[Frontiers in Bioscience 9, 1382-1397, May 1, 2004]

MANGANESE-DEPENDENT PROTEIN O-PHOSPHATASES IN PROKARYOTES AND THEIR BIOLOGICAL FUNCTIONS

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1. ABSTRACT

During the past decade, numerous Mn^{2+}-dependent protein serine, threonine and/or tyrosine phosphatases (O-phosphatases) from prokaryotes have been characterized. Based on their amino acid sequences, they belong to PPP, PPM or PHP superfamilies. Both the PPP and PPM families of protein phosphatases are metalloenzymes which active centers contain two metal ions that function as cofactors. Results from sequence analysis also suggest that PHP family protein phosphatase is a metalloenzyme. The identified functions for PPP family protein phosphatases from different prokaryotic organisms include regulation of stress-response, nitrogen fixation and vegetative growth. At least one phosphatase, PrpB from *Escherichia coli*, is also implicated in bacterial pathogenesis. Prokaryotic PPM family protein phosphatases are involved in controlling spore formation, stress-response, cell density during stationary phase, carbon and nitrogen assimilation, vegetative growth, development of fruiting bodies and cell segregation. The function of CpsB, a PHP family protein tyrosine phosphatase from *Streptococcus pneumonia*, is to regulate biosynthesis of capsular polysaccharide, an important virulence determinant. Thus, this group of functionally diverse protein phosphatases plays an important role in prokaryotes. Discovery of Mn^{2+}-dependent prokaryotic protein O-phosphatases and their functions also contributes to new insight into Mn^{2+} homeostasis and many roles played by Mn^{2+} and protein O-phosphorylation in prokaryotic cells.

2. INTRODUCTION

Manganese (Mn) is a trace element essential for both prokaryotes and eukaryotes (1). One of the major physiological roles that Mn plays is its use as an enzyme cofactor. However, only a few Mn-dependent enzymes have been identified (2). Among them are a group of protein serine, threonine and/or tyrosine phosphatases (O-phosphatases) recently characterized from prokaryotic cells (Table 1). Based on their amino acid sequences, these protein phosphatases belong to three different superfamilies: the phosphoprotein phosphatase (PPP) family, the Mg^{2+} or Mn^{2+}-dependent protein phosphatase (PPM) family and the polymerase and histidinol

Table 1. Mn^{2+}-dependent protein O-phosphatases from prokaryotes

Name	Organism	Molecular Weight (kDa)	Domain	Gene Family	Activating Metals	Physiological Substrates	Functions
PP-lambda[14-16]	Lambda phage	25		PPP	Mn^{2+}, Ni^{2+}	Unknown	Unknown
[a]PrpE[40]	*B. subtilis*	27	*Bacteria*	PPP	Ni^{2+}	Unknown	Unknown
EC-PrpA[41]	*E. coli*	24	*Bacteria*	PPP	Mn^{2+}	Unknown	Stress response
EC-PrpB[41]	*E. coli*	24	*Bacteria*	PPP	Mn^{2+}	Unknown	Stress response
ST-PrpA[42]	*S. typhimurium*	26	*Bacteria*	PPP	Mn^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}	Unknown	Stress response
ST-PrpB[42]	*S. typhimurium*	25	*Bacteria*	PPP	Mn^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}	Unknown	Stress response
PP1-arch1[43]	*S. solfataricus*	34	*Archaea*	PPP	Mn^{2+}, Ni^{2+}, Co^{2+}, Mg^{2+}	Unknown	Unknown
PP1-arch2[44]	*M. thermophila* TM-1	31	*Archaea*	PPP	Mn^{2+}, Ni^{2+}, Co^{2+}	Unknown	Unknown
Py-PP1[45]	*P. abyssi* TAG11	33	*Archaea*	PPP	Mn^{2+}, Ni^{2+}, Co^{2+}	Unknown	Unknown
PP1-cyano1[46]	*M. aeruginosa* PCC7820	30	*Bacteria*	PPP	Mn^{2+}, Mg^{2+}	Unknown	Unknown
PP1-cyano2[47]	*M. aeruginosa* UTEX2063	30	*Bacteria*	PPP	Mn^{2+}, Mg^{2+}	Unknown	Unknown
Sll1837[48]	*Synechocystis* sp PCC6803	28	*Bacteria*	PPP	Mn^{2+}, Mg^{2+}	Unknown	Unknown
SppA[49]	*S. coelicolor* A3(2)	39	*Bacteria*	PPP	Mn^{2+}, Ni^{2+}	Unknown	Vegetative growth & formation of hyphae and spores
PrpA[50]	*Anabaena* sp PCC7120	96	*Bacteria*	PPP	Mn^{2+}	Unknown	Heterocyst formation & nitrogen fixation
IcfG[48]	*Synechocystis* sp PCC6803	71	*Bacteria*	PPM	Mn^{2+}, Mg^{2+}	Slr1856-PS[b]	Carbon & glucose metabolism
SpoIIE[26]	*B. subtilis*	92	*Bacteria*	PPM	Mn^{2+}	SpoIIAA-PS	Sporulation
RsbP[28]	*B. subtilis*	46	*Bacteria*	PPM	Mn^{2+}	RsbV-PS	Energy stress response
RsbX[62]	*B. subtilis*	22	*Bacteria*	PPM	Mn^{2+}	RsbS-PS	Environmental stress response
RsbU[62]	*B. subtilis*	39	*Bacteria*	PPM	Mn^{2+}	RsbV-PS	Environmental stress response
PA-Stp1[63]	*P. aeruginosa*	27	*Bacteria*	PPM	Mn^{2+}	Unknown	Unknown
SA-Stp1[64]	*S. agalactiae*	27	*Bacteria*	PPM	Mn^{2+}	Stk1-PS, PpaC-PS	Cell segregation
Php1[65]	*M. xanthus*	25	*Bacteria*	PPM	Mn^{2+}, Mg^{2+}	Unknown	Vegetative growth & formation of fruiting body
PrpC[66]	*B. subtilis*	28	*Bacteria*	PPM	Mn^{2+}	PrkC-PT, EF-G-PT	Stationary-phase physiology
PhpA[67]	*Synechocystis* sp PCC6803	28	*Bacteria*	PPM	Mn^{2+}, Mg^{2+}	P_{II}-PS	Nitrogen assimilation
CpsB[30]	*S. pneumonia*	28	*Bacteria*	PHP	Mn^{2+}, Co^{2+}	CpsD-PY	Capsule biosynthesis

