Chapter 5

Inositol in Bacteria and Archaea

Mary F. Roberts
Department of Chemistry, Boston College, Chestnut Hill, MA 02467, USA

1. INTRODUCTION

Phosphorylated *myo*-inositol is an important moiety in biochemistry as the basis for signal transduction pathways in eukaryotes. Inositol also occurs in bacteria and archaea, not as part of a signaling pathway, but with diverse and unique uses. *myo*-Inositol occurs as part of mycothiol, a molecule comparable to glutathione in mycobacteria, as part of an unusual osmolyte in hyperthermophiles [*e.g.*, di-*myo*-inositol-1,1′-phosphate (DIP)], and as the lipid headgroup anchor for a series of glycosylated lipids in mycobacteria that are critical in the interaction of pathogenic mycobacteria with mammalian cells.

myo-Inositol biosynthesis occurs by the same steps in all organisms, and comparisons of bacterial/archaeal enzymes to their eukaryotic counterparts often yield interesting surprises. The inositol moiety can be converted to a soluble molecule with specific roles in cells or it can be fixed into membrane components. When unique inositol-containing products are generated in pathogens, these can be targets for drug development. Bacteria have also evolved enzymes that specifically degrade inositol-containing compounds: phosphatidylinositol (PI)-specific phospholipase C would hydrolyze the PI in target membranes, while phytases hydrolyze inositol hexakisphosphate (phytate) to supply cells with inorganic phosphate.

This review aims to present some of the more interesting molecules containing inositol synthesized by bacterial and archaeal cells, and to describe what is known about their biosynthesis. A brief review of bacterial and archaeal inositol biosynthesis and catabolic activities is also provided.

A. Lahiri Majumder and B. B. Biswas (eds.), Biology of Inositols and Phosphoinositides, 103–134

2. IDENTITY OF INOSITOL-CONTAINING COMPOUNDS FOUND IN CELLS

In bacteria and archaea, inositol-containing molecules are not ubiquitous but restricted to certain classes of organisms. Inositol solutes involved in osmotic balance have been detected in hyperthermophilic archaea and Thermotoga sp. Several archaea have also been shown to contain phospholipids containing D-*myo*-inositol 1-phosphate. However, mycobacteria contain the largest variety of inositol compounds including mycothiol, a glutathione analogue, as well as an array of diacylglycerol (DAG)-based glycosylinositols and glycosylinositol phosphorylceramides (GIPCs). Structures of these unusual metabolites are presented in Table 1.

2.1 Soluble inositol solutes – what does and does not occur in microorganisms

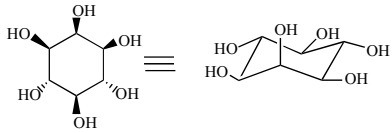
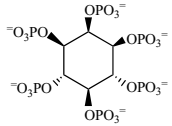
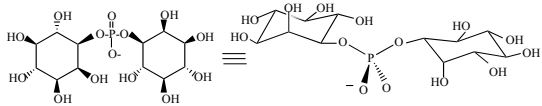
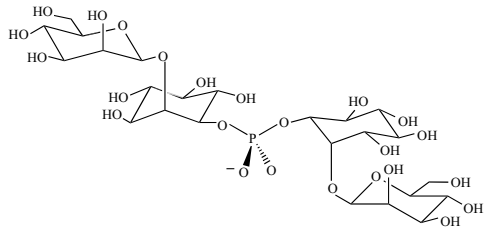
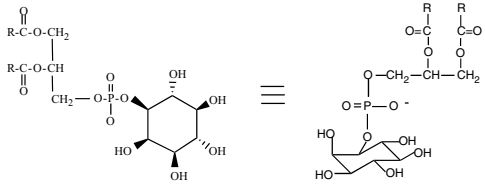
myo-Inositol occurs and accumulates in many eukaryotic cells. In mammals, it often functions as an osmolyte, a role well established in the CNS (Fisher *et al.*, 2002). There are no reports of it accumulating in bacteria at detectable concentrations. Instead *myo*-inositol is converted to specialized soluble phosphate esters with unique roles in protecting cells against stress and to various phospholipids, many of which are unique to bacteria and aid in infectivity of an organism.

Inositol phosphates are key components of eukaryotic signaling pathways that do not occur in bacteria and archaea. Thus, molecules such as inositol-1-phosphate or polyphosphorylated inositols (IP_2, IP_3) have not been detected in bacteria and archaea. Nonetheless, these organisms have evolved pathways to degrade phosphoinositides as a way of scavenging inorganic phosphate. *myo*-Inositol hexakisphosphate, or phytate, is an abundant plant constituent and is the main storage form of phosphate in seeds. It is also found in other eukaryotes where its metabolism is a basic component of cellular housekeeping (see Raboy, 2003, for review). While there are no reports of phytate synthesis in bacteria, many microorganisms have evolved a class of specialized phosphatases to degrade this compound and thus provide the cells with inorganic phosphate. Bacterial phytases have industrial uses. For example, in the baking industry, lactic acid bacteria possessing phytases enhance demineralization of flour. Phytate may also play other roles in cells. For example, IP_6 appears to mediate iron transport in *Pseudomonas aeruginosa* (Hirst *et al.*, 1999), although mechanistic details are not clear at this time.

2.1.1 DIP, DIP-isomers, and mannosyl-DIP derivatives

DIP is a complex solute that was first noted as a major solute in *Pyrococcus woesei* (Scholz *et al*., 1992) and *Methanococcus igneus* (Ciulla *et al*., 1994). In *P. woesei*, DIP accumulates in quantities comparable to intracellular

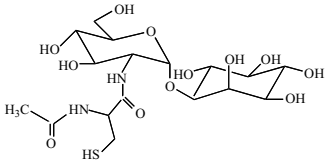
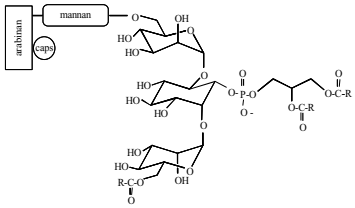
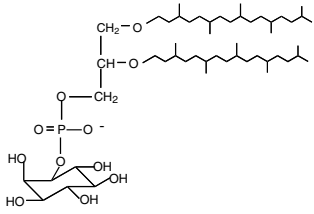
Table 1. Structures of inositol compounds found in (or utilized by) bacteria and archaea

Solute	Roles
myo-Inositol 	Free *myo*-inositol is not observed to accumulate in bacteria or archaea; it is fixed in unusual solute or lipids
Inositol hexakisphosphate (phytate) 	Synthesized by plants; good source of inorganic phosphate for bacteria who have evolved efficient phytases
Di-L,L-*myo*-1,1′-inositol phosphate (DIP) 	Synthesized in hyperthermophic archaea and Thermotoga sp. in response to osmotic and thermal stress
Di-2-*O*-β-mannosyl-di-L,L-*myo*-1,1-phosphate (mannosyl-DIP) 	Synthesized in Thermotoga sp. in response to osmotic and thermal stress
Phosphatidylinositol (PI) 	Minor membrane component in most bacteria; base of GPI-anchored proteins and unusual cell wall structures in bacteria

(*continued*)

K^+ (500–600 mM). DIP in *M. igneus* is chiral and composed of L-inositol-1-phosphate (L-I-1-P) units (Chen *et al*., 1998); in *Thermotoga neapolitana*, a second set of ^1H and ^{13}C resonances is consistent with a DIP molecule formed from both L- and D-I-1-P moieties (Martins *et al*., 1996). DIP functions as an osmolyte since the intracellular concentration increases with increasing

Table 1. (continued)

Solute	Roles
Glycosylinositol phosphorylceramides: *e.g.*, Manα1 → 3 Man α1→ 6 GlcNH$_2$ α1 → 2 Ins1→ P→1Cer Manα1 →3 Manα1→Ins1→P→1Cer	Unusual bacterial phospholipids based on inositol ceramide
Mycothiol 	Reducing agent, much like glutathione, in mycobacteria
Lipoarabinomannan 	PI-anchored antigen in mycobacteria
2-*O*-(3′-Hydroxy)phytanyl-3-*O*-phytanyl-sn-glycerol phospho-*myo*-inositol (hydroxyarchaetidyl-*myo*-inositol) 	Membrane component of archaea

external NaCl in these cells. However, DIP is detected only when hyperthermophilic Archaea and Thermotogales cells are grown above 75 °C (Chen et al., 1998; Ciulla *et al*., 1994; Martin *et al*., 1999; Martins *et al*., 1996). Two related biosynthetic pathways have been proposed for DIP (Chen *et al*., 1998; Scholz *et al*., 1998). The more likely four-step synthesis (Figure 1) includes: (i) conversion of D-glucose-6-phosphate to L-I-1-P catalyzed by the enzyme inositol-1-phosphate synthase (*myo*-inositol-1-phosphate synthase, MIPS); (ii) generation of *myo*-inositol from the I-1-P by an inositol monophosphatase (IMPase); (iii) activation of the I-1-P with CTP to form CDP-inositol (CDP-inositol cytidylyltransferase); and (iv) condensation of CDP-inositol with *myo*-inositol

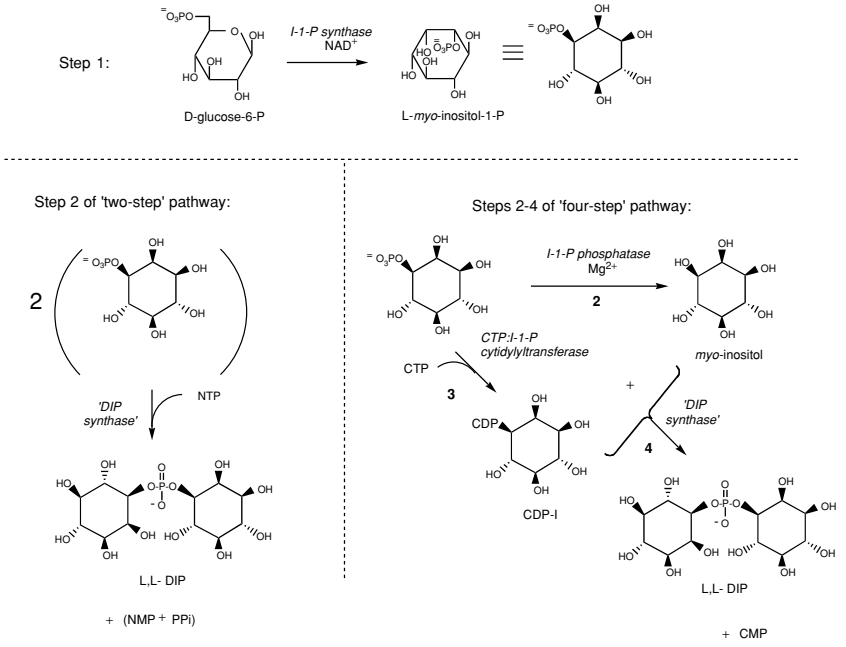


Figure 1. Proposed biosynthetic pathways for DIP.

(DIP synthase) to form DIP (Chen *et al*., 1998). The two-step mechanism does not utilize *myo*-inositol (Scholz *et al*., 1998), although direct condensation of two L-I-1-P molecules to form a phosphodiester, even with NTP, is an unprecedented activity for a single enzyme. Furthermore, the two-step mechanism cannot explain forming DIP with both L- and D-I-1-P units as is observed in *T. neapolitana* (Martins *et al*., 1996). *Archaeoglobus fulgidus* is one of the archaea that accumulates DIP when grown above 75 °C (Martins *et al*., 1997). In this archaeon, there is no other known use of inositol (*e.g*., no inositol-containing lipids), so that production of L-I-1-P from D-glucose-6-phosphate (via MIPS) directs carbon resources to DIP only. Since MIPS is the first and committed step in DIP production, it is likely that MIPS protein expression is coupled to growth temperature. A structure of this archaeal enzyme with NAD^+ and Pi bound has been completed recently (Stieglitz *et al*., 2005) and may shed light on other mechanisms for regulating MIPS activity and DIP production.

T. neapolitana also adds mannose moieties onto the inositol rings, and this new phosphodiester, di-2-*O*-β-mannosyl-di-*myo*-inositol-1,1′-phosphate (Table 1) also appears to accumulate at high growth temperatures. However, DIP is still the major solute accumulated in *T. neapolitana* at supraoptimal growth temperatures (Martins *et al.*, 1996). The addition of mannose to DIP produces a very unique phosphodiester, yet we know very little about why it is synthesized and accumulated. The involvement of these inositol solutes in thermoprotection

as well as osmoadaptation could involve modulation of water structure. Perhaps the rigid inositol rings serve to stabilize water hydrogen bonding networks at high temperatures.

