked dimer, with each 80-kDa monomer containing four structural domains. The PPK active site is located in a tunnel, which contains a unique ATP-binding pocket and may accommodate the translocation of synthesized poly P (Zhu et al. 2005). The two closely related carboxy-terminal domains (C1 and C2 domains) of PPK are structurally similar to the catalytic domain of phospholipase D (PLD). It is interesting to note that the first step of the PLD reaction is the transfer of a phosphate moiety to a PLD histidine residue (Zhu et al. 2005). PPK1 can catalyze, in addition to the synthesis of poly P from ATP, the conversion of ADP back to ATP, and the conversion of other nucleotides, especially GDP to GTP (nucleoside diphosphate kinase activity, Eq. 6). Another reaction is the transfer of a pyrophosphoryl group from poly P to GDP to form the linear guanosine 5′-tetraphosphate (Eq. 7) (ppppG; Kornberg et al. 1999).

$$\text{Poly P}_n + n\text{GDP} \rightarrow \text{ Poly P}_{n-1} + n\text{GTP} \tag{6}$$

$$\text{Poly P}_n + \text{ GDP} \rightarrow \text{ Poly P}_{n-2} + \text{pppG} \tag{7}$$

PPK1 associates to the *E. coli* degradosome (Blum et al. 1997). This is a multienzyme complex with four major components: the endoribonuclease RNase E, the exoribonuclease polynucleotide phosphorylase (PNPase), the RNA helicase RhlB, and enolase. The first three of these proteins have important functions in mesenger RNA (mRNA) processing and degradation. By virtue of its RNA binding activity PPK1 might promote the assembly of the degradosomes or its interaction with the RNA to be degraded. In addition, since it converts poly P to ATP using ADP, it would remove poly P, which is known to be a potent inhibitor of mRNA degradation, and ADP, which is a potent inhibitor of PNPase, in the degradosome. Mutations in PPK1 increased mRNA stability. However, since PPK1 is not essential it probably

plays a modulatory role rather than a core role in mRNA degradation (Blum et al. 1997).

PPK2 has been shown to differ from PPK1 is several aspects: synthesis of poly P from GTP or ATP, a preference for Mn^{2+} over Mg^{2+}, and stimulation by poly P. The reverse reaction, a poly P-driven nucleoside diphosphate kinase synthesis of GTP from GDP, is 75-fold faster than the forward reaction (Zhang et al. 2002; Ishige et al. 2002).

PPX is one of the most important enzymes involved in poly P degradation in bacteria. The PPX of *E. coli* is a homodimer of a 58-kDa subunit and, as PPK, is membrane-bound (Akiyama et al. 1993). The gene encoding PPX (*ppx*) is in the same operon as the gene encoding PPK (*ppk*) in *E. coli* and in other but not all bacteria (Kornberg et al. 1999). PPX splits P_i from the end of long-chain poly P in a processive manner (Eq. 8):

$$\text{Poly } P_n + (n-2)H_2O \rightarrow (n-2)P_i + PP_i\,. \tag{8}$$

Another *E. coli* PPX was shown to be responsible for the conversion of pppGpp to the tetraphosphate (ppGpp), which is the most active nucleotide involved in the stringent cellular response to deficiencies in amino acids and P_i (Keasling et al. 1993). The structural characterization of *Aquifex aeolicus* PPX/guanosine pentaphosphate phosphohydrolase (GPPA) has been determined (Kristensen et al. 2004). The protein has a two-domain structure with an active site located in the interdomain cleft. A calcium ion was observed at the center of the active site, substantiating that PPK/GPPA enzymes use metal ions for catalysis.

A specific tripolyphosphatase was purified from *Methanobacterium thermoautotrophicum* (van Alebeek et al. 1994), and the *S*-adenosyl methionine synthetase, first isolated from *E. coli*, is known to catalyze cleavage of tripolyphosphate (poly P_3), a reaction that is stimulated by adenosylmethionine (AdoMet). Both enzymatic activities require a divalent metal ion and are markedly stimulated by certain monovalent cations. AdoMet synthesis also takes place if adenyl-5′yl imidodiphosphate (AMP-PNP) is substituted for ATP. The imidotriphosphate (PPNP) formed is not hydrolyzed, permitting dissociation of AdoMet formation from poly P_3 cleavage (Markham et al. 1980). Two isofoms were found in *Sulfolobus solfataricus* but the tripolyphosphatase activity of only only one of them is stimulated by AdoMet (Porcelli et al. 1988).