[a]: PrpE, a N^{2+}-dependent PPP family protein phosphatase, is also listed in this Table for the purpose of comparison [b]: PS: phosphorylated serine; PT: phosphorylated threonine; PY: phosphorylated tyrosine.

phosphatase (PHP) family (3-4). They are found in both *Bacteria* and *Archaea* and have diverse physiological functions. This review will briefly discuss the catalytic mechanism of PPP and PPM families of protein phosphatases and our current understanding of PHP family phosphatases and then focus on what we have learned from those protein phosphatases characterized from prokaryotes, especially their biological functions, during the past decade.

2.1. Catalytic mechanism of PPP family protein phosphatases

PPP family protein phosphatases are originally identified and characterized from eukaryotic cells where they are the most abundant protein serine/threonine phosphatases. Eukaryotic PPP family protein phosphatases include PP1, PP2A, PP2B (calcineurin) and related protein phosphatases. PP1/PP2A/PP2B are holoenzymes with different subunits. They only show high degree of sequence similarity with each other over a common catalytic domain of about 280 amino acid residues. Outside their catalytic domains, sequence similarity is significantly more varied. The enzymatic activity, substrate specificity, and physiological functions of PP1/PP2A/PP2B are mainly controlled by their regulatory and targeting subunits that interact with the catalytic subunits through their different and relatively poorly conserved noncatalytic domains (5-7). Evolutionary variation in the non-catalytic domains allows the phosphatases to interact with a variety of regulatory proteins and thus be adapted to provide regulatory functions for a wide spread of physiological roles. It is still unclear whether prokaryotic PPP family protein phosphatases possess any regulatory or target subunit functionally similar to those for eukaryotic PP1/PP2A/PP2B phosphatases.

The crystal structures of the catalytic subunits for PP1 and PP2B have been determined (8-11). PP1 and PP2B share an identical catalytic domain structure with two metal ions at the center of active site. The metal ions found in the active site from structural studies are Mn^{2+} and Fe^{2+} (or Fe^{3+}) in PP1 and Zn^{2+} and Fe^{2+} in PP2B (5-6). Data from crystal structure suggest that most conserved amino acid residues found in the signature sequence motifs of PPP family protein phosphatases (Figure 1) are involved in metal binding. PP1 and PP2B dephosphorylate their substrates in a single step reaction. During this reaction, a metal-activated water molecule is used as a nucleophile to attack the phosphorus atom in an S_N2 mechanism. The roles of metal ions in catalysis are twofold: to act as Lewis acid to increase the nucleophilicity of the metal-activated water, and to enhance the electrophilicity of the phosphorus atom. The side chain of the conserved histidine residue at the motif 3 (Figure 1) may assist the reaction by donating a proton to the leaving-group oxygen of a serine or threonine side chain (5-6).