2.1.2 Mycothiol

Inositol is an important component of mycothiol [2-(*N*-acetylcysteinyl)amido-2-deoxy-α-D-glucopyranosyl-(1→1)-*myo*-insoitol] (Newton *et al.*, 1995; Spies and Steenkamp, 1994), the major low molecular weight solute in mycobacteria. Mycothiol (for structure see Table 1) is synthesized by attachment of *N*-acetyl-glucosamine to *myo*-inositol, deacetylation of the glucosamine moiety and ligation to L-cysteine, followed by transacetylation of the cysteinyl residue by acetyl-CoA to produce mycothiol (for review see Newton and Fahey, 2002). Mycothiol is thought to protect *Mycobacterium tuberculosis* from inactivation by the host during infections via its antioxidant activity (similar to glutathione) as well as its ability to detoxify a variety of toxic thiol-reactive compounds. Consistent with this protective role, a mutant lacking the Rv1170 gene (which codes for the deacetylase activity needed for mycothiol production) had increased sensitivity to the toxic oxidant cumene hydroperoxide and to the antibiotic rifampin (Buchmeier *et al.*, 2003).

2.2 Inositol-containing lipids

The major use of inositol in bacteria is in generating PIs, versatile membrane components that can be derivatized to anchor proteins or complex carbohydrates to the cell surface. In some microorganisms, the complex carbohydrate structure attached to the PI anchor in the cell envelope is part of the way the microorganism recognizes or binds to target cell components.

2.2.1 Phosphatidylinositol

Gram-negative bacteria normally do not contain much, if any, PI in their membranes. When it does occur, it often correlates with unusual properties of a microorganism. For example, microorganisms that have ice-nucleation activity in supercooled water have been shown to accumulate PI in their membranes (*e.g.*, 0.1–1.0% of total phospholipids in *Escheria coli* K-12 Ice+ strains) (Kozloff *et al.*, 1991a). Corresponding Ice− *E. coli* strains also contained PI, but at 2–30% of the level found in the Ice+ *E. coli* strains. Treatment of these cells with a PI-specific phospholipase C (PI-PLC), which cleaves PI to diacylglcyerol and inositol-1-phosphate, destroyed the ice-nucleating ability. Thus, the functioning of the ice gene apparently increased both the PI synthase activity and the PI content of Ice+ strains from low endogenous levels. These results strongly indicate that PI plays an important role in ice nucleation at −4 °C or above, although it is not thought to be a direct interaction with water. Rather, it has been proposed

(Kozloff *et al.*, 1991a) that PI serves to anchor the appropriate nucleation protein to the cell membrane. The ice-nucleation gene product appears to be attached to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor. The protein is coupled to mannose residues via an *N*-glycan bond to the amide nitrogen; the mannose residues are attached to PI (Kozloff *et al.*, 1991b). This GPI-linked protein structure is the critical element in the class A nucleating structure. The PI mannoside moiety has also been identified as a mycobacterial adhesin mediating binding to non-phagocytic cells (Hoppe *et al.*, 1997).

The distribution of PI in other bacteria appears confined to actinomycetes (*Mycobacterium, Corynebacterium, Nocardia, Micromonospora, Streptomyces, and Propionobacterium*) (Brennan and Ballou, 1968; Brennan and Lehane, 1971; Goren, 1984; Kataoka and Nojima, 1967; Tabaud *et al.*, 1971; Yano *et al.*, 1969), myxobacteria (Elsbach and Weiss, 1988), and *Treponema* (Belisle *et al.*, 1994). The linkages become quite complex, for example the triacylphosphatidylinositol dimannosides (Ac(3)PIM(2)) in *Corynebacterium amycolatum, Corynebacterium jeikeium,* and *Corynebacterium urealyticum* (Yague *et al.*, 2003) and glycosylphosphoinositol mannosides found in Mycobacteria (*e.g.*, Manα1$\rightarrow$2Ins1-P-1Cer and Manα1$\rightarrow$3Manα1$\rightarrow$2Ins1-P-1Cer). For review of cell wall structure and function in *Mycobacterium tuberculosis*, see Brennan (2003).

2.2.2 Inositol-sphingolipids

Mycobacteria have unusual acidic GIPCs as well as GPI-anchors in its cell envelope. The sphingolipids in mycopathogens (Toledo *et al.*, 2001) often have the same linkage and structure found in GPIs of Mycobacteria. Unusual core linkages in *Sporothrix schenckii* (Manα1$\rightarrow$6Ins) and *Paracoccidiodes brasiliensis* (Levery *et al.*, 1998) (Manα1$\rightarrow$2Ins) have also been seen. The synthesis of these GIPCs that are not found in mammalian cells is required for viability of fungi. Indeed, inositol phosphorylceramide (IPC) synthase, the first step in GIPC biosynthesis, could be a potential drug target (Nagiec *et al.*, 1997).

2.2.3 Archaeal inositol-containing lipids

Inositol-containing lipids appear to be more common in archaea than in bacteria; they have been best characterized in methanogens. Archaeal lipids have ether linkages rather than ester linkages; the alkyl chains are appended to the 2- and 3-positions of glycerol rather than the 1- and 2-positions modified in eukaryotic and bacterial lipids. *Methanosarcina barkeri* contains 2-*O*-(3′-hydroxy)phytanyl-3-*O*-phytanyl-sn-glycerol phospho-*myo*-inositol, otherwise known as hydroxyarchaetidyl-*myo*-inositol (Table 1) (Nishihara and Koga, 1991), which can be further modified. A related phosphoglycolipid has also been detected in *Aeropyrum pernix* (Morii *et al*., 1999). The stereochemical configuration of the phospho-*myo*-inositol residue of glucosaminyl archaetidylinositol

was determined to be 1-D-*myo*-inositol 1-phosphate (Nishihara *et al*., 1992). Since MIPS generates L-I-1-P, the synthesis must use an activated pyrophosphate ester that is attacked by *myo*-inositol; L-I-1-P cannot be the direct precursor. The inositol headgroups of both diether and tetraether polar lipids appear oriented to the cytoplasmic surface of the membrane in *Methanobacterium thermoautotrophicum* (Morii and Koga, 1994); this is similar to mammalian cells where PI molecules are on the cytoplasmic leaflet.

2.3 Cell wall components (lipomannan and lipoarabinomannan from mycobacterium)

In mycobacterial cell envelopes, there are specific cell wall components, lipoarabinomannan (LAM), containing inositol. LAMs (Table 1) have three structural domains: two homopolysaccharides (D-mannan and D-arabinan) constitute the carbohydrate backbone; the mannan core is terminated by a GPI-anchor and arabinan capped by GPI. These phosphatidylmannosides (PIMs) are mediators of adhesion. Tuberculosis is caused by *Mycobacterium tuberculosis*, a facultative intracellular pathogen of alveolar macrophages in the lung. Two human pulmonary surfactant proteins play key roles in the pathogenicity of the organism: hSP-A and hSP-D. hSP-A promotes attachment of *M. tuberculosis* to phagocytes (Downing *et al.*, 1995), while hSP-D reduces uptake of macrophages (Ferguson *et al.*, 1999). hSP-D appears to bind to mannose-capped LAM (Ferguson *et al.*, 1999). hSP-A binds tightly to both pathogenic and non-pathogenic Mycobacterium sp. through specific interactions with the major lipoglycans (mannosylated LAM and lipomannan) (Sidobre *et al.*, 2000). The PI anchor (along with terminal mannose residues) is required for the high affinity of LAMs to the surfactant proteins. PI synthesis in mycobacteria has been reviewed by Salman *et al*., 1999.

3. ENZYMES OF *MYO*-INOSITOL BIOSYNTHESIS

Inositol is synthesized by the same route in all cells. D-Glucose-6-phosphate (D-G-6-P) is converted to L-*myo*-inositol-1-phosphate (L-I-1-P), which can also be termed D-*myo*-inositol-3-phosphate. The L-I-1-P is then hydrolyzed to *myo*-inositol by a relatively specific phosphatase. As we will see, enzymes that do these reactions in bacteria and archaea can have quite different characteristics from the eukaryotic enzymes.

3.1 myo-Inositol-1-phosphate synthase

MIPS is the rate-limiting step in *myo*-inositol biosynthesis. Regulation of the enzyme from yeast has been examined in detail since there are multiple ways to

modulate this activity in cells (Majumder *et al.*, 1997); the eukaryotic enzyme is also of interest since the mammalian homologue may be a potential drug target for bipolar disease (Agam *et al.*, 2002). The well-studied yeast MIPS is a homotetramer (60 kDa subunits) that is activated by ammonium ions (Majumder *et al.*, 1997). It converts D-G-6-P to L-I-1-P with NAD^+ as a cofactor (Loewus, 1977; Loewus *et al.*, 1980; Tian *et al.*, 1999). There are three distinct steps of the reaction with intermediates tightly bound to the protein: (i) D-G-6-P is oxidized to 5-keto-D-G-6-P concomitant with NAD^+ reduction to NADH; (ii) after enolization of the 5-keto-D-G-6-P, an aldol condensation reaction occurs to form the new carbon–carbon bond and yield 2-inososе-1-phosphate; (iii) the inosose compound is reduced to L-I-1-P by NADH regenerating NAD^+ at the active site. The *ino1* gene and its product, MIPS, are found in most eukaryotes but scattered in prokaryotes (Bachawat and Mande, 2000). Sequence homology searches readily identify a putative *ino1* gene product in hyperthermophilic Archaea, *Aquifex aeolicus*, and Thermotoga species; several of these organisms use DIP for osmotic balance or use inositol-containing lipids in their membranes. MIPS activities have been identified in high (G+C) Gram-positive mesophilic bacteria such as Mycobacteria and Streptomyces (Bachawarat and Mande, 1999, 2000), both of which use inositol in cell wall or antibiotic production (in Streptomyces, MIPS is needed for generating *myo*-inositol that is used in synthesizing antibiotics, Walker, 1995). Sequence analyses suggest bacteria recruited the *ino1* gene from archaea (Nesbo *et al.*, 2001), for example it is likely that there was lateral *ino* gene transfer to *T. martima* from *P. horikoshii*. However, it appears that two distinct bacterial lineages appear to have acquired *ino1* from different archaea (Majumdar *et al*., 2003). Another chapter in this volume provides a detailed review of MIPS structure and mechanism (Chapter 7).

3.1.1 *Mycobacterium tuberculosis* MIPS

myo-Inositol is critical for synthesis of the cell wall lipoglycans of *M. tuberculosis*, and the MIPS commits resources to inositol biosynthesis. Recently, an *ino1*-deficient mutant of *M. tuberculosis* was constructed (Movahedzadeh *et al.*, 2004). The mutant was only viable with high *myo*-inositol in the medium. When this mutant was grown and then incubated in inositol-free medium, levels of mycothiol were reduced while PI mannoside, lipomannan, and LAM levels were not altered. Its infectivity of macrophages was attenuated as well.

The crystal structure of *M. tuberculosis* MIPS has been solved to 1.95 Å (Norman *et al.*, 2002). The most striking difference from the yeast MIPS structure (Stein and Geiger, 2002) was the presence of a metal ion, identified as Zn^{2+}, in the active site in the vicinity of the NAD^+. The observation of a metal ion was surprising because EDTA does not appear to affect the activity of this enzyme. Mutagenesis of four active site residues (D197A, K284A, D310A, and K346A) generated inactive enzyme as detected by the lack of growth when plasmids bearing one of these mutant *ino1* genes was introduced into *M. tuberculosis*

containing a defective *ino1* gene (Movahedzadeh *et al.*, 2004). Asp310 was a ligand of the Zn^{2+} ion observed in the crystal structure. Structural studies of D310N caused a loss of the Zn^{2+} ion and a conformational change in the NAD^+ cofactor. This strongly implies that the Zn^{2+} is critical for activity.