5
Functions of Poly P Discovered by the Manipulation of the Expression of Genes Involved in Their Synthesis and Degradation

Disruption of the *ppk* gene in *E. coli* led to a disruption of the *ppx* gene (located in the same operon) and to a drastic reduction in the poly P content.

Poly P deficient cells exhibited a striking phenotype characterized by their failure to survive in the stationary phase and loss of resistance to heat, oxidants, and osmotic challenge (Crooke et al. 1994; Rao and Kornberg 1996). High poly P levels were also associated with reduced survival (Crooke et al. 1994). Lack of *ppk* in *E. coli* also impaired the induction of amino acid biosynthetic enzymes and the cells were defective in their adaptation to nutritional deprivation (Kuroda et al. 1999). Poly P levels in *E. coli* have also been reduced by overexpression of yeast PPX, leading to decreased resistance to H_2O_2 (Shiba et al. 1997). The expression of *rpoS*, the gene encoding the stationary-phase-specific RNA polymerase σ factor that governs the expression of many genes is affected by the lack of poly P (Shiba et al. 1997). The demonstration of a functional interaction between *E. coli* RNA polymerase and poly P has led to the suggestion that poly P may play a role in the promoter selectivity control of RNA polymerase (Kusano and Ishihama 1997).

Disruption of the *ppk* gene in six bacterial pathogens greatly reduced their motility on semisolid agar plates likely owing to altered functioning of the flagella (Rashid et al. 2000). In *Pseudomonas aeruginosa* this disruption rendered them unable to form a thick and differentiated biofilm, caused alterations in quorum sensing and virulence (Rashid et al. 2000b), and affected their swimming, swarming, and twitching motilities (Rashid and Kornberg 2000). Disruption of *ppk* in *Porphyromonas gingivalis*, one of the agents causing periodontitis, also resulted in deficient biofilm formation (Chen et al. 2002). Disruption of *ppk* in *Shigella* sp. and *Salmonella* spp. produced growth defects, defective responses to stress and starvation, intolerance to acid and heat, and diminished invasiveness in epithelial cells (Kim et al. 2002), while disruption of *ppk* in *B. cereus* resulted in addition to defects in motility and biofilm formation, in a defective sporulation (Shi et al. 2004). The *null* mutant of *ppk1* in *Myxococcus xanthus* was defective in social motility, overproduced pilin protein on the cell surface, was delayed in fruiting body formation, produced fewer spores, was delayed in germination, and had reduced predation, while the *null* mutant in poly P : AMP phosphotransferase (*pap*) showed only slightly reduced abilities in development and predation (Zhang et al. 2005). In agreement with all these results it has been shown that a modification in the *ppk* gene of *H. pylori* that led to higher enzymatic activity of PPK resulted in a better capacity of the bacteria to colonize mice, suggesting that PPK is an important virulence factor (Ayraud et al. 2003). In contrast with these results, although *ppk1* inactivation caused the expected near-complete absence of poly P in all strains, it had phenotypic effects that differed markedly among unrelated strains of *H. pylori* (Tan et al. 2005).

Recent work has shown that an insertion mutation in *ppk* casues a decrease in adaptive mutation in *E. coli* strain FC40. The results suggest a novel mechanism involving poly P that directly or indirectly regulates DNA polymerase activity or fidelity (Stumpf and Foster 2005).

6 Conclusions

Acidocalcisomes or poly P granules were found in bacteria more than 100 years ago but their study, as well as the study of the main constituent, poly P, has been neglected for many years. The conservation of this organelle from bacteria to humans implies important functions that await discovery. Further studies are necessary to understand the biogenesis and function of acidocalcisomes in bacteria, and how widely distributed the organelle is.