The crystal structure of PP-lambda, a prokaryotic PPP family protein phosphatase encoded by the genome of bacterial phage, has also been determined (12). PP-lambda has only 221 residues (13), which sequence is 28% identical to the catalytic domain of PP1 (9). The three dimensional structure and catalytic mechanism of PP-lambda appear very similar to those for PP1 and PP2B, and two Mn^{2+} ions are found in its active site (12). This is consistent with results from biochemical studies showing that PP-lambda is a Mn^{2+}-dependent protein phosphatase (14-16). Recent studies also show that Mn^{2+} is a native activating metal ion for PP-lambda in *Escherichia coli* (17) and that PP-lambda binds the two Mn^{2+} ions with different affinities, indicating that PP-lambda might bind only one Mn^{2+} ion under physiological conditions. It is proposed that the second Mn^{2+} ion forms complex with the substrate before entering the active site. The resting PP-lambda with only one Mn^{2+} ion then binds to the substrate complexed with the second Mn^{2+} ion to form dinuclear metal center (18). If this hypothesis is correct, it may explain why PP-lambda and probably all characterized prokaryotic PPP

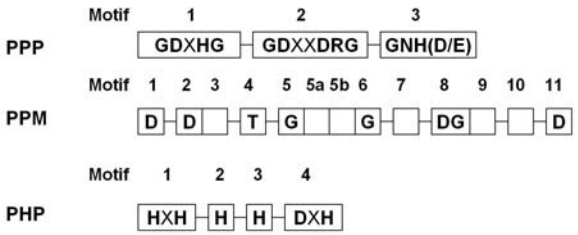

Figure 1. Schematic representation of signature sequence motifs of the PPP, PPM and PHP families of phosphatases. The sizes of these motifs are not drawn to scale.

family protein phosphatases require exogenous metal ions for their phosphatase activity, different from eukaryotic PPP family protein phosphatases that usually bind metal ions tightly enough so that they do not require exogenous metal ions to activate their phosphatase activity.

2.2. Catalytic mechanism of PPM family protein phosphatases

Like PPP family protein phosphatases, PPM family protein phosphatases are also first found in eukaryotes. They are a group of diverse Mg^{2+} or Mn^{2+}-dependent protein serine or threonine phosphatases that include PP2C, pyruvate dehydrogenase phosphatase and related enzymes. All share a common catalytic domain that spans a region of approximately 290 amino acid residues in length and contains eleven signature sequence motifs with eight conserved amino acid residues (Figure 1) (19-20). The crystal structure of a human PP2C has been determined at 2.0 Å resolution (21). Although the PPM and PPP phosphatase families share no sequence similarity, the molecular structure of catalytic domain of PP2C is very similar to those of the PPP family members PP1 and PP2A (5-6). The active center contains two Mn^{2+} ions coordinated by four conserved aspartic acid residues, a non-conserved glutamic acid residue and six water molecules. The catalytic mechanism of PP2C is also similar to that of the PPP family protein phosphatases. To remove the phosphoryl group from a protein substrate, a metal-activated water molecule acts as a nucleophile to attack the phosphorus atom in an S_N2 mechanism. A second water molecule protonates the oxygen atom of seryl leaving group (21). Similarities between PPP and PPM families protein phosphatases in their crystal structures and catalytic mechanisms may be the result of convergent evolution (5-6).

Many PPM family protein phosphatases also contain additional domains with distinct functions. The Kapp phosphatase from *Arabidopsis* has three domains: an N-terminal membrane localization domain, followed by a kinase interaction domain and a C-terminal phosphatase catalytic domain (22). ABI1, another PPM family phosphatase from *Arabidopsis*, possesses a Ca^{2+}-binding EF hand close to its N-terminus (23). Pyruvate dehydrogenase phosphatase also contains a Ca^{2+}-binding site close to its N-terminus. Its protein serine phosphatase activity is Mg^{2+}-dependent and stimulated by Ca^{2+} (24). Most interestingly, fungal adenylate cyclases contain a phosphatase catalytic domain in an area between the leucine-rich repeats and the cyclase catalytic domain. SpoIIE from *Bacillus subtilis* has a 10-membrane-spanning domain in its N-terminal region, a protein-protein interaction domain in the middle that regulates the oligomerization of SpoIIE as well as its interaction with cell division protein FtsZ, and a catalytic domain in its C-terminal region (25-26). RsbP, also from *B. subtilis,* contains a PAS [named after proteins PER (*per*iod clock protein), ARNT (*a*ryl hydrocarbon *r*eceptor *n*uclear *t*ranslocator), and SIM (*si*ngle-*m*inded protein), which amino acid sequences contain common imperfect repeats (27)] domain at its N-terminal region, which may be used to detect the change of intracellular redox potential (28).