3.1.2 *Archaeoglobus fulgidus MIPS*

An *ino1* gene, identified in this hyperthermophilic archaeon by sequence homology to yeast MIPS, was cloned and overexpressed in *E. coli* (Chen *et al.*, 2000). The archaeal MIPS subunit is 44 kDa, considerably smaller than the yeast enzyme (60 kDa). Like the yeast and mycobacterial enzymes, *A. fulgidus* MIPS is a tetramer. Not surprisingly, it is very thermostable: at 90 °C, K_m is 0.12 mM for G-6-P, 5.1 μM for NAD^+ , and $k_{cat} = 9.6\ s^{-1}$. Use of D-[5-^{13}C] G-6-P has clearly shown that the product is L-I-1-P (Chen *et al.*, 2000). What appears unique about this MIPS compared to the other ones examined thus far is that it absolutely requires metal ions for activity, with Zn^{2+} and Mn^{2+} optimum but Mg^{2+} also effective. EDTA inhibits the enzyme and halts the reaction after oxidation of G-6-P by NAD^+ to form 5-keto-glucose-6-phosphate and NADH. The latter two compounds are generated in 1:1 stoichiometry with the enzyme subunits (Chen *et al.*, 2000). This suggests that this archaeal MIPS is a class II aldolase (Figure 2). A structure of the *A. fulgidus* MIPS crystallized without any added NAD^+ or divalent metal ions has recently been described (Stieglitz *et al.*, 2005). That enzyme has NAD^+ and Pi bound to each subunit and two of the subunits have a density that has characteristics more consistent with K^+ as the metal ion. Subunits with this "crystallographic" ion, which occupies a position similar to the putative Zn^{2+} in the *M. tuberculosis* MIPS, have altered NAD^+ conformations from those subunits that do not have a metal ion present. While this structure will provide a good comparison to both the yeast and *M. tuberculosis* enzymes, what is really needed is the *A. fulgidus* MIPS structure with an activating metal ion, since this type of cation is absolutely necessary for catalytic activity.

Not all MIPS enzymes from thermophiles may have divalent metal ion-dependent activities. While crude protein extracts of *A. fulgidus* exhibited a MIPS activity that absolutely required divalent metal ions and was totally inhibited by 1 mM EDTA, the production of I-1-P from G-6-P (carried out ≥75 °C) by protein extracts from *M. igneus* and *T. maritima* was observed in the presence of 1 mM EDTA. This observation (Chen and Roberts, unpublished results) suggests that either the MIPS from these particular archaea is not divalent metal ion-dependent or that the metal ions bind very tightly and are not easily removed. None of the MIPS activities from thermophiles are affected by the addition of NH_4^+. Since the divalent cation in *A. fulgidus* MIPS has been suggested to aid in the aldol condensation and since this is supposedly done by NH_4^+ in the yeast enzyme, it is of interest how this step occurs in the other archaea and bacteria.

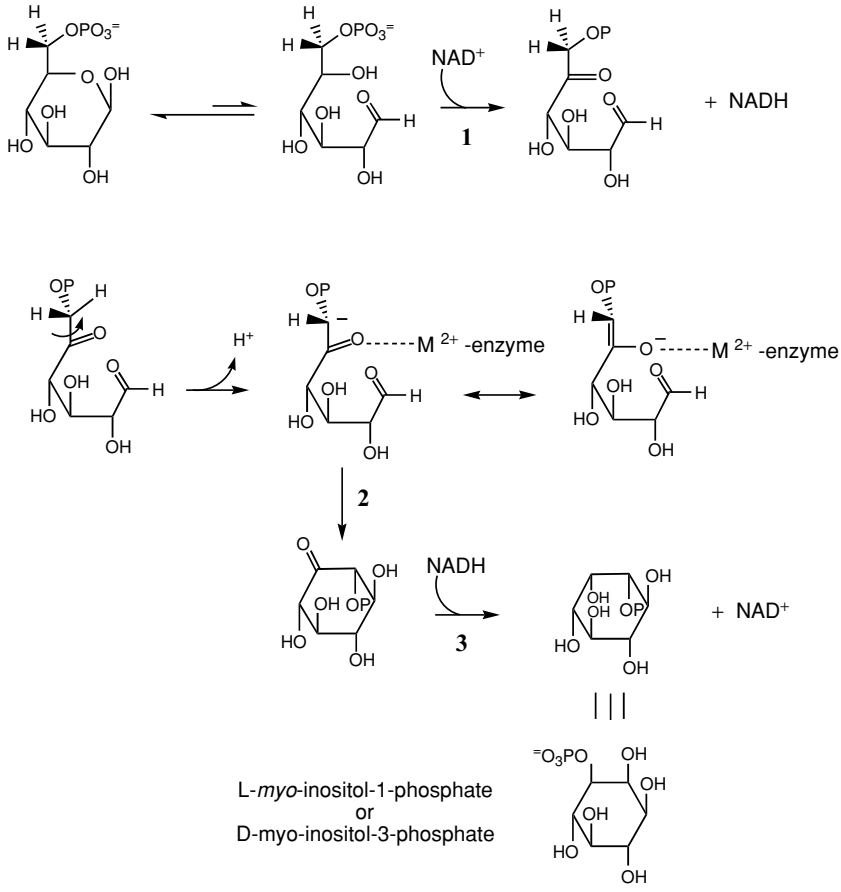


Figure 2. Sequence of MIPS reactions for the enzyme from *A. fulgidus*.

3.2 Inositol monophosphatase

In the bacteria that use PI derivatives (*e.g.*, lipomannan biosynthesis in Mycobacteria), IMPase activities are required. However, in some bacteria that do not use significant PI in their membranes, IMPase activities have been identified and characterized. The biological function of an IMPase in a non-PI containing organism must be something other than generating *myo*-inositol. A good example is the *E. coli suhB* gene product (SuhB), which has high sequence homology to mammalian IMPases and exhibits IMPase activity (PI is not a significant *E. coli* phospholipid). In archaea, IMPase activities are required for DIP biosynthesis (Chen *et al.*, 1998). However, homologues occur in all sequenced archaea whether or not they accumulate DIP or have PI analogues in their membranes. As will be discussed, one of the more unusual

features of these IMPases is their dual fructose 1,6-bisphosphatase (FBPase) activity (Stec *et al.*, 2000).

In general there are three phosphatase families: alkaline, acid, and protein phosphatases. Alkaline phosphatases are typically dimers that contain three metal ions per subunit and have a pH optimum pH above 8. Acid phosphatases exhibit an optimum pH$<$7 and are usually divided into three classes: low molecular weight acid phosphatases ($<$20 kDa), high molecular weight acid phosphatases (50–60 kDa), and purple acid phosphatases (which contain an Fe–Fe or Fe–Zn center at the active site). Phosphatases specific for I-1-P appear to be most similar (in kinetic characteristics but not in mechanism) to the alkaline phosphatases, but their structures define a superfamily that also includes inositol polyphosphate 1-phosphatase, fructose 1, 6-bisphosphatase, and Hal2. The members of this superfamily share a common structural core of 5 α-helices and 11 β-strands. Many are Li^+-sensitive (York *et al.*, 1995), and more recent structures of archaeal IMPase proteins suggest the Li^+ -sensitivity is related to the disposition of a flexible loop near the active site (Stieglitz *et al.*, 2002).

3.2.1 *E. coli* SuhB

The *suhB* gene of *E. coli* was identified by isolating mutants that suppressed a variety of temperature-sensitive defects including specific mutations in protein secretion, DNA synthesis (Chang *et al*., 1991), and the heat shock response (Yano *et al*., 1990). The SuhB protein has significant sequence similarity to human IMPase and was shown to exhibit Li^+ -sensitive IMPase activity (Matsuhisa *et al*., 1995) similar to the mammalian enzyme (see Table 2). Since there are essentially no *myo*-inositol-containing phospholipids in most *E. coli*, the occurrence of an IMPase activity strongly suggests that this enzyme must either use another specific substrate or possess another type of "activity." Insight into the function of SuhB and other bacterial homologues has been provided by analysis of the suppressor behavior of the *suhB* gene. *E. coli* mutations in *rpoH* and *dnaB* cause the cells to grow only at 30 °C but not at 42 °C. If *suhB* is also mutated, the cells now grow only at 42 °C. Introducing the SuhB product back into the double mutants via a plasmid allows the cells to grow at 30 °C once again. Thus, mutant *suhB* is a suppressor of mutant *rpoH* or *dnaB* phenotypes. The *E. coli* mutant with *suhB* missing or inactivated is cold-sensitive with defects in protein synthesis (Inada and Nakamura, 1995). Interestingly, RNA cleavage mutations restore minimal protein synthesis to *suhB* mutants (Inada and Nakamura, 1995). Thus, it has been suggested that that the SuhB protein/IMPase can affect a number of genes at the translational level (Chang *et al*., 1991).

One possibility is that the double-strand RNA-processing activity of RNase III, an endoribonuclease involved in the rate-limiting first cleavage step of mRNA degradation (Chang *et al.*, 1991), is potentially lethal to *E. coli*, and the

Table 2. Characteristics of IMPase activities from bacteria and archaea

Source[a]	M_n	Assay T (°C)	K_m (I-1-P) mM[b]	Unusual substrates	K_D (Mg^{2+}) mM	IC_{50} (Li^+) mM	k_{cat} (s^{-1})	References
Ec	M_1[c]	37	0.068	–	0.5	2	4.4	Chen and Roberts (2000)
Mt	M_2	37	0.18	–	6.0[d]	0.9	3.6	Nigou *et al.* (2002)
Rl		37?	0.23	–				Janczarek and Skorupska (2001)
Af	M_2	85	0.11	PNPP, G-1-P, 2′-AMP, FBP	15[e]	290	4.3	Stieglitz *et al.* (2002)
Mi	M_2	85	0.3	PNPP, G-1-P, 2′-AMP, FBP	6–8	160		
Mj	M_2	85	0.09	2′-AMP best substrate, FBP	3–4	200	4.2	Chen and Roberts (1998)
Tm	M_4	95	0.13	D-I-1-P is 20 times better than L-I-1-P	5–10	100	210	Chen and Roberts (1999)

[a]Ec, *E. coli*; Mt, *Mycobacterium tuberculosis*; Rl, *Rhizobium leguminosa;* Af, *Archaeoglobus fulgidus*; Mi, *Methanococcus igneus*; Mj, *M. jannaschii;* Tm, *Thermotoga maritima. Other abbreviations: PNPP, p-nitrophenylphosphate; G-1-P, glucose-1-phosphate; FBP, fructose-1,6-bisphosphate.*
[b]For D-I-1-P as the substrate unless otherwise noted.
[c]SuhB aggregates, but this can be suppressed with KCl >0.2 M.
[d]The optimum Mg^{2+} concentration is provided instead of a K_D.
[e]The apparent K_D for Mg^{2+} depends on the substrate; this is an average value.

normal function of SuhB is to modulate the lethal action of RNase III. SuhB could alter mRNA stability by (1) modulating RNase III activity or (2) binding to target RNA molecules and protecting them from degradation by RNase III. The first possibility is based on the observation that the RNase III activity was stimulated when the protein was phosphorylated, and SuhB acting as a protein phosphatase could dephosphorylate RNase III. For this to be the mechanism, the phosphatase activity of SuhB must be intact. However, work by Chen and Roberts (2000) demonstrated that the phosphatase activity is absolutely unrelated to the SuhB function in the *dnaB*121 mutant. SuhB functioning in RNA protection appears a more plausible explanation at this point. Suppose high temperature (42 °C) destabilizes double-strand RNA (dsRNA) structure, while at lower temperatures, RNase III, specific for dsRNA, forms a complex with its target RNA and degrades the RNA. SuhB binding to dsRNA could protect it from RNase III degradation. This implies that at the lower temperature, wild type SuhB is required to compensate for the lethal RNase III activity, and loss of SuhB would always generate a cold-sensitive phenotype. Since dsRNA

is destabilized at the higher growth temperature, RNase III cannot degrade it and the cells are able to grow.

The observations with the *dnaB* (*dnaB*121) mutation can be understood in this context (Figure 3). At 42 °C, the mutant *dnaB* gene product is not able to form the functional hexamer (Yano *et al.*, 1990) that participates in initiation and elongation of *E. coli* replication by interacting with dnaC and other *E. coli* proteins (Inada and Nakamura, 1996). Without functional dnaB protein, DNA replication is blocked and cells cannot grow. At 30 °C, the mutant dnaB product can still form functional hexamer and wild type SuhB protects the dsRNA from degradation by RNase III. Under these conditions, the cells can grow. The *dnaB* and *suhB* double mutant, although capable of forming functional dnaB (mutant) hexamer at 30 °C, cannot grow because of unregulated RNase III activity at this temperature. At 42 °C, RNase III activity is reduced because of the smaller population of dsRNA; the presence of mutant SuhB must make the mutant dnaB functional at this temperature, although the mechanism for this is unknown.