References

Akiyama M, Crooke E, Kornberg A (1992) The polyphosphate kinase gene of *Escherichia coli*. Isolation and sequence of the ppk gene and membrane location of the protein. J Biol Chem 267:22556–22561

Akiyama M, Crooke E, Kornberg A (1993) An exopolyphosphatase of *Escherichia coli*. The enzyme and its *ppx* gene in a polyphosphate operopn. J Biol Chem 268:633–639

Archibald FS, Fridovich I (1982) Investigations of the state of the manganese in *Lactobacillus plantarum*. Arch Biochem Biophys 215:589–596

Ayraud S, Janvier B, Salaun L, Fauchere JL (2003) Modification in the *ppk* gene of *Helicobacter pylori* during single and multiple experimental murine infections. Infect Immun 71:1733–1739

Babes V (1985) Beoachtungen über die metachromatischen Körperchen, Sporenbildung, Verzweigung, Kolben- und Kapselbildung pathogener Bakterien. Z Hyg Infektkr 20:412–420

Baltscheffsky H, Von Sterdingk LV, Heldt HW, Klingenberg M (1966) Inorganic pyrophosphate: formation in bacterial photophosphorylation. Science 153:1120–1122

Baltscheffsky M, Schultz A, Baltscheffsky H (1999) H^+-PPases: a tightly membrane-bound family. FEBS Lett 457:527–533

Baxter M, Jensen T (1980) Uptake of magnesium, strontium, barium, and manganese by *Plectonema boryanum* (Cyanophyceae) with special reference to polyphosphate bodies. Protoplasma 104:81–89

Belogurov GA, Lahti R (2002) A lysine substitute for K^+. A46K mutation elim inates K^+ dependence in H^+-pyrophosphatase of *Carboxydothermus hydrogenoformans*. J Biol Chem 277:49651–49654

Blum E, Py B, Carpousis AJ, Higgins CF (1997) Polyphosphate kinase is a component of the *Escherichia coli* RNA degradosome. Mol Microbiol 26:387–398

Bonting CFC, Korstee GJJ, Zehnder AJB (1991) Properties of polyphosphate:AMP phosphotransferase of *Acinetobacter* strain 210A. J Bacteriol 173:6484–6488

Cagen LM, Friedmann HC (1972) Enzymatic phosphorylation of serine. J Biol Chem 247:3382–3392

Castuma CE, Huang R, Kornberg A, Reusch RN (1995) Inorganic polyphosphates in the acquisition of competence in *Escherichia coli*. J Biol Chem 270:12980–12983

Chapman AG, Atkinson DE (1977) Adenine nucleotide concentrations and turnover rates. The correlation with biological activity in bacteria and yeast. Adv Microbiol Physiol 15:253–306

Chen W, Palmer RJ, Kuramitsu HK (2002) Role of polyphosphate kinase in biofilm formation by *Porphyromonas gingivalis*. Infect Immun 70:4708–4715

Crooke E, Akiyama M, Rao NN, Kornberg A (1994) Genetically altered levels of inorganic polyphosphate in *Escherichia coli*. J Biol Chem 269:6290–6295

Deinema MH, VanLoosdrecht M, Scholten A (1985) Some physiological characteristics of *Acitenobacter spp*. accumulating large amounts of phosphate. Water Sci Technol 17:119–125

Docampo R, Scott DS, Vercesi AE, Moreno SNJ (1995) Intracellular Ca^{2+} storage in acidocalcisomes of *Trypanosoma cruzi*. Biochem J 310:1005–1012

Docampo R, de Souza W, Miranda K, Rohloff P, Moreno SNJ (2005) Acidocalcisomes—conserved from bacteria to man. Nat Rev Microbiol 3:251–261

Erhart R, Bradford D, Sevior RJ, Amann R, Blackall LL (1997) Development and use of fluorescent in situ hybridization probes for the detection and identification of *Micotrix parvicella* in activated sludge. Syst Appl Microbiol 20:310–318