2.3. PHP family phosphatases

Unlike the PPP and PPM families of protein phosphatases, PHP family phosphatases are much less studied and none of their molecular structures has been determined. Its members include (A) α subunits of bacterial DNA polymerase III (pol III) and the proteins with high degree of sequence similarity to the PHP domains found in α subunits of bacterial DNA pol III; (B) family X DNA polymerase from *B. subtilis*, *Aquifex aeolicus* and *Methanobacterium thermoautotrophicum* and the proteins with high degree of sequence similarity to the PHP domains of family X DNA polymerase, (C) histidinol phosphatase from yeast and its homologs in *Lactococcus lactis* and *B. subtilis*; and (D) CpsB and its homologs in *Streptococcus*, *Staphylococcus*, *B. subtilis* and *Lactococcus lactis*. All share a predicted catalytic domain of four signature sequence motifs containing five conserved histidine residues and one conserved aspartic acid residue that are likely involved in the metal-dependent catalysis of phosphoester bond hydrolysis (Figure 1) (4).

Most PHP family members are not protein phosphatases. The PHP catalytic domains found in bacterial DNA pol III α subunits and the family X DNA polymerase are speculated to hydrolyze inorganic pyrophosphate, a by-product produced during the DNA polymerization. Removal of the pyrophosphate when it is produced will help to shift the equilibrium of the reaction to the direction of polymerization (4). The function of yeast histidinol phosphatase is to remove the phosphate group from histidinol phosphate during histidine biosynthesis. Yeast histidinol phosphatase is also the one of only two characterized members from PHP superfamily. Interestingly, the enzymatic activity of purified yeast histidinol phosphatase is neither metal-dependent nor enhanced by Mn^{2+} (29). However, this observation does not rule out the possibility that yeast histidinol phosphatase is a metalloenzyme. Like eukaryotic PPP family protein phosphatases, yeast histidinol phosphatase might have a very high binding affinity to the metal(s) and thus not need exogenous metal(s) for its phosphatase activity after it was purified directly from yeast cells. CpsB from *Streptococcus pneumonia* is the only member of PHP family characterized so far to function as a Mn^{2+}-dependent protein phosphatase. It is a novel protein tyrosine phosphatase and shows no sequence similarity to the members of well studied protein tyrosine phosphatase (PTP) superfamily (30). All PTP family protein phosphatases share a characteristic active site signature