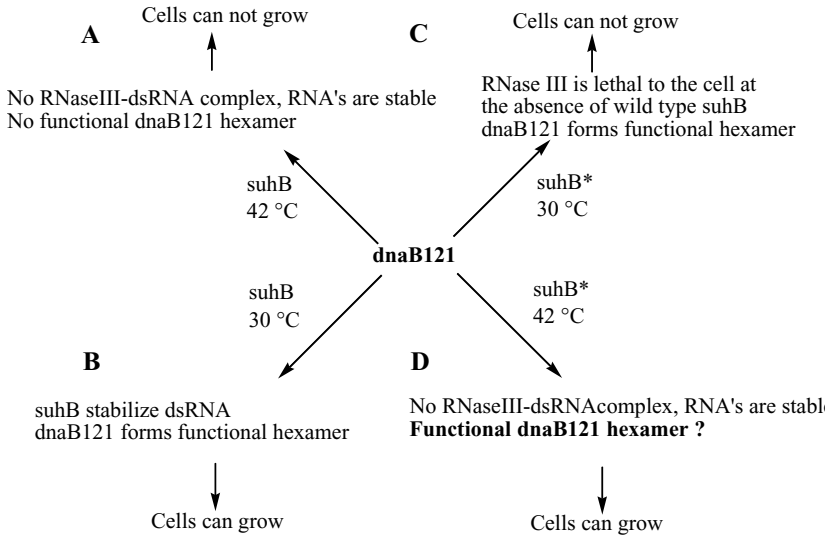


Figure 3. Suppressor effect of mutant *suhB* on the *dnaB121* mutant of *E. coli*.

3.2.2 *Mycobacterium tuberculosis* IMPase

Since inositol lipids are so prevalent in the cell membranes of mycobacteria, significant IMPase activity is needed. There are four ORFs in the *M. tuberculosis* genome with the IMPase signature DPIDGT and WDXAAG found in the superfamily of lithium-sensitive phosphatases (Neuwald *et al*., 1991), of which three are close to other known genes: *suhB*, *impA*, and *csyQ* (Nigou *et al*., 2002). The one with closest homology to human IMPase was identified as the

suhB gene product. Cloning, expression, purification, and characterization of the IMPase protein showed it was very similar to the *E. coli* protein in terms of physical characteristics (Table 2). Similar to *E. coli* SuhB, the *M. tuberculosis* IMPase migrated anomalously on gels and exhibited a native molecular mass suggesting it was a trimer. Kinetic characteristics for this IMPase were very similar to those for *E. coli* SuhB. Substrate specificity studies indicated that I-1-P was the preferred substrate; glycerol-2-phosphate, 2′-AMP, mannitol-1-phosphate were not as effectively hydrolyzed. Although *M. tuberculosis*, as a human pathogen, has an optimal growth temperature of 37 °C, the IMPase is quite stable with an optimum assay temperature of 80 °C. Site-directed mutagenesis indicated that Glu83, Asp104, Asp107, Asp-235, and Trp234, which aligned with human IMPase active site residues, were necessary for catalysis. The residue Ile68, which is comparable to Val70 in HAL2 (a yeast PAPase), was shown to affect Li^+-sensitivity: L81A exhibited a 10-fold increase in the IC_{50} for Li^+.

How critical is the IMPase in mycobacteria? In a related but non-pathogenic organism, *M. smegmatis*, a mutant constructed to have a defective IMPase had altered cell envelope permeability (Parish *et al.*, 1997). Certainly, mutating the IMPase gene should affect the distribution of LAMs since the PI moiety is the basic scaffold, and in the *M. smegmatis* mutant, there was a decrease in PIM2 consistent with altered cell permeability. The same mutant also indirectly provides information on mycothiol production. Mycothiol has an inositol unit that could be incorporated via I-1-P or from *myo*-inositol. The IMPase mutants show dramatically decreased mycothiol levels, consistent with *myo*-inositol as the direct precursor of the inositol unit in mycothiol.

3.2.3 *Rhizobium leguminosarum* bv. Trifoli pssB gene product

A protein of 284 residues, the *pssB* gene product, has been shown to have IMPase activity (Janczarek and Skorupska, 2001). The phosphatase activity of PssB (Table 2) is suggested to have a regulatory function in exopolysaccharide (EPS) synthesis. Mutation of *pssB* caused EPS overproduction, while introducing *pssB* in the wild type strain reduced the levels of EPS. These alterations in EPS production could be correlated with a non-nitrogen-fixing phenotype of rhizobia.

3.2.4 Archaeal IMPase homologues

Hyperthermophilic archaea possess genes whose translated products have considerable sequence homology to human IMPase. Recombinant IMPase activities from *Methanococcus jannaschii*, *Archaeoglobus fulgidus*, and the closely related bacterium *Thermotoga maritima* have been expressed in *E. coli* (Chen and Roberts, 1998, 1999; Stieglitz *et al.*, 2002). The occurrence of an IMPase in archaea that accumulate DIP would be expected. However, IMPase homologues appear to be ubiquitous in archaeal genomes, so that this protein is likely to have another role. In fact, in most archaeal genomes, the genes homologous to

IMPase are annotated as coding for SuhB homologues. MJ0109 in *M. jannaschii*, the archaeal protein first identified by sequence homology as an IMPase, exhibits slightly broader substrate specificity than human or *E. coli* IMPases (*e.g.*, *p*-nitrophenylphosphate is a substrate), but is poorly inhibited by Li^+ (Table 2) (Chen and Roberts, 1998). The structure of this archaeal IMPase solved by molecular replacement using the human IMPase structure (Johnson *et al.*, 2001; Stec *et al.*, 2000), clearly belongs to the IMPase superfamily (Figure 4A) but it actually has some features reminiscent of pig kidney FBPase, a member of the IMPase superfamily that is a tetramer (a dimer of dimers). In particular, active site loop 1 in the archaeal IMPase (MJ0109) was quite different from the corresponding loop in the human I-1-Pase (Figure 4B) (Johnson *et al.*, 2001). The disposition of this loop was much closer to that in pig kidney FBPase (Stec *et al.*, 2000).

Indeed, MJ0109 can catalyze the hydrolysis of the phosphate at C-1 of fructose 1,6-bisphosphate (FBP) generating inorganic phosphate and fructose-6-phosphate (F-6-P) (Stec *et al.*, 2000). G-6-P and F-6-P are not substrates for MJ0109. The FBPase-specific activity exhibited by MJ0109 is comparable to the IMPase-specific activity, making MJ0109 a dual phosphatase that can process substrates in completely different pathways (Stec *et al.*, 2000). FBPase activity gives the protein a potential role in gluconeogenesis, although another protein in *M. jannaschii* with FBPase and not IMPase activity has been partially purified and cloned (Rashid *et al.*, 2002). The IMPase activity links the protein to inositol biosynthesis and stress responses, although *M. jannaschii* does not accumulate DIP and has no *ino1* homologue. The IMPases from *T. maritima* and *A. fulgidus*, both of which accumulate DIP, have also been shown to be bifunctional IMPase/FBPase enzymes (Stec *et al.*, 2000). These IMPases from hyperthermophiles are functionally quite different from *E. coli* SuhB, which cannot hydrolyze FBP. Detailed analyses of the crystal structures of MJ0109 (Johnson *et al.*, 2001) and the homologue from *A. fulgidus*, AF2372 (Stieglitz *et al.*, 2002), with substrates or products and metal ions bound suggest these phosphatases work using a three metal ion-assisted catalytic mechanism. *In vivo* regulation mechanisms for these enzymes are not known, although the IMPase from *A. fulgidus* has two closely spaced cysteine sidechains, Cys150 and Cys186, that inactive the enzyme when they are oxidized to form an intramolecular disulfide (Stieglitz, 2003). Such a mode of phosphatase regulation occurs in chloroplast FBPase enzymes (Balmer *et al.*, 2001; Jacquot *et al.*, 1997).

3.2.5 Glucose-1-phosphatase has specific phytase activity

The glucose-1-phosphatase from *E. coli* hydrolyzes phytate as well as glucose-1-phosphate. This enzyme is a member of the histidine acid phosphatase family (Lee *et al.*, 2003). Glucose-1-phosphatase cleaves only the 3-phosphate from phytate, and no further hydrolysis takes place. It is the only phosphatase

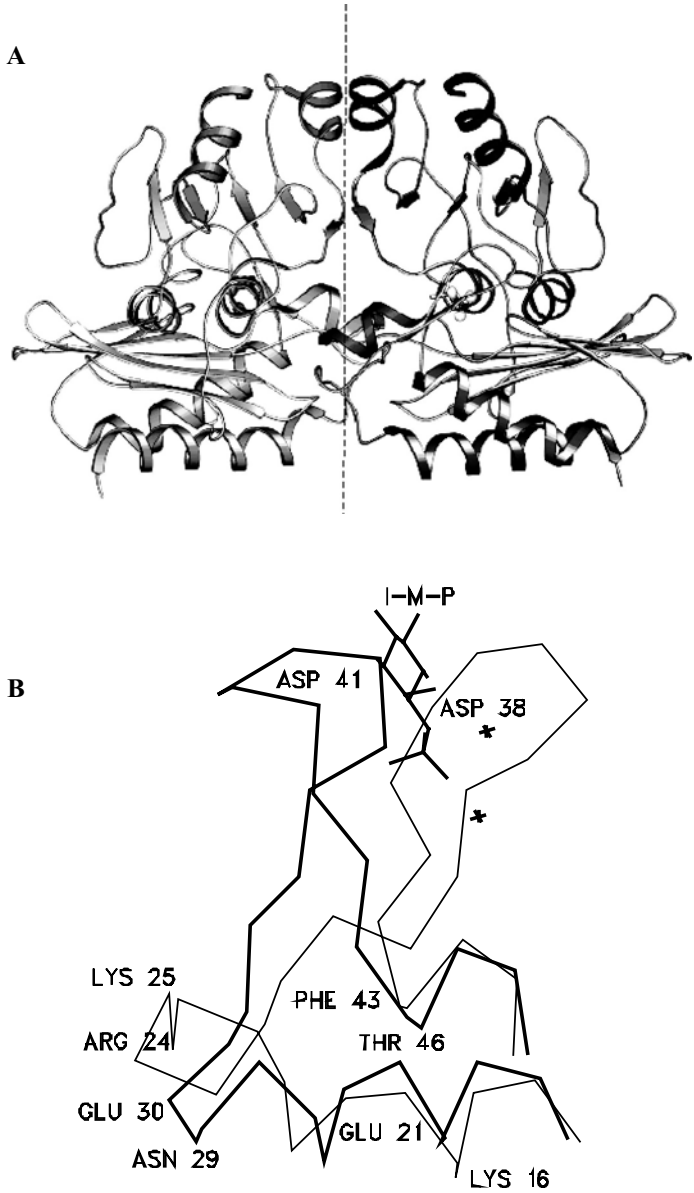


Figure 4. (A) Ribbon diagram showing the structure of the MJ0109 IMPase/FBPase dimer. (B) Comparison of the catalytic loop 1 in MJ0109 (thin line) compared to human IMPase (thick line) with I-1-P and metal ions (+) shown. Reproduced with permission from (Johnson *et al.*, 2001).

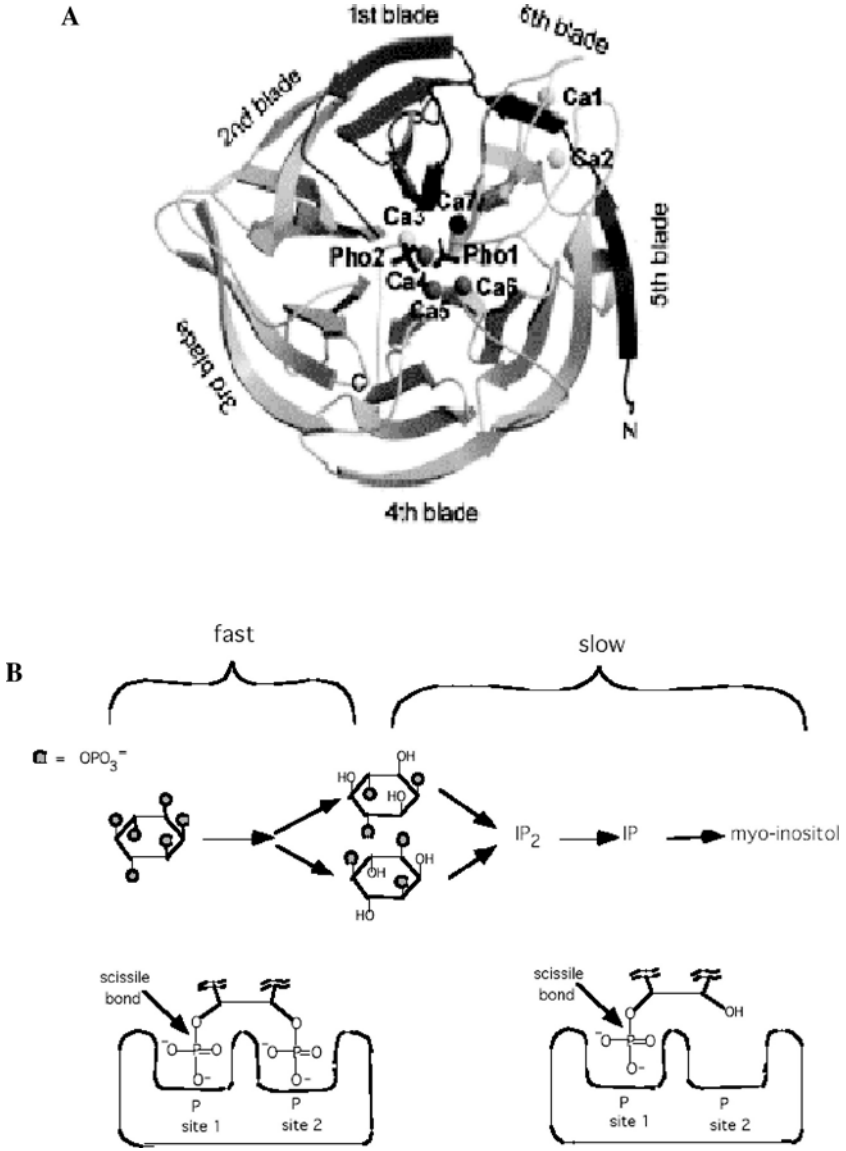


Figure 5. (A) Phytase crystal structure and (B) model for two phosphate binding sites (from Shin *et al.*, 2001).

known to possess this unique specificity. The enzyme, secreted into the host cell, may be involved in the pathogenic inositol phosphate signal transduction pathways.