Evans HJ, Wood HG (1968) The mechanism of the pyruvate, phosphate dikinase reaction. Proc Natl Acad Sci USA 61:1448–1453

Friedberg I, Avigad G (1968) Structures containing polyphosphate in *Micrococcus lysodeikticus*. J Bacteriol 96:544–553

Garavaglia S, Galizzi A, Rizzi M (2003) Allosteric regulation of *Bacillus subtilis* NAD kinase by quinolinic acid. J Bacteriol 185:4844–4850

Gonzalez H, Jensen TE (1998) Nickel sequestration by polyphosphate bodies in *Staphylococcus aureus*. Microbios 93:179–185

Hardoyo, Yamada K, Shinjo H, Kato J, Ohtake H (1994) Production and release of polyphosphate by a genetically engineered strain of *Escherichia coli*. Appl Environm Microbiol 60:3485–3490

Harold FM (1966) Inorganic polyphosphates in biology: structure, metabolism, and function. Bacteriol Rev 30:772–794

Hensgens CM, Santos H, Zhang C, Kruizinga WH, Hansen TA (1996) Electron-dense granules in *Desulfibrio gigas* do not consist of inorganic triphosphate but of a glucose pentakis(diphosphate). Eur J Biochem 242:327–331

Hsieh P-C, Shenoy BC, Jentoft JE, Phillips NFB (1993) Purification of polyphosphate and ATP glucose phosphotransferase from *Mycobacterium tuberculosis* $H_{37}Ra$: evidence that poly (P) and ATP glucokinase activities are catalyzed by the same enzyme. Protein Expr Purif 4:76–84

Huang RP, Reusch RN (1995) Genetic competence in Escherichia coli requires poly-beta-hydroxybutirate calcium polyphosphate membrane complex and certain divalent cations. J Bacteriol 177:486–490

Ishige K, Noguchi T (2000) Inorganic polyphosphate kinase and adenylate kinase participate in the polyphosphate:AMP phosphotransferase activity of *Escherichia coli*. Proc Natl Acad Sci USA 97:14168–14171

Ishige K, Noguchi T (2001) Polyphosphate:AMP phosphotransferase and polyphosphate:ADP phosphotransferase activities in *Pseudomonas aeruginosa*. Biochem Biophys Res Commun 281:821–826

Ishige K, Zhang H, Kornberg A (2002) Polyphosphate kinase (PPK2), a potent polyphosphate-driven generator of GTP. Proc Natl Acad Sci USA 99:16684–16688

Jensen TE (1968) Electron microscopy of polyphosphate bodies in a blue-green alga, *Nostoc pruniforme*. Arch Mikrobiol 62:144–152

Jensen TE, Rachlin JW, Jani V, Warkentine B (1982) An X-ray energy dispersive study of cellular compartmentalization of lead and zinc in *Chlorella saccharophila* (Chlorophyta), *Navicula incerta* and *Nitzschia closterium* (Bacillariophyta). Environ Exp Bot 22:319–328

Kato J, Yamada K, Muramatsu A, Hardoyo T, Ohtake H (1993) Genetic improvement of Escherichia coli for the enhanced biological removal of phosphate. Appl Environ Microbiol 59:3744–3749

Kawai S, Mori S, Mukai T, Suzuki S, Yamada T, Hashimoto W, Murata K (2000) Inorganic polyphosphate/ATP-NAD kinase of *Micrococcus flavus* and *Mycobacterium tuberculosis* H_{37}Rv. Biochem Biophys Res Commun 276:57–63

Keasling JD (1997) Regulation of intracellular toxic metals and other cations by hydrolysis of polyphosphate. Ann New York Acad Sci 829:243–249

Keasling JD, Bertsch LR, Kornberg A (1993) Guanosine pentaphosphate phosphohydrolase of *Escherichia coli* is a long chain exopolyphosphatase. Proc Natl Acad Sci USA 90:7029–7033

Keasling JD, Hupf GA (1996) Genetic manipulation of polyphosphate metabolism affects cadmium tolerance in *Escherichia coli*. Appl Environ Microbiol 62:743–746