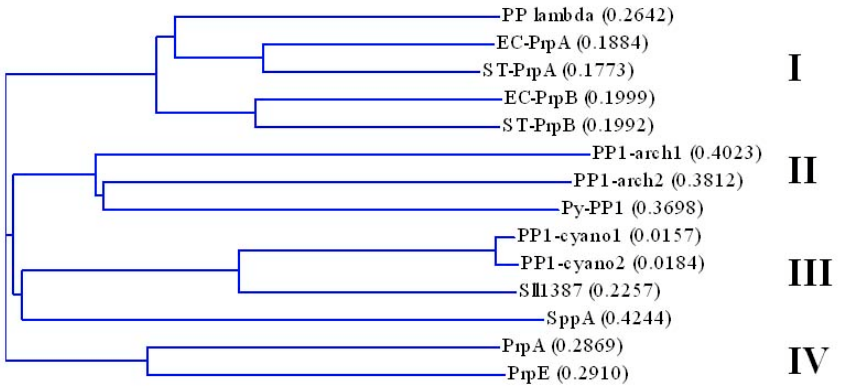

Figure 2. Phylogenetic analysis of characterized PPP family protein phosphatases from prokaryotes. A phylogenetic tree was constructed with AlgnX program of Vector NTI from InforMax (Frederick, MD), based on the amino acid sequences of characterized PPP family protein phosphatases from prokaryotes. The numbers in parenthesis after the phosphatase names were their calculated evolutionary distances. Different branches of these phosphatases were labeled on the right. Abbreviation used included: (Branch I) PP lambda, protein phosphatase lambda from bacteriophage lambda (14-16); EC-PrpA, protein phosphatase A from *E. coli* (41); ST-PrpA, protein phosphatase A from *S. typhimurium* (42); EC-PrpB, protein phosphatase B from *E. coli* (41); ST-PrpB, protein phosphatase B from *S. typhimurium* (42); (Branch II) PP1-arch1, protein phosphatase 1 from *S. solfataricus* (43); PP1-arch2, protein phosphatase 1 from *M. thermophila* TM-1 (44); Py-PP1, protein phosphatase 1 from *P. abyssi* (45); (Branch III) PP1-cyano1, protein phosphatase 1 from *M. aeruginosa* PCC 7820 (46); PP1-cyano 2, protein phosphatase 1 from *M. aeruginosa* UTEX 2063 (46-47); Sll1387, protein phosphatase 1 from *Synechocystis* sp PCC 6803 (48); SppA, protein phosphatase A from *S. coelicolor* A3(2) (49); (Branch IV) PrpA, protein phosphatase A from *Anabaena* sp PCC 7120 (50); PrpE, protein phosphatase E from *B. subtilis* (40).

sequence motif, CX_5R, and their phosphatase activity are metal-independent. The active site cysteine acts as a nucleophile to attack the protein-bound phosphoryl group, resulting displacement of the dephosphorylated protein and formation of a cysteinyl-phosphate enzyme intermediate (31-32). The conserved arginine forms salt bridges with phoshporyl group to promote substrate binding (33) and to stabilize the phosphoenzyme intermediate (34). The catalytic cycle is complete when a water molecule enters the region vacated by the dephosphorylated protein, hydrolyzing the cysteinyl-phosphate moiety to generate free enzyme and inorganic phosphate. A conserved aspartic acid residue is also involved in catalysis. It serves as a general acid-base. During the initial nucleophilic attack on the phosphorylated substrate, the aspartic acid protonates the leaving group alcohol on the protein as it is displaced. Later, the resulting conjugate base abstract a proton from the entering water molecule to enhance its nucleophilicity and accelerate the hydrolysis of the phosphoenzyme intermediate (35). Thus, the catalysis of PTP family protein phosphatases does not require any metal as a cofactor. PTP family protein phosphatases are found in both eukaryotic and prokaryotic cells. The biochemical properties and biological function of prokaryotic PTP family protein phosphatases and their distribution in prokaryotic genomes have been thoroughly reviewed before (20, 36-39). The properties and function of CpsB from PHP superfamily will be discussed in detail in this review.

3. PROKARYOTIC PPP FAMILY PROTEIN PHOSPHATASES

3.1. Overview

Not all prokaryotic genomes have PPP family protein phosphatases. Recent results showed that only 11 of 35 (32%) surveyed prokaryotic genomes contained the open reading frame (ORF) encoded possible PPP-type protein phosphatases (39). Almost all of them have only single ORF. *E. coli* and *Salmonella typhimurium* are the only prokaryotes studied so far to have two PPP family protein phosphatase genes. Fourteen prokaryotic PPP family protein phosphatases have been characterized (Table 1). Mn^{2+} activates early all of their phosphatase activity. The only exception is PrpE from *B. subtilis* (40), which activity requires Ni^{2+}. Ni^{2+} is also found to activate most these phosphatases, but with less efficiency. In addition, Fe^{2+}, Co^{2+} and Mg^{2+} stimulate a small degree of the activity of some these phosphatases. The molecular weights of these protein phosphatases vary from 24 to 96 kDa (Table 1). Their presumed evolutionary relationship is displayed in Figure 2, a computer-generated phylogenetic tree of characterized PPP family protein phosphatases from prokaryotes. It appears that they are grouped into four main branches. Branch I includes PP-lambda (14-16) and PrpA/B from *E. coli* (EC-PrpA and EC-PrpB) (41) and *S. typhimurium* (ST-PrpA and ST-PrpB) (42). PP1-arch1 (43), PP1-arch2 (44) and Py-PP1 (45) from *Archaea* form Branch II. Three cyanobacterial PPP family protein phosphatases, PP1-cyano1 (46), PP1-cyano2 (47) and Sll1387 (48), and SppA from *Streptomyces coelicolor* A3(2)(49) are found in Branch III. Branch IV includes PrpA from the cyanobacterium *Anabaena* sp PCC 7120 (50) and PrpE (40). None of them have their physiological substrates identified and more than half of them have no known biological functions (Table 1).