3.2.6 Phytases

Although phytate is not accumulated in microorganisms, its hydrolysis can provide a large amount of inorganic phosphate to bacterial cells. While a number of bacterial phytases (*e.g.*, from *E. coli*, Bacillus sp.) have been studied in depth, new bacterial phytases are characterized frequently, in part for their biotechnology usefulness. For example, a phytase recently been purified from *Pseudomonas syringae MOK1* (45 kDa) has an optimal activity at pH 5.5 and is inhibited by EDTA. It exhibits a high specificity for phytate and little or no activity for other inositol phosphates (Cho *et al.*, 2003). Strong phytase activity associated with a number of lactic acid bacteria has commercial importance as this enzyme is a useful additive that aids in demineralization of whole grain flours (Vohra and Satyanarayana, 2003).

The stepwise degradation of phytate has been shown to proceed via initial removal of the 3-phosphate to form D-I(1,2,4,5,6)P_5, then dephosphorylation to I(2,4,5,6)P_4, then to I(2,4,6)P_3 or I(2,5,6)P_3. A second pathway is suggested by the observation that I(1,2,4,5,6)P_5 can be degraded to I(1,2,5,6)P_4 to finally I(1,2,6)P_3. Further degradation to I-2-P can occur after prolonged incubation times (Greiner *et al.*, 2002). Insight into the mechanism for phytate hydrolysis has been provided by crystal structures (Shin *et al.*, 2001). The thermostable, calcium-dependent Bacillus phytase adopts a β-propeller fold. The structure shows two phosphates and four Ca^{2+} in the active site (Figure 5A). The non-equivalent phosphate sites represent a "cleavage site" and an "affinity site" that increases the binding affinity for substrates containing adjacent phosphate groups (Figure 5B). The two phosphate binding sites explain the puzzling formation of the alternately dephosphorylated *myo*-inositol triphosphates from phytate and the much slower hydrolysis of *myo*-inositol monophosphates. A structure of *E. coli* phytase with phytate (Lim *et al.*, 2000) suggested that the mechanism of that particular phytase is similar to histidine acid phosphatase, although no pronounced sequence homology was found.

4. BIOSYNTHESIS AND DEGRADATION OF PI

The detection of PI in bacterial and archaeal systems implies that PI synthase, which is an integral membrane protein, is present. Once PI is available, kinases could add phosphates to generate various phosphoinositides, although to date this chemistry has only been demonstrated to occur in eukaryotes. Bacterial catabolic activities specific to PI have also been identified. In most cases these are secreted soluble proteins that can cleave PI and in some cases GPI-anchored

proteins to DAG. These phospholipase activities often play a role in infectivity of the bacteria.

4.1 PI Synthase

The enzyme that converts CDP-DAG and *myo*-inositol to PI belongs to the CDP-alcohol phosphatidyltransferase class-I family. In yeast, the enzyme is located in the outer mitochondrial membranes and microsomes (Nikawa and Yamashita, 1997). In *M. tuberculosis*, the *pgsA* gene was identified as encoding a PI synthase enzyme by construction of a conditional mutant. The strain would not grow under non-permissive conditions. There was also a noted loss of cell viability when PI and PI dimannoside dropped to 30% and 50% of what occurs in wild type cells. Therefore PI and the machinery to make it are essential for survival of mycobacteria (Jackson *et al.*, 2000).

Using a eukaryotic PI synthase as query, one can easily identify homologues in mycobacteria as well as in some archaea. The bulk of each PI synthase sequence contains the local, conserved region found in enzymes catalyzing the transfer of the phosphoalcohol moiety from CDP-alcohol, such as phosphatidylserine synthase, cholinephosphotransferase, and phosphatidylglycerolphosphate synthase (Figure 6). The *M. bovis* PI synthase gene codes for a 217-residue membrane protein with an N-terminal hydrophobic segment likely to span the membrane and the region from 58 to 201 constituting the CDP-alcohol transferase domain. Similar sequences in *Streptomyces coelicolor* and even in archaeal genes (*e.g.*, *Pyrococcus furiosus*, *Methanosarcina acetivorans*, *Methanobacterium thermoautotrophicum*) can be identified. There is a very strong alignment of the initial half of the CDP-alcohol transferase domain among these proteins. Whether or not the archaeal genes code for PI synthase activity or another activity that has a CDP-alcohol domain (*e.g.*, the DIP synthase proposed in the biosynthesis of DIP (Chen *et al.*, 1998) awaits heterologous expression of these genes, so far a very difficult task for archaeal membrane proteins.

4.2 Enzymes of inositol phospholipid degradation: PI-specific phospholipase C

Bacterial PI-PLC enzymes have quite a different role from the eukaryotic enzymes involved in signal transduction. A variety of Gram-positive bacteria secrete PI-PLC that is highly specific for non-phosphorylated PI and does not require Ca^{2+} (required by all the mammalian PI-PLC enzymes). The PI-PLC enzyme in a subset of these organisms can also cleave the glycerol-phosphate bond of glycosyl-PI-anchored proteins (Ferguson *et al.*, 1985; Sharom and Lehto, 2002). Secreted PI-PLCs often contribute to the virulence of a microorganism. In *M. tuberculosis*, the gene for PI-PLC is upregulated during the initial 24 h of macrophage infection (Raynaud *et al.*, 2002). Often the PLC enzymes aid in the microorganism avoiding antibacterial host factors as it negotiates

```
My.b.   1   MSKLPFLSRAAFARITTPIARGLLRVGLTPDVVTILGTTASVAGALTLFP  50
Ms.a.   4                    PFARS---VPLSPNTLTLLGFAVSVAAGVA-FA  32
Mb.t.  13           RPVIRRFIDPIAD---RIALPADYITLTGFLVACAASAG-YA  50
Pyr.f.  2   LSNLRPLAKKPLEKIAEPFSK----LGITPNQLTMVGFFLSLLASYEYYL  47
                   +  + ++  P++     + +  + +T++G++++  ++   +

My.b.  51   MGKLFAGACVVWFFVLFDMLDGAMARERGGGTRFGAVLDATCDRISDGAV 100
Ms.a.  33   LGKPFEGGFLILFSGVFDILDGGVARAKGRITPFGGVLDSVCDRYSDGLM  82
Mb.t.  51   SGSLITGAALLAASGFIDVLDGAVARRRFRPTAFGGFLDSTLDRLSDGII 100
Pyr.f.48   NNQVF-GSLILLLGAFLDALDGSLARLTGRVTKFGGFLDSTMDRLSDAAI  96
             + + G++++++++++D+LDG++AR     T FG++LD++ DR+SD+++

My.b. 101   FCGLL------WWIAF-HMRDRPLVI-----ATLICLVTSQVISYIKARA 138
Ms.a.  83   FLGIM------AGAIYGRLSFAPFLGVEGWLWAGFALIGSFLVSYTRARA 126
Mb.t. 101   IIGIT-------------AGGFTGLL-----TGLLALHSGLMVSYVRARA 132
Pyr.f. 97   IFGIALGELVNWKVAF-----------------LALIGSYMVSYTRCRA 128
            ++G++                            ++L   +++SY ++RA


My.b. 139   EASGLRG-DG----GFIERPERLIIVLTGAGVSDFPFVPWPPALSVGMWL 183
Ms.a. 127   ESAGCRKLSV----GIAERTERMVILALGA-LSGF--------LGWALVL 163
Mb.t. 133   ESLGIEC-AV----GIAERAERIIIILAGSLAGYLIHPW--FMDAAIIVL 175
Pyr.f.129   E---LAG-SGTLAVGIAERGERLLILVI-AGLFGI--------IDIGVYL 165
            E   +          G  ER ER++I+   +     +          ++L


My.b.  184  LAVASVITCVQRL---HTVWTSPGAID         207
Ms.a.  164  IAVFSHITMIQRV                       176
Mb.t.  176  AALGYFTMI-QRM---IYVW                191
Pyr.f. 166  VAILSWITFLQRV---Y                   179
            +A+ +  +  QR+
```

Figure 6. Alignment of archaeal PI synthase homologues with the *M. bovis* PI synthase (My.b.): Ms.a., *Methanosarcina acetivorans*; Mb.t., *Methanobacterium thermoautotrophicum*; Py.f., *Pyrococcus furiosus*. The transmembrane sequence of the *M. bovis* enzyme is in italics while the CDP-alcohol transferase domain is underlined.

different compartments of cells (*e.g.*, *Bacillus cereus*, *B. thuringiensis*, *B. anthracis*). In *B. anthracis*, the *plcR* gene, which regulates expression of phospholipases and other lytic factors, is expressed under anaerobic conditions (Klichko *et al.*, 2003).

For all the PI-PLC enzymes examined thus far, PI hydrolysis occurs in two sequential reactions as shown in Figure 7 (Griffith and Ryan 1999): (i) an intramolecular phosphotransferase reaction at a phospholipid/aggregate surface to produce DAG and water-soluble 1,2-cyclic phosphate, cIP (or cIP_x in the case of a phosphoinositide substrate), followed by (ii) a phosphodiesterase reaction where the cIP is hydrolyzed to inositol-1-phosphate (or IP_x). The second reaction can be studied separately; it occurs with a soluble monomeric substrate and is much slower (both lower k_{cat} and higher K_m) than the first step (Volwerk *et al.*, 1990; Zhou *et al.*, 1997). It is an excellent probe for allosteric effects of membranes on catalysis (Zhou *et al.*, 1997; Wu *et al.*, 1997).

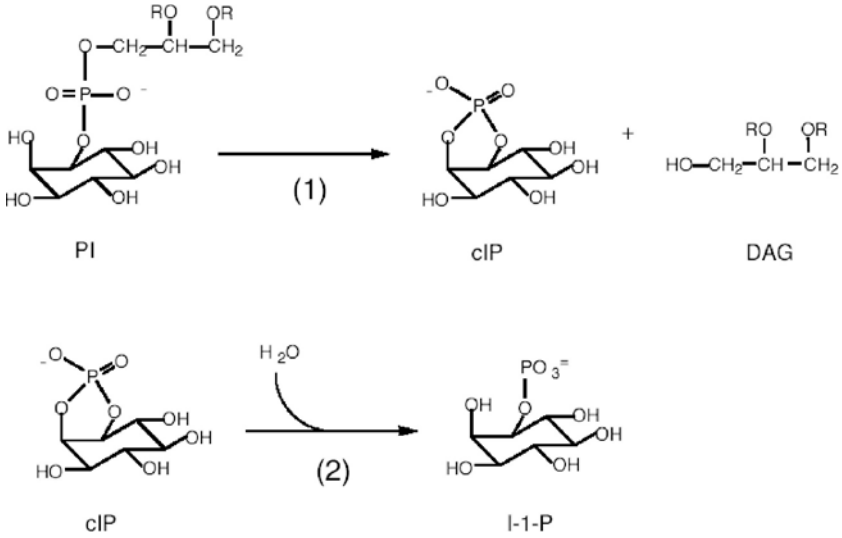


Figure 7. Reactions catalyzed by PI-PLC enzymes.