Kim KS, Rao NN, Fraley CD, Kornberg A (2002) Inorganic polyphosphate is essential for long-term survival and virulence factors in *Shigella* and *Salmonella* spp. Proc Natl Acad Sci USA 99:7675–7680

Kornberg A (1995) Inorganic polyphosphate: toward making a forgotten polymer unforgettable. J Bacteriol 177:491–496

Kornberg A, Rao NN, Ault-Riché D (1999) Inorganic polyphosphate: a molecule of many functions. Annu Rev Biochem 68:89–125

Kristensen O, Laurberg M, Liljas A, Kastrup JS, Gajhede M (2004) Structural characterization of the stringent response related exopolyphosphatase/guanosine pentaphosphate phosphohydrolase protein family. Biochem 43:8894–8900

Kulaev I, Kulakovskaya T (2000) Polyphosphate and phosphate pump. Annu Rev Microbiol 54:709–734

Kulaev I, Vagabov V, Kulakovskaya T (1999) New aspects of inorganic polyphosphate metabolism and function. J Biosci Bioeng 88:111–129

Kuroda A, Murphy M, Cashel M, Kornberg A (1997) Guanosine tetra- and pentaphosphate promote accumulation of inorganic polyphosphate in *Escherichia coli*. J Biol Chem 272:21240–21243

Kuroda A, Ohtake H (2000) Molecular analysis of polyphosphate accumulation in bacteria. Biochemistry (Moscow) 65:304–308

Kuroda A, Tanaka S, Ikeda T, Kato J, Takiguchi N, Ohtake H (1999) Inorganic polyphosphate kinase is required to stimulate protein degradation and the adaptation to amino acid starvation in *Escherichia coli*. Proc Natl Acad Sci USA 96:14264–14269

Kuroda A, Nomura K, Ohtomo R, Kato J, Ikeda T, Takiguchi N, Ohtake H, Kornberg A (2001) Role of inorganic polyphosphate in promoting ribosomal protein degradation by the Lon protease in *E. coli*. Science 293:705–708

Kusano S, Ishihama A (1997) Functional interaction of *Escherichia coli* RNA polymerase with inorganic polyphosphate. Genes Cells 2:433–441

Markham GD, Hafner EW, Tabor CW, Tabor H (1980) *S*-Adenosylmethionine synthetase from *Escherichia coli*. J Biol Chem 255:9082–9092

Meyer A (1904) Orientierende Untersuchungen über Verbreitung, Morphologie und Chemie des Volutins. Bot Z 62:113–152

Morohoshi T, Maruo T, Shirai Y, Kato J, Ikeda T, Takiguchi N, Ohtake H, Kuroda A (2002) Accumulation of inorganic polyphosphate in *phoU* mutants of *Escherichia coli* and *Synechocystis* sp. strain PCC6803. Appl Environm Microbiol 68:4107–4110

Mukai T, Kawai S, Matsukawa H, Matuo Y, Murata K (2003) Characterization and molecular cloning of a novel enzyme, inorganic polyphosphate/ATP-glucomannokinase, of *Arthrobacter* sp. strain KM. App Environm Microbiol 69:3849–3857

Mukai T, Kawai S, Mori S, Mikami B, Murata K (2004) Crystal structure of bacterial inorganic polyphosphate/ATP-glucomannokinase. Insights into kinase evolution. J Biol Chem 279:50591–50600

Nakamura K, Hiraishi A, Yoshimi Y, Kawaharasaki M, Masuda K, Kamagata Y (1995) *Microlunatus phosphovorus* gen. nov. sp. nov., a new gram-positive polyphosphate-accumulating bacterium isolated form activated sludge. Int J Syst Bacteriol 45:17–22

Nesmeyanova MA, Gonina SA, Kulaev IS (1975) Bionsynthesis of poly P and alkaline phosphatase in *E. coli* is under control of integrated regulatory genes. Dokl Akad Nauk SSSR 224:710–712