3.2. Enterobacterial PrpA and PrpB

3.2.1. Genomics

EC-PrpA and EC-PrpB are the first two PPP family protein phosphatases that are identified and

characterized from a bacterial genome. Both EC-PrpA and EC-PrpB have 218 residues and show certain degrees of sequence similarity to the catalytic domains of PP1/PP2A/PP2B and even higher sequence similarity to PP-lambda (41). Recently, their respective homologs from *S. typhimurium*, ST-PrpA and ST-PrpB, have also been characterized. ST-PrpA has 222 amino acid residues, while ST-PrpB has 218. They show only 41% identity to each other, and, strikingly, only 61% identity to the corresponding *E. coli* proteins. The percent identity between *S. typhimurium* and *E. coli* for other Mn^{2+}-dependent enzymes such as SodA, GpmM, SpoT and PepP are all above 90% (42). This difference suggests that the *E. coli* and *S. typhimurium* phosphatases may have originated in another more distantly related bacterium and been acquired by horizontal transfer.

3.2.2. Phosphatase activity

Like PP-lambda, PrpA and PrpB from both *E. coli* and *S. typhimurium* are functional protein phosphatases (41-42). The phosphatase activities of ST-PrpA and ST-PrpB have been shown to be divalent metal ion-dependent. Addition of Ni^{2+}, Co^{2+} and Fe^{2+} stimulate a certain degree of phosphatase activity; however, Mn^{2+} gives the greatest stimulation by far (42). Although Mn^{2+} activates considerable enzymatic activity from EC-PrpA and EC-PrpB, no other cations have been tested so the degree of their Mn^{2+}-dependence has not been determined (41). Interestingly, ST-PrpA and ST-PrpB display different affinities to Mn^{2+}. Half-maximal activation (K_a) of ST-PrpA for Mn^{2+} is 65 μM, which is more than 50 times higher than the Mn^{2+} K_a of ST-PrpB at 1.3 μM. The *Ka* values of Ni^{2+}, Co^{2+}, and Fe^{2+} for ST-PrpA and ST-PrpB are in the mM ranges, clearly indicating that Mn^{2+} is the physiologically relevant cation (42). PP-lambda, EC-PrpA and ST-PrpA are relatively heat resistant (14, 41-42). For example, EC-PrpA maintains 90% of its activity at 65 ^{0}C (41). Temperature optima are 42-45 ^{0}C for PP-lambda (14) and 45-55 ^{0}C for ST-PrpA (42). In contrast, the optimal temperature of 30-37 ^{0}C for ST-PrpB is lower than that for ST-PrpA (42), and EC-PrpB loses 90% of its activity at 65 ^{o}C (41). pH optima are pH 7.8-8.5 for PP-lambda (14), pH 6.5 for ST-PrpA and pH 8 for ST-PrpB (42); the pH optima for EC-PrpA and EC-PrpB have not been determined. These distinct biochemical properties suggest that ST-PrpA and ST-PrpB function in *S. typhimurium* under different growth or environmental conditions (42). *In vitro*, bacterial PrpA and PrpB and PP-lambda dephosphorylate not only phosphoseryl and phosphothreonyl but also phosphotyrosyl protein and peptide substrates (14-16, 41-42). This contrasts with the eukaryotic PP1/PP2A/PP2B phosphatases that only dephosphorylate phosphoseryl and phosphothreonyl residues (7). PP-lambda has even broader specificity in that it can also act as a protein histidine phosphatase (15). It has not been determined whether the enterobacterial PrpA/B phosphatases can dephosphorylate a phosphohistidyl moiety.