Crystal structures exist of two bacterial PI-PLC enzymes, the protein from *B. cereus* (Heinz *et al.*, 1995), which can cleave GPI-anchors, and the PI-PLC from *Listeria monocytogenes* (Moser *et al.*, 1997), which is not able to effectively release GPI-anchored proteins. While the sequence homology of these two proteins is limited, the structures are very similar. The bacterial PI-PLC proteins are folded into a distorted TIM-barrel, where the parallel β-strands form an inner circular and closed barrel with α-helices located on the outside between neighboring β-strands, that is structurally very similar to the catalytic domain of PLCδ_1, the only mammalian PI-PLC for which there is a structure (Essen *et al.*, 1996; Heinz *et al.*, 1998). The availability of structures and results of mutagenesis provide details on the catalytic mechanism for this type of enzyme (for review and more extensive references see Mihai *et al.* (2003)).

In the bacterial PI-PLC structures, the top of the barrel rim has several hydrophobic residues that are fully exposed to solvent and poorly defined in the crystal structures (implying significant mobility). The active site of PI-PLC is accessible and well-hydrated, and these mobile elements at the top of the barrel offer a different motif for interactions of the protein with phospholipid interfaces. The PI-PLC from *B. thuringiensis* (nearly identical in sequence to the enzyme from *B. cereus* whose crystal structure was determined) exhibits the property of interfacial activation, where enhanced activity is observed when the substrate PI is present in an interface compared to monomeric substrate (Lewis *et al.*, 1993). However, other non-substrate lipids such as phosphatidylcholine (PC), phosphatidic acid (PA), and other anionic lipids have an effect on the activity of PI-PLC toward both substrates PI and water-soluble cIP (Zhou *et al.*, 1997). In particular, the presence of PC enhances the catalytic activity of

the enzyme toward PI as well as cIP. Two residues at the top of the barrel – Trp47 in short helix B and Trp242 in a flexible loop (Figure 8) – are required for the protein to bind to activating PC surfaces (Feng *et al.*, 2002). Each tryptophan interacts with a PC molecule (Zhang *et al.*, 2004), and in so doing must alter the active site so that PI cleavage and cIP hydrolysis occur with enhanced k_{cat} and decreased apparent K_m (Zhou and Roberts, 1998; Zhou *et al.*, 1997). Removal of both tryptophan residues generates an enzyme that no longer effectively binds to membranes. The PI-PLC from *L. monocytogenes* also has aromatic residues fully exposed to solvent at the top of the barrel (Moser *et al.*, 1997) that are likely to be involved in surface binding of the enzyme. However, as discussed below, the interaction of this region of the protein with a net neutral amphiphile is different for the Listeria protein, and the differences from the Bacillus PI-PLC may be an important key to the role of PI-PLC in *L. monocytogenes* infectivity or mammalian cells.

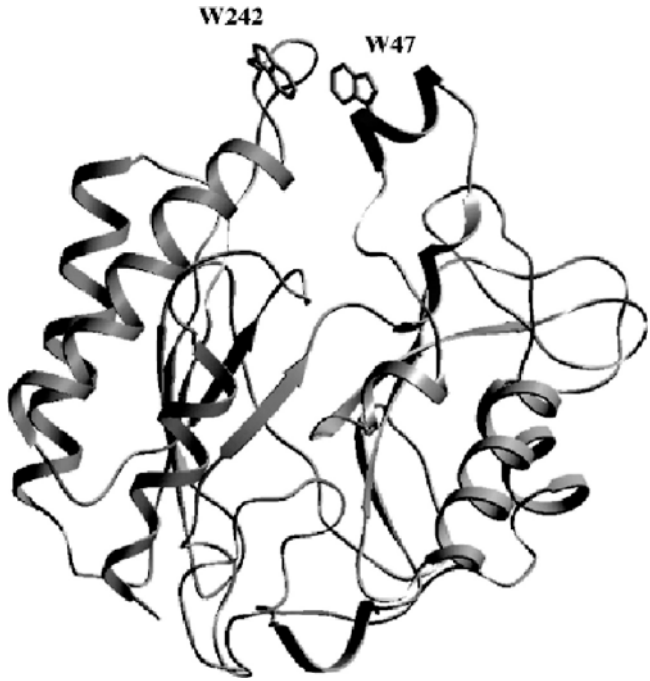


Figure 8. Structure of *B. cereus* PI-PLC.

4.3 Role of inositol enzymes in infection and virulence: *Listeria monocytogenes* PI-PLC

In bacteria, the biological role of secreted PI-PLC enyzmes, along with other non-specific phospholipases, is to aid in survival of the microorganism.

Bacterial PI-PLC enzymes that cleave GPI-anchored proteins, such as the PI-PLC secreted by *B. thuringiensis*, *B. cereus*, and *Staphylococcus aureus*, could target those membrane components in the extracellular leaflet of the plasma membrane, which is rich in the zwitterionic lipids PC and sphingomyelin. However, secreted PI-PLC enzymes whose targets are PI and not GPI-anchors, must follow a different path to aid bacterial survival since PI is not found in the external leaflet of most organisms. *Listeria monocytogenes* is a foodborne pathogen of humans and animals that can cause serious infections in immunocompromised individuals, pregnant women, and the elderly. The substrate for this PI-PLC is the PI (not GPI-anchors) that is on the inner monolayer of the plasma membrane of mammalian cells. When *L. monocytogenes* is internalized into macrophages, both listeriolysin O and PI-PLC expression are upregulated. PI-PLC presumably gains access to host PI by means of phagosome permeabilization and destruction caused by listeriolysin O (Sibelius *et al.*, 1999; Wadsworth and Goldfine, 2002). When this PI-PLC hydrolyzes PI and generates DAG, host PKCβ is activated. This along with elevated intracellular calcium plays an important role in escape of the bacterium from the phagosome.

While the *L. monocytogenes* PI-PLC has a similar structure to the *B. cereus* enzyme, its regulation of activity by amphiphiles and ionic strength is quite different. The Listeria enzyme, like the Bacillus PI-PLC, binds very tightly to vesicles of negatively charged phospholipids, and the surface interaction appears to cause aggregation of the enzyme on the vesicle surface. Unlike the *B. thuringiensis* PI-PLC, the *L. monocytogenes* PI-PLC has a very weak affinity for PC vesicles, although it can bind to short-chain PC micelles. Neutral amphiphiles such as PC or a detergent such as Triton X-100 as well as increased ionic strength "activate" the enzyme by appearing to shift the equilibrium toward monomeric protein, possibly by preventing anionic lipid aggregation of the protein (W. Chen and M.F. Roberts, unpublished results). The inner leaflet of the vacuole membrane, presumably like the external leaflet of the plasma membrane, is rich in PC or sphingomyelin and has a low content of anionic phospholipids. Assuming this bacterial PI-PLC has weak affinity for PC (and presumably for sphingomyelin and PE as well) under the moderate ionic strengths in cells, it will stay in the vacuolar fluid and be easily dispersed into the cytoplasm. Once there it will partition with the negatively charged components of the target membrane. As long as there are some zwitterionic/neutral lipids around, it is likely the enzyme can bind and partially insert into the bilayer in a way that allows it to effectively hydrolyze PI and generate DAG.

5. PERSPECTIVES FOR THE FUTURE

While inositol compounds are not ubiquitous in bacteria and archaea, they do occur and often have critical roles in those organisms. Inositol-containing

phospholipids do occur, but in these two domains of life as opposed to Eukarya, they are not components of signaling pathways. Often PI (or the sphingolipid counterpart) serves as a membrane anchor of complex glycolipids and surface proteins. These lipid units are absolutely critical to the integrity and infectivity of mycobacteria. Soluble inositol compounds are also not very common, but those that have been detected have rather unusual roles in microorganisms. Intracellular concentrations of DIP and related solutes are modulated by external NaCl, indicating they are part of the osmotic response of the cells. Their biosynthesis and accumulation is also modulated by the growth temperature of the organisms. Mycothiol is used as a redox agent in mycobacteria – in essence it is a more complex glutathione analogue. Clearly, there are many areas that are ripe for further investigation.

The occurrence of PI anchors in mycobacteria is important for growth and infectivity of these cells, although the inositol unit itself does not play a direct role in the infectivity. Rather the membrane-anchored inositol unit is a structural scaffold. If a unique step in inositol biosynthesis can be inhibited, new drugs might be available against these organisms. The *M. tuberculosis* MIPS is not likely to be a good target since its structure is so similar to the yeast (and presumably human) enzyme. IMPases are likely to be difficult targets since there may be several activities that can carry out this chemistry in *M. tuberculosis*. One of the more promising targets has been suggested to be IPC synthase, since these particular inositol-containing lipids are unique to mycobacteria. Several natural products (*e.g.*, khafrefungin, Nakamura *et al.*, 2003, and galbonolide, Sakoh *et al.*, 2004) that target IPC synthase have been identified, but improving these as inhibitors of IPC synthase and ensuring that they have no effect on mammalian enzymes is likely to require a structure of an IPC synthase. The deacetylase activity needed for mycothiol production might also be a target since mycothiol helps the bacterium fend off oxidative stresses of the host. A recent structure of the deacetylase from *M. tuberculosis* that is absolutely critical for mycothiol production, while providing detailed information of the mechanism for this enzyme, also indicates it may be a representative of a wider family of enzymes, including eukaryotic ones, that work on *N*-acetylglucosamine residues (McCarthy *et al.*, 2004). However, the availability of the structure may allow researchers to develop very specific inhibitors of this enzyme.

DIP and its derivatives are interesting compounds for hyperthermophilic cells to synthesize in response to salt and/or temperature stress. Significant cell resources are necessary to make this molecule, particularly when the intracellular concentration is >0.5 M. With this in mind, one can pose two major questions. (i) What is there about the DIP molecule that makes it a particularly useful solute at high ionic strength and high temperature? (ii) How is DIP accumulation at supraoptimal temperatures regulated? For the first of these, studies will be needed to see how DIP affects water structure around proteins or other macromolecules. The second awaits the development of genetic systems for hyperthermophiles, a non-trivial task at this point.

Inositol enzymology is also ripe for future studies. The recent MIPS structures have certainly aided in our appreciation of the chemistry this enzyme enables. However, they also point out that a significant conformational change is likely to be necessary for the cyclization step of the reaction. Structures with linear G-6-P analogues or transition intermediate mimics fail to show how C1 and C6 are moved close enough for the aldol condensation reaction to occur. While it is not clear how to generate and characterize enzyme intermediate complexes on an atomic level, doing so would open up our understanding of this interesting enzyme. Whether or not metal ions are needed for all MIPS is another intriguing question at this point, since recent structural work with the yeast enzyme suggests it too has a metal ion in the vicinity of the NAD^+.

Continued structural characterization of inositol enzymes could shed light on some of their more uncommon roles. For example, we already know that SuhB does not need its IMPase activity to suppress diverse temperature-sensitive mutants in *E. coli* (Chen and Roberts, 2000). What are its targets in *E. coli*– RNAse III, mRNA molecules, or something else? The availability of a structure for this enzyme for comparison to all the other members of the IMPase superfamily might supply the key to unraveling this other biological activity. And perhaps more intriguingly, we can ask if other bacterial IMPase enzymes exhibit behavior like SuhB such that their inactivation gives rise to a cold-sensitive phenotype, or is this a property solely of the *E. coli* enzyme? The archaeal IMPase/FBPase duality also serves as an interesting entry into how specific activities in this class of phosphatases, members of the IMPase superfamily, evolved. Any details on PI synthases would aid in our understanding of this step in PI biosynthesis. The possible identity of archaeal PI synthase sequences could also provide new targets for expression and characterization that might be better suited for crystallization and structural studies.

ACKNOWLEDGMENTS

The author would like to thank the DOE Department of Energy Biosciences Division DE-FG02-91ER20025 and NIH GM60418 for support of some of the research discussed in this review article.

REFERENCES

Agam, G., Shamir, A., Shaltiel, G., and Greenberg, M.L., 2002, *myo*-Inositol-1-phosphate (MIP) synthase: A possible target for antibipolar drugs. *Bipolar Disord.* **4**(Suppl 1): 15–20.

Bachawarat, N., and Mande, S.C., 1999, Identification of the INO1 gene of *Mycobacterium tuberculosis* H37Rv reveals a novel class of inositol-1-phosphate synthase enzyme. *J. Mol. Biol*. **291:** 531–536.

Bachawarat, N., and Mande, S.C., 2000, Complex evolution of the inositol-1-phosphate synthase gene among archaea and eubacteria. *Trends Genet.* **16:** 111–113.

Balmer, Y., Stritt-Etter, A., Hirasawa, M., Jacquot, J.P., Keryer, E., Knaff, D.B., and Schurmann, P., 2001, Oxidation-reduction and activation properties of chloroplast fructose 1,6-bisphosphatase with mutated regulatory site. *Biochemistry* **40:** 15444–15450.