Nierzwicki-Bauer SA, Balkwill DL, Stevens SE Jr (1983) Three-dimensional ultrastructure of a unicellular cyanobacterium. J Cell Biol 97:713–722

Nomura K, Kato J, Takiguchi N, Ohtake H, Kuroda A (2004) Effects of inorganic polyphosphate on the proteolytic and DNA-binding activities of Lon in *Escherichia coli*. J Biol Chem 279:34406–34410

O'Brien WE, Bowien S, Wood HG (1975) Isolation and characterization of a pyrophosphate-dependent phosphofructokinase from *Propionobacterium shermanii*. J Biol Chem 250:8690–8695

Ohtake H, Yamada K, Hardoyo, Muramatsu A, Anbe Y, Kato J, Shinjo H (1994) Genetic approach to enhanced biological phosphorous removal. Wat Sci Technol 30:185–192

Ohtake H, Kato J, Kuroda A, Ikeda T (1998) Regulation of bacterial phosphate taxis and polyphosphate accumulation in response to phosphate starvation stress. J Biosci 23:491–499

Pan-Hou H, Kiyono M, Omura H, Omura T, Endo G (2002) Polyphosphate produced in recombinant *Escherichia coli* confers mercury resistance. FEMS Microbiol Lett 10325:159–164

Porcelli M, Cacciapuoti G, Carteni-Farina M, Gambacorta A (1988) *S*-adenosylmethionine in the thrermophilic archaebacterium *Sulfolobus solfataricus*. Purification and characterization of two isoforms. Eur J Biochem 177:273–280

Rachlin JW, Jensen TE, Warkentine B (1985) Morphometric analysis of the response of *Anabena flos-aquae* and *Anabena variabilis* (Cyanophyceae) to selected concentrations of zinc. Arch Enrivon Contam Toxicol 14:395–400

Rao NN, Kornberg A (1996) Inorganic polyphosphate supports resistance and survival of stationary-phase *Escherichia coli*. J Bacteriol 1778:1394–1400

Rashid MH, Kornberg A (2000) Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 97:4885–4890

Rashid MH, Rao NN, Kornberg A (2000) Inorganic polyphosphate is required for motility of bacterial pathogens. J Bacteriol 182:225–227

Rashid MH, Rumbaugh K, Passador L, Davies DG, Hamood AN, Iglewski BH, Kornberg A (2000b) Poplyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 97:9636–9641

Reeves RE (1968) A new enzyme with the glycolytic function of pyruvate kinase. J Biol Chem 243:3202–3204

Reusch RN (1999) Polyphosphate/poly-(R)-3-hydroxybutyrate ion channels in cell membranes. In: Schröder HC, Müller WEG (eds) Progress in molecular and subcellular biology, vol 23. Springer, Berlin Heidelberg New York, pp 151–182

Reusch RN, Sadoff HL (1988) Putative structure and functions of poly-beta-hydroxybutyrate/calcium polyphosphate channel in bacterial plasma membranes. Proc. Natl Acad Sci USA 85:4176–4180

Serrano A (2004) Comparative functional and structural genomics of inorganic pyrophosphatase reveal dynamic evolutionary stories. In: White SA (ed) Recent advances in inorganic pyrophosphatase research. The University of Birmingham, Birmingham, pp 12–17

Seufferheld M, Vieira MCF, Ruiz FA, Rodrigues CO, Moreno SNJ, Docampo R (2003) Identification in bacteria of organelles similar to acidocalcisomes of unicellular eukaryotes. J Biol Chem 278:29971–29978

Seufferheld M, Lea C, Vieira M, Oldfield E, Docampo R (2004) The H^+-pyrophosphatase of *Rhodospirillum rubrum* is predominantly located in polyphosphate-rich acidocalcisomes. J Biol Chem 279:51193–51202

Shi X, Rao NN, Kornberg A (2004) Inorganic polyphosphate in *Bacillus cereus*: motility, biofilm formation, and sporulation. Proc Natl Acad Sci 101:17061–17065

Shiba T, Tsutsumi K, Yano H, Ihara Y, Kamedaa A, Tanaka K, Takahashi H, Munekata M, Rao NN, Kornberg A (1997) Inorganic polyphosphate and the induction of *rpoS* expression. Proc Natl Acad Sci USA 94:11210–11215