3.2.3. Biological functions

EC-PrpA and EC-PrpB are also the first prokaryotic PPP family protein phosphatases with their biological functions determined. Both EC-PrpA and EC-PrpB regulate transcription of *htrA* (*degP*) gene (41). *htrA* encodes a periplasmic protease that degrades misfolding proteins to avoid potential toxicity to the cell envelope. At least two different signal transduction pathways control the transcription of *htr*A in *E. coli*. One is mediated by sigma–E (sigma-24), an alternative sigma factor, signal transduction pathway, and another is *via* the CpxA/CpxR two-component system. CpxA is a membrane protein and serves as a sensor kinase. It becomes autophosphorylated at a conserved histidine residue located in cytoplasm when misfolding proteins accumulates in periplasm after treatments such as heat-shock. The phosphate group from the phosphorylated histidine residue of CpxA is then transferred to a conserved aspartic acid residue in CpxR, and CpxR becomes activated. Activated CpxR binds to the promoter region of *htrA* gene to up-regulate its expression (51). Both EC-PrpA and EC-PrpB increase *htrA* expression acting through or in concert with the CpxA/CpxR pathway by changing the phosphorylation level of CpxA/CpxR. It is still unclear how EC-PrpA and EC-PrpB regulate the phosphorylation level of CpxA/CpxR. Although they are proposed to function as histidine or aspartate phosphatases *in vivo* (41), these activities have not been biochemically demonstrated. Nonetheless, given that PP-lambda has activity against phosphohistidyl residues, this is a reasonable speculation. EC-PrpA is also a heat shock protein. Heat-shock treatment increases *prpA* transcription. Conversely, overexpression of EC-PrpA induces a heat shock response (41). Whether EC-PrpB is also a heat shock protein remains to be determined. Thus, at least one of the physiological roles of EC-PrpA and EC-PrpB is stress-related. Furthermore, EC-PrpB is implicated in bacterial pathogenesis. During a murine model of septicemic infection, *prpB* transcription is induced in an *E. coli* septicemia strain and a *prpB* mutant strain does not compete effectively against wild type strain, indicating that EC-PrpB is involved in survival inside the host body during infection (52).

ST-PrpA and ST-PrpB are also stress-related proteins. Under normal laboratory growth condition (37^{0}C, LB broth), the growth rate of wild type, *prpA* and *prpB* mutant strains are the same. Their ability to use 190 different carbon, 95 different nitrogen, 59 different phosphorus and 35 different sulfur sources has been tested with Phenotype MicroArrays™ from BIOLOG (Hayward, CA), but no difference is found between wild type and *prpA* or *prpB* mutant strains. When grown at 47 ^{0}C, however, growth rates for *prpA* and *prpB* mutant strains are significantly lower than that of wild type cells. Compared to wild type cells, *prpA* and *prpB* mutant strains are also more susceptible to heat shock (55 ^{0}C) or peroxide challenges. Like *prpA* from *E. coli*, mild heat shock treatment at 42 ^{0}C increases *prpA* promoter activity in *S. typhimurium* cells. Same treatment, however, has no significant effects on *prpB* promoter. Whether ST-PrpA and ST-PrpB also regulate *htrA* expression has not been determined (L. Shi & M.E. Maguire, unpublished results). The role of ST-PrpB in *S. typhimurium* pathogenesis is uncertain. *prpB* is located next to SPI-1 pathogenicity island (53-54). It is not clear if *prpB* is actually part of SPI-

1. Interestingly, *prpB* is on the 5' side of *mutS* in *S. typhimurium*, but on the 3' side of *mutS* in *E. coli*, suggesting that *prpB* was acquired independently by *S. typhimurium* and *E. coli* (54). The biological role of PP-lambda in *E. coli* cells is unknown. Infection of *E. coli* cells by bacteriophage lambda gt10 whose genome contains a complete PP-lambda gene is accompanied with a surge of protein phosphatase activity. However, lambda gt11, which carries a truncated PP-lambda gene, appears to infect *E. coli* and grow normally in the absence of any increase in phosphatase activity (13).

3.3. Cyanobacterial PPPs

PPP family protein phosphatases have been identified and characterized from several species of cyanobacteria. PP1-cyano1 is from a microcystin-producing strain *Microcystis aeruginosa* PCC 7820, while PP1-cyano2 is from a non-producing strain, *M. aeruginosa* UTEX 2063. PP1-cyano1 and PP1-cyano2, each has 264 amino acid residues, are 98% identical to each other. However, they show less than 20% identity to PrpA, PrpB and PP-lambda (46). PP1-cyano1 and PP1-cyano2 are both divalent metal ion-dependent protein phosphatases with nearly identical biochemical properties. Among the metal ions tested, Mn^{2+} gives the greatest activation. The apparent K_a of both phosphatases for Mn^{2+} is 200 μM, 3 times higher than that of ST-PrpA and more than 100 times higher than for ST-PrpB (42). Mg^{2+} also gives a small degree of stimulation of PP1-cyano1 and PP1-cynao2, while Co^{2+} and Ni^{2+} inhibit their Mn^{2+}-dependent protein phosphatase activity. Like PrpA/B from *E. coli* and *S. typhimurium* and PP-lambda, PP1-cyano1 and PP1-cyano2 are protein serine/threonine/tyrosine phosphatases. *In vitro* they also exhibit phosphohydrolase activity towards 3-phosphohistidine and phospholysine- containing amino acid homopolymers (46). PP1-cyano1 and PP1-cyano2 are the first PPP family protein phosphatases showing symmetrical phosphatase activity against diadenosine tetraphosphate. This is not surprising because symmetrical diadenosine tetraphosphatases share conserved sequence features with PPP family protein phosphatases. The symmetrical diadenosine tetraphosphatase activity of PP1-cyano1 and PP1-cyano2 strongly suggests that these enzymes share a common catalytic mechanism (46).