Belisle, J.T., Brandt, M.E., Radolf, J.D., and Norgard, M.V., 1994, Fatty acids of *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins. *J. Bacteriol.* **176:** 2151–2157.

Brennan, P.J., 2003, Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. *Tuberculosis* **83:** 91–97.

Brennan, P.J., and Ballou, C.E., 1968, Phosphatidylmyoinositol monomannoside in *Propionibacterium shermanii*. *Biochem. Biophys. Res. Commun.* **30:** 69–75.

Brennan, P.J., and Lehane, D.P., 1971, The phospholipids of corynebacteria. *Lipids* **6:** 401–409.

Buchmeier, N.A., Newton, G.L., Koledin, T., and Fahey, R.C., 2003, Association of mycothiol with protection of *Mycobacterium tuberculosis* from toxic oxidants and antibiotics. *Mol. Microbiol.* **47:** 1723–1732.

Chang, S.F., Ng, D., Baird, L., and Georgopoulos, C., 1991, Analysis of an *Escherichia coli dnaB* temperature-sensitive insertion mutation and its cold-sensitive extragenic suppressor. *J. Biol. Chem.* **266:** 3654–3660.

Chen, L., and Roberts, M.F., 1998, Cloning and expression of the inositol monophosphatase gene from *Methanococcus jannaschii* and characterization of the enzyme. *Appl. Environ. Microbiol.* **64:** 2609–2615.

Chen, L., and Roberts, M.F., 1999, Characterization of a tetrameric inositol monophosphatase from the hyperthermophilic bacterium *Thermotoga maritima*. *Appl. Environ. Microbiol.* **65**: 4559–4567.

Chen, L., and Roberts, M.F., 2000, Overexpression, purification, and analysis of complementation behavior of *E. coli* SuhB protein: Comparison with bacterial and archaeal inositol monophosphatases. *Biochemistry* **39:** 4145–4153.

Chen, L., Spiliotis, E., and Roberts, M.F., 1998, Biosynthesis of Di-*myo*-inositol-1,1′-phosphate, a novel osmolyte in hyperthermophilic archaea. *J. Bacteriol.* **180:** 3785–3792.

Chen, L., Zhou, C., Yang, H., and Roberts, M.F., 2000, Inositol-1-phosphate synthase from *Archaeoglobus fulgidus* is a class II aldolase. *Biochemistry* **39:** 12415–12423.

Chi, H., Tiller, G.E., Dasouki, M.J., Romano, P.R., Wang, J., O'Keefe, R.J., Puzas, J.E., Rosier, R.N., and Reynolds, P.R., 1999, Multiple inositol polyphosphate phosphatase: evolution as a distinct group within the histidine phosphatase family and chromosomal localization of the human and mouse genes to chromosomes 10–23 and 19. *Genomics* **56:** 324–336.

Cho, J.S., Lee, C.W., Kang, S.H., Lee, J.C., Bok, J.D., Moon, Y.S., Lee, H.G., Kim, S.C., and Choi, Y.J., 2003, Purification and characterization of a phytase from *Pseudomonas syringae* MOK1. *Curr. Microbiol.* **47:** 290–294.

Ciulla, R.A., Burggraf, S., Stetter, K O., and Roberts, M.F., 1994, Occurrence and role of di-*myo*-inositol-1,1′-phosphate in *Methanococcus igneus*. *Appl. Environ. Microbiol.* **60:** 3660–3664.

Downing, J.F., Pasula, R., Wright, J.R., Twigg, H.L., III, and Martin, W.J., II, 1995 Surfactant protein A promotes attachment of *Mycobacterium tuberculosis* to alveolar macrophages during infection with human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **92:** 4848–4852.

Elsbach, P., and Weiss, J., 1988, Phagocytosis of bacteria and phospholipid degradation. *Biochim. Biophys. Acta* **947:** 29–52.

Essen, L.O., Perisic, O., Cheung, R., Katan, M., and Williams, R.L., 1996, Crystal structure of a mammalian phosphoinositide-specific phospholipase C δ. *Nature* **380:** 595–602.

Feng, J., Wehbi, H., and Roberts, M.F., 2002, Role of tryptophan residues in interfacial binding of phosphatidylinositol-specific phospholipase C. *J. Biol. Chem.* **277:** 19867–19875.

Ferguson, J.S., Voelker, D.R., McCormack, F.X., and Schlessinger, L.S., 1999, Surfactant protein D binds to *Mycobacterium tuberculosis* bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. *J. Immunol.* **163:** 312–321.

Ferguson, M.A., Low, M.G., and Cross, G.A., 1985, Glycosyl-sn-1,2-dimyristylphosphatidylinositol is covalently linked to *Trypanosoma brucei* variant surface glycoprotein. *J. Biol. Chem.* **260:** 14547–14555.

Fisher, S.K., Novak, J.E., and Agranoff, W., 2002, Inositol and higher inositol phosphates in neural tissues: Homeostasis, metabolism and functional significance. *J. Neurochem.* **82:** 736–754.

Goren, M.B., 1984. Biosynthesis and structures of phospholipids and sulfatides. In: Kubica, G.P., and Wayne, L.G. (eds.), The Mycobacteria: A Sourcebook. Marcel Dekker, Inc., New York, pp. 379–415.

Greiner, R., Farouk, A., Alminger, M.L., and Carlsson, N.G., 2002, The pathway of dephosphorylation of *myo*-inositol hexakisphosphate by phytate-degrading enzymes of different Bacillus sp. *Can. J. Microbiol.* **48:** 986–994.

Griffith, O.H., and Ryan, M., 1999, Bacterial phosphatidylinositol-specific phospholipase C: Structure, function, and interaction with lipids. *Biochim. Biophys. Acta* **1441:** 237–254.

Heinz, D.W., Essen, L.O., and Williams, R.L., 1998, Structural and mechanistic comparison of prokaryotic and eukaryotic phosphoinositide-specific phospholipases C. *J. Mol. Biol*. **275:** 635–650.

Heinz, D.W., Ryan, M., Bullock, T.L., and Griffith, O.H., 1995, Crystal structure of the phosphatidylinositol-specific phospholipase C from *Bacillus cereus* in complex with *myo*-inositol. *EMBO J.* **14:** 3855–3863.

Hirst, P.H., Riley, A.M., Mills, S.J., Spiers, I.D., Poyner, D.R., Freeman, S., Potter, B.V., Smith, A.W., 1999, Inositol polyphosphate-mediated iron transport in *Pseudomonas aeruginosa*. *J. Appl. Microbiol.* **86:** 537–543.

Hoppe, H.C., de Wet, B.J., Cywes, C., Daffe, M., and Ehlers, M.R., 1997, Identification of phosphatidylinositol mannoside as a mycobacterial adhesin mediating both direct and opsonic binding to nonphagocytic mammalian cells. *Infect. Immun.* **65:** 3896—3905.

Inada, T., and Nakamura, Y., 1995, Lethal double-stranded RNA processing activity of ribonuclease III in the absence of suhB protein of *Escherichia coli*. *Biochimie* **77:** 294–302.

Inada, T., and Nakamura, Y., 1996, Autogenous control of the suhB gene expression of *Escherichia coli*. *Biochimie* **78:** 209–212.

Jackson, M., Crick, D.C., and Brennan, P.J., 2000, Phosphatidylinositol is an essential phospholipids of mycobacteria. *J. Biol. Chem.* **275:** 30092–30099.

Jacquot, J.P., Lopez-Jaramillo, J., Miginiac-Maslow, M., Lemaire, S., Cherfils, J., Chueca, A., and Lopez, J., 1997, Cysteine-153 is required for redox regulation of pea chloroplast fructose-1,6-bisphosphatase. *FEBS Lett*. **401:** 143–147.

Janczarek, M., and Skorupska, A., 2001, The *Rhizobium leguminosarum* bv. Trifolii *pssB* gene product is an inositol monophosphatase that influences exopolysaccharide synthesis. *Arch. Microbiol.* **175:** 143–151.

Johnson, K.A., Chen, L., Yang, H., Roberts, M.F., and Stec, B., 2001, Crystal structure and catalytic mechanism of the MJ0109 gene product: A bifunctional enzyme with inositol monophosphatase and fructose 1,6-bisphosphatase activities. *Biochemistry* **40:** 618–630.

Kataoka, T., and Nojima, S., 1967, The phospholipid compositions of some Actinomycetes. *Biochim. Biophys. Acta* **144:** 681–683.

Klichko, V.I., Miller, J., Wu, A., Popv, S.G., and Alibekk, K., 2003, Anaerobic induction of *Bacillus anthracis* hemolytic activity. *Biochem. Biophys. Res. Commun.* **303:** 855–862.

Kozloff, L.M., Turner, M.A., and Arellanno, F., 1991a, Formation of bacterial membrane ice-nucleating lipoglcoprotein complexes. *J. Bacteriol.* **173:** 6528–6536.

Kozloff, L.M., Turner, M.A., Arellanno, F., and Lute, M., 1991b, Phosphatidylinositol, a phospholipid of ice-nucleating bacteria. *J. Bacteriol.* **173:** 2053–2060.

Lee, D.C., Cottrill, M.A., Forsberg, C.W., and Jia, Z., 2003, Functional insights revealed by the crystal structures of *Escherichia coli* glucose-1-phosphatase. *J. Biol. Chem.* **278:** 31412–31418.

Levery, S.B., Toledo, M.S., Straus, A.H., and Takahashi, H.K., 1998, Structure elucidation of sphingolipids from the mycopathogen *Paracoccidiodes brasiliensis*: An immunodominant β-galactofuranose residue is carried by a novel glycosylinositol phosphorylceramide antigen. *Biochemistry* **37:** 8764–8775.

Lewis, K., Garigapati, V., Zhou, C., and Roberts, M.F., 1993, Substrate requirements of bacterial phosphatidylinositol-specific phospholipase C. *Biochemistry* **32:** 8836–8841.

Lim, D., Golovan, S., Forsberg, C.W., and Jia, Z., 2000, Crystal structures of *Escherichia coli* phytase and its complex with phytate. *Nat. Struct. Biol.* **7:** 108–113.

Loewus, M.W., 1977, Hydrogen isotope effects in the cyclization of D-glucose 6-phosphate by *myo*-inositol-1-phosphate synthase. *J. Biol. Chem.* **252:** 7221–7223.

Loewus, M.W., Loewus, F.A., Brillinger, G.U., Otsuka, H., and Floss, H.G., 1980, Stereochemistry of the *myo*-inositol-1-phosphate synthase reaction. *J. Biol. Chem.* **255:** 11710–11712.

Majumdar, A.L., Chatterjee, A., Dastidar, K.G., and Majee, M., 2003, Diversification and evolution of L-*myo*-inositol 1-phosphate synthase. *FEBS Lett.* **553:** 3–10.

Majumder, A.L., Johnson, M.D., and Henry, S.A., 1997, 1L-*myo*-inositol-1-phosphate synthase. *Biochim. Biophys. Acta* **1348:** 245–256.

Martin, D.D., Ciulla, R.A., and Roberts, M.F., 1999, Osmoadaptation in archaea. *Appl. Environ. Microbiol.* **65:** 1815–1825.

Martins, L.O., Carreto, L.S., Da Costa, M.S., and Santos, H., 1996, New compatible solutes related to di-*myo*-inositol-phosphate in members of the order Thermotogales. *J. Bacteriol.* **178:** 5644–5651.

Martins, L.O., Huber, R., Huber, H., Stetter, K.O., da Costa, M.S., and Santos, H., 1997, Organic solutes in hyperthermophilic *Archaea. Appl. Environ. Microbiol.* **63:** 896–902.

Matsuhisa, A., Suzuki, N., Noda, T., and Shiba, K., 1995, Inositol monophosphatase activity from the *Escherichia coli* suhB gene product. *J. Bacteriol.* **177:** 200–205.

McCarthy, A.A., Peterson, N.A., Knijff, R., and Baker, E.N., 2004, Crystal structure of MshB from *Mycobacterium tuberculosis*, a deacetylase involved in mycothiol biosynthesis. *J. Mol. Biol.* **335:** 1131–1141.

Mihai, C., Kravchuk, A.V., Tsai, M.D., and Bruzik, K.S., 2003, Application of Bronsted-type LFER in the study of the phospholipase C mechanism. *J. Amer. Chem. Soc.* **125:** 3236–3242.

Morii, H., Yagi, H., Akutsu, H., Nomura, N., Sako, Y., and Koga, Y., 1999, A novel phosphoglycolipid archaetidyl(glucosyl)inositol with two sesterterpanyl chains from the aerobic hyperthermophilic archaeon *Aeropyrum pernix* K1.