Shintani T, Uchiumi T, Yonezawa T, Salminen A, Baykov AA, Lahti R, Hachimori A (1998) Cloning and expression of a unique inorganic pyrophosphatase from *Bacillus subtilis*: evidence for a new family of enzymes. FEBS Lett 439:263–266

Shirai M, Kakada J, Shibata K, Morshed MG, Matsushita T, Nakazawa T (2000) Accumulation of polyphosphate granules in *Helicobacter pylori* cells under anaerobic conditions. J Med Microbiol 49:513–519

Shively JM (1974) Inclusion bodies of prokaryotes. Annu Rev Microbiol 28:167–187

Shively JM, Bryant DA, Fuller RC, Konopka AE, Stevens SE Jr, Strohl WR (1988) Functional inclusions in prokaryotic cells. Int Rev Cytol 113:35–100

Skorko R (1989) Polyphosphate as a source of phosphoryl group in protein modification in the archaebacterium *Sulfolobus acidocaldarius*. Biochemie 71:1089–1093

Stumpf JD, Foster PL (2005) Polyphosphate kinase regulates error-prone replication by DNA polymerase IV in *Escherichia coli*. Mol Microbiol 57:751–761

Tan S, Fraley CD, Zhang M, Dailidiene D, Kornberg A, Berg DE (2005) Diverse phenotypes resulting from polyphosphate kinase gene (*ppk1*) inactivation in different strains of *Helicobacter pylori*. J Bacteriol 187:7687–7695

Tiensley CR, Manjula BN, Gotschlich EC (1993) Purification and characterization of polyphosphate kinase from *Neisseria meningitides*. Infect Immun 61:3703–3710

Torres M, Goldberg J, Jensen TE (1998) Heavy metal uptake by polyphosphate bodies in living and killed cells of *Plectonema boryanum* (Cianophycae). Microbios 96:141–147

Van Alebeek GJWM, Kejens JT, van der Drift C (1994) Tripolyphosphatase from *Methanobacterium thermoautotrophicum* (strain DH). FEMS Microbiol Lett 117:263–268

Vercesi AE, Moreno SNJ, Docampo R (1994) Ca^{2+}/H^+ exchange in acidic vacuoles of *Trypanosoma brucei*. Biochem J 304:227–233

Wiame JH (1947) Etude d'une substance polyphosphorée, basophile et m'tachromatique chez les levures. Biochim Biophys Acta 1:234–255

Wood HG (1977) Some reactions in which inorganic pyrophosphate replaces ATP and serves as a source of energy. Fed Proc 36:2197–2205

Young TW, Kuhn NJ, Wadeson A, Ward S, Burges D, Cooke GD (1998) *Bacillus subtilis* ORF yybQ encodes a manganese-dependent inorganic pyrophosphatase with distinctive properties: the first of a new class of soluble pyrophosphatase? Microbiology 144:2563–2571

Zakharian E, Reusch RN (2004) Functional evidence for a supramolecular structure for the *Streptomyces lividans* potassium channel KcsA. Biochem Biophys Res Commun 322:1059–1065

Zhang H, Ishige K, Kornberg A (2002) A polyphosphate kinase (PPK2) widely conserved in bacteria. Proc Natl Acad Sci USA 99:16678–16683

Zhang H, Rao NN, Shiba T, Kornberg A (2005) Inorganic polyphosphate in the social life of *Myxococcus xanthus*: motility, development, and predation. Proc Natl Acad Sci USA 102:13416–13420

Zhu Y, Huang W, Lee SSK, Xu W (2005) Crystal structure of a polyphosphate kinase and its implications for polyphosphate synthesis. EMBO Rep 6:681–687

http://www.springer.com/978-3-540-26205-3

Inclusions in Prokaryotes
Shively, J.M. (Ed.)
2006, XII, 349 p. 68 illus., 11 illus. in color., Hardcover
ISBN: 978-3-540-26205-3