Morii, H., and Koga, Y., 1994, Asymmetrical topology of diether- and tetraether-type polar lipids in membranes of *Methanobacterium thermoautotrophicum* cells. *J. Biol. Chem.* **269:** 10492–10497.

Moser, J., Gerstel, B., Meyer, J.E., Chakraborty, T., Wehland, J., and Heinz, D.W., 1997, Crystal structure of the phosphatidylinositol-specific phospholipase C from the human pathogen *Listeria monocytogenes*. *J. Mol. Biol.* **273:** 269–282.

Movahedzadeh, F., Smith, D.A., Norman, R.A., Dinadayala, P., Murray-Rust, J., Russell, D.G., Kendall, S.L., Rison, S.C., McAlister, M.S., Bancroft, G.J., McDonald, N.Q., Daffe, M., Av-Gay, Y., and Stoker, N.G., 2004, The *Mycobacterium tuberculosis ino1* gene is essential for growth and virulence. *Mol. Microbiol.* **51:** 1003–1014.

Nagiec, M.M., Nagiec, E.E., Baltisberger, J.A., Wells, G.B., Lester, R.L., and Dickson, R.C., 1997, Sphingolipid synthesis as a target for antifungal drugs. Complementation of the insoitol phosphorylceramide synthase defect in a mutant strain of *Saccharomyces cerevisiae* by the *AUR1* gene. *J. Biol. Chem.* **272:** 9809–9817.

Nakamura, M., Mori, Y., Okuyama, K., Tanikawa, K., Yasuda, S., Hanada, K., and Kobayashi, S., 2003, Chemistry and biology of khafrefungin. Large-scale synthesis, design, and structure-activity relationship of khafrefungin, an antifungal agent. *Org. Biomol. Chem.* **1:** 3362–3376.

Nesbo, C.L., L'Haridon, S., Stetter, K.O., and Doolittle, W.F., 2001, Phylogenetic analyses of two "archaeal" genes in *Thermotoga maritima* reveal multiple transfers between archaea and bacteria. *Mol. Biol. Evol.* **18:** 362–375.

Neuwald, A.F., York, J.D., and Majerus, P.W., 1991, Diverse proteins homologous to inositol monophosphatase. *FEBS Lett.* **294:** 16–18.

Newton, G.L., Bewley, C.A., Dwyer, T.J., Horn, R., Abaromowitz, Y., Cohen, G., Davies, J., Faulkner, D.J., and Fahey, R.C., 1995, The structure of U17 isolated from *Streptomyces clavuligerus* and its properties as an antioxidant thiol. *Eur. J. Biochem.* **230:** 821–825.

Newton, G.L., and Fahey, R.C., 2002, Mycothiol biochemistry. *Arch. Microbiol.* **178:** 388–394.

Nigou, J., Dover, L.G., and Besra, G.S., 2002, Purification and biochemical characterization of *Mycobacterium tuberculosis* SuhB, an inositol monophosphatase involved in inositol biosynthesis. *Biochemistry* **41:** 4392–4398.

Nikawa, J., and Yamashita, S., 1997, Phosphatidylinositol synthase from yeast. *Biochim. Biophys. Acta* **1348:** 173–178.

Nishihara, M., and Koga, Y., 1991, Hydroxyarchaetidylserine and hydroxyarchaetidyl-*myo*-inositol in *Methanosarcina barkeri*: Polar lipids with a new ether core portion. *Biochim. Biophys. Acta* **1082:** 211–217.

Nishihara, M., Utagawa, M., Akutsu, H., and Koga, Y., 1992, Archaea contain a novel diether phosphoglycolipid with a polar head group identical to the conserved core of eucaryal glycosyl phosphatidylinositol. *J. Biol. Chem.* **267:** 12432–12435.

Norman, R.A., McAlister, M.S., Murray-Rust, J., Movahedzadeh, F., Stoker, N.G., and McDonald, N.Q., 2002, Crystal structure of inositol 1-phosphate synthase from *Mycobacterium tuberculosis*, a key enzyme in phosphatidylinositol synthesis. *Structure* **10:** 393–402.

Parish, T., Liu, J., Nikaido, H., and Stoker, N.G., 1997, A *Mycobacterium smegmatis* mutant with a defective inositol monophosphate phosphatase gene homolog has altered cell envelope permeability. *J. Bacteriol.* **179:** 7827–7833.

Raboy, V., 2003, *myo*-Inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry* **64:** 1033–1043.

Rashid, N., Imanaka, H., Kanai, T., Fukui, T., Atomi, H., and Imanaka, T., 2002, A novel candidate for the true fructose-1,6-bisphosphatase in archaea. *J. Biol. Chem.* **277:** 30649–30655.

Raynaud, C., Guilhot, C., Rauzier, J., Bordat, Y., Pelicic, V., Manganelli, R., Smith, I., Gicquel, B., and Jackson, M., 2002, Phospholipases C are involved in the virulence of *Mycobacterium tuberculosis*. *Mol. Microbiol.* **45:** 203–217.

Sakoh, H., Sugimoto, Y., Imamura, H., Sakuraba, S., Jona, H., Bamba-Nagano, R., Yamada, K., Hashizume, T., and Morishima, H., 2004, Novel galbonolide derivatives as IPC synthase inhibitors: Design, synthesis and in vitro antifungal activities. *Bioorg. Med. Chem. Lett.* **14:** 143–145.

Salman, M., Lonsdale, J.T., Besra, G.S., and Brennan, P.J., 1999, Phosphatidylinositol synthesis in mycobacteria. *Biochim. Biophys. Acta* **1436:** 437–450.

Scholz, S., Sonnenbichler, J., Schafer, W., and Hensel, R., 1992, Di-*myo*-inositol-1,1′-phosphate: A new inositol phosphate isolated from *Pyrococcus woesei*. *FEBS Lett.* **306:** 239–242.

Scholz, S., Wolff, S., and Hensel, R., 1998, The biosynthesis pathway of di-*myo*-inositol-1,1′-phosphate in *Pyrococcus woesei*. *FEMS Microbiol. Lett.* **168:** 37–42.

Sharom, F.J., and Lehto, M.T., 2002, Glycosylphosphatidylinositol-anchored proteins: Structure, function, and cleavage by phosphatidylinositol-specific phospholipase C. *Biochem. Cell. Biol.* **80:** 535–549.

Shin, S., Ha, N.C., Oh, B.C., Oh, T.K., and Oh, B.H., 2001, Enzyme mechanism and catalytic property of beta propeller phytase. *Structure* **9:** 851–858.

Sibelius, U., Schulz, E.C., Rose, F., Hattar, K., Jacobs, T., Weiss, S., Chakraborty, T., Seeger, W., and Grimminger, F., 1999, Role of *Listeria monocytogenes* exotoxins listeriolysin and phosphatidylinositol-specific phospholipase C in activation of human neutrophils. *Infect. Immun.* **67:** 1125–1130

Sidobre, S., Nigou, J., Puzo, G., and Riviere, M., 2000, Lipoglycans are putative ligands for the human pulmonary surfactant protein A attachment to mycobacteria. *J. Biol. Chem.* **275:** 2415–2422.

Spies, H.S.C., and Steenkamp, D.J., 1994, Thiols of intracellular pathogens. Identification of ovothiol A in *Leishmania donovani* and structural analysis of a novel thiol from *Mycobacterium bovis*. *Eur. J. Biochem.* **224:** 203–213.

Stec, B., Yang, H., Johnson, K.A., Chen, L., and Roberts, M.F., 2000, MJ0109 is an enzyme that is both an inositol monophosphatase and the 'missing' archaeal fructose-1,6-bisphosphatase. *Nat. Struct. Biol.* **7:** 1046–1050.

Stein, A.J., and Geiger, J.H., 2002, The crystal structure and mechanism of 1-L-*myo*-inositol-1-phosphate synthase. *J. Biol. Chem.* **277:** 9484–9491.

Stieglitz, K.A., Johnson, K.A., Yang, H., Roberts, M.F., Seaton, B.A., Head, J.F., and Stec, B., 2002, Crystal structure of a dual activity IMPase/FBPase (AF2372) from *Archaeoglobus fulgidus*. The story of a mobile loop. *J. Biol. Chem.* **277:** 22863–22874.

Stieglitz, K.A., Yang, H., Roberts, M.F., and Stec, B., 2005, Reaching for mechanistic consensus across life kingdoms: Structure and insights into catalysis of the inositol-1-phosphate synthase (MIPS) from *Archaeoglobus fulgidus*. *Biochemistry* **44:** 213–224.

Stieglitz, K.A., Seaton, B.A., Head, J.F., Stec, B., and Roberts, M.F., 2003, Unexpected similarity in regulation between an archaeal inositol monophosphatase/fructose bisphosphatase and chloroplast fructose bisphosphatase. *Protein Sci.* **12:** 760–767.

Tabaud, H., Tisnovska, H., and Vilkas, E., 1971, Phospholipids and glycolipids of a *Micromonospora* strain. *Biochimie* **53:** 55–61.

Tian, F., Migaud, M.E., and Frost, J.W., 1999, Stereochemistry of the *myo*-inositol-1-phosphate synthase reaction. *J. Biol. Chem.* **255:** 11710–11712.

Toledo, M.S., Levery, S.B., Glushka, J., Straus, A.H., and Takahashi, H.K., 2001, Structure elucidation of sphingolipids from the mycopathogen *Sporothrix schenckii*: Identification of novel glycosylinositol phosphorylceramides with core Manα1$\rightarrow$6Ins linkage. *Biochem. Biophys. Res. Commun.* **280:** 19–24.

Vohra, A., and Satyanarayana, T., 2003, Phytases: Microbial sources, production, purification, and potential biotechnological applications. *Crit. Rev. Biotechnol.* **23:** 29–60.

Volwerk, J.J., Shashidhar, M.S., Kuppe, A., and Griffith, O.H., 1990, Phosphatidylinositol-specific phospholipase C from *Bacillus cereus* combines intrinsic phosphotransferase and cyclic phosphodiesterase activities: A ^{31}P NMR study. *Biochemistry* **29:** 8056–8062.

Wadsworth, S.J., and Goldfine, H., 2002, Mobilization of protein kinase C in macrophages induced by *Listeria monocytogenes* affects its internalization and escape from the phagosome. *Infect. Immun.* **70:** 4650–4660.

Walker, J.B., 1995, Enzymatic synthesis of aminocyclitol moieties of aminoglycoside antibiotics from inositol by *Streptomyces* spp.: Detection of glutamine-aminocyclitol aminotransferase and diaminocyclitol aminotransferase activities in a spectinomycin producer. *J. Bacteriol.* **177:** 818–822.

Wu, Y., Perisic, O., Williams, R.L., Katan, M., and Roberts, M.F., 1997, Phosphoinositide-specific phospholipase C δ1 activity toward micellar substrates, inositol 1,2-cyclic phosphate, and other water-soluble substrates: A sequential mechanism and allosteric activation. *Biochemistry* **36:** 11223–11233.

Yague, G., Segovia, M., and Valero-Guillen, P.L., 2003, Phospholipid composition of several clinically relevant Corynebacterium species as determined by mass spectrometry: An unusual fatty acyl moiety is present in inositol-containing phospholipids of *Corynebacterium urealyticum*. *Microbiology* **149:** 1675–1685.

Yano, I., Furukawa, Y., and Kusunose, M., 1969, Phospholipids of *Nocardia coeliaca*. *J. Bacteriol.* **98:** 124–130.

Yano, R., Nagai, H., Shiba, K., and Yura, T., 1990, A mutation that enhances synthesis of σ^{32} and suppresses temperature-sensitive growth of the *rpoH15* mutant of *Escherichia coli*. *J. Bacteriol.* **172:** 2124–2130.

York, J.D., Ponder, J.W., and Majerus, P.W., 1995, Definition of a metal-dependent/Li^+-inhibited phosphomonoesterase protein family based upon a conserved three-dimensional core structure. *Proc. Natl. Acad. Sci. USA* **92:** 5149–5153.

Zhang, X., Wehbi, H., and Roberts, M.F., 2004, Crosslinking phosphatidylinositol-specific phospholipase C traps two activating phosphatidylcholine molecules on the enzyme. *J. Biol. Chem.* **279:** 20490–20500.

Zhou, C., and Roberts, M.F., 1998, Nonessential activation and competitive inhibition of bacterial phosphatidylinositol-specific phospholipase C by short-chain phospholipids and analogues. *Biochemistry* **37:** 16430–16439.

Zhou, C., Wu, Y., and Roberts, M.F., 1997, Activation of phosphatidylinositol-specific phospholipase C toward inositol 1,2-(cyclic)-phosphate. *Biochemistry* **36:** 347–355.