PP1-cyano1 and PP1-cyano2 are also resistant to microcystin-LR as they lack apparent microcystin binding motifs in their amino acid sequences (46). Microcystins are group cyclic peptides produced by some aquatic cyanobacteria. Although their physiological functions inside cyanobacterial cell are unknown, microcystins are potent inhibitors of eukaryotic PP1/PP2A (55). Microcystins bind to the catalytic subunits of PP1/PP2A near nanomolar affinity, but their affinity is more than 1000 times poorer for PP2B (56). Microcystin-LR, one of the most common microcystins, directly blocks substrate binding by interacting with two metal-bound water molecules in the catalytic center of PP1. Microcystin-LR also forms a covalent bond to PP1 through a conserved cysteine residue in a region called the "microcystin binding motif". The conformations of microcystin-LR in solution and in complex with PP1 are nearly identical, which reduces entropy lost during binding and results in a high affinity of PP1 for microcystin-LR (9). Unlike eukaryotic PP1/PP2A, bacterial PPP family protein phosphatases are resistant to microcystin-LR because they do not have the microcystin binding motifs found in the eukaryotic phosphatases (14-15, 41-42). Insensitivity of PP1-cyano1 to microcystin-LR renders the *M. aeruginosa* PCC 7820 immune to the effects of its own endogenous toxin (46), which accumulates at various locations, including the thylakoid and nuclear regions inside cyanobacterial cells (57). Resistance of PP1-cyano2, which is from non-toxin producing strain *M. aeruginosa* UTEX 2063, to microcystin-LR also suggests that if microcystins serve as a means for the molecular defense of environmental habitat, they are not directed against the encroachments of other cyanobacteria (46). Consistent with these results, Sll1387, a Mn^{2+}-dependent phosphatase homolog of PP1-cyano1/PP1-cyano2 from a non-microcystin producing cyanobacterium *Synechocystis* sp PCC 6803 (48), is also resistant to microcystin-LR. Transcription of *sll1387* is induced by a change in nitrogen source but not by heat-shock treatment, suggesting that physiological function of Sll1387 may be different from those for PrpA/B from *E. coli* and *S. typhimurium* (L. Shi & P. J. Kennelly, unpublished results). The physiological substrates and thus function(s) of PP1-cyano1, PP1-cyano2 and Sll1387 are still unknown.

A PPP family protein phosphatase has been identified in the cyanobacterium *Anabaena* sp PCC 7120 and also named PrpA. PrpA has 858 amino acid residues and displays Mn^{2+}-dependent phosphatase activity toward *p*-nitrophenyl phosphate. The catalytic domain is found at the region from amino acid residue 158 to 518. Within this region, PrpA shows 16 to 23% identity to eukaryotic PPPs. It is still unknown whether its non-catalytic regions have any functional roles. In the genome of *Anabaena* sp PCC 7120, *prpA* is located 301base pairs (bp) upstream of *pknE*, a eukaryotic like protein kinase gene, although the genes are not cotranscribed (50). Unlike *M. aeruginosa* and *Synechocystis* sp PCC 6803, which are unicellular cyanobacteria, *Anabaena* sp PCC 7120 is a multi-cellular cyanobacterium and some of its cells can differentiate into heterocysts to fix nitrogen when the nitrogen source is changed from nitrate to dinitrogen. PrpA is constitutively expressed and a switch from nitrate to dinitrogen does not affect its expression level. In contrast, the expression level of PknE is high when cells grow with nitrate. PknE expression initially decreases upon switching from nitrate to dinitrogen, but its expression returns to normal eight hours after switch. Mutation of either *prpA* or *pknE* has no effect on cell growth when nitrate is abundant. If nitrate is limiting and dinitrogen must be used, *prp*A and *pkn*E mutants grow much slower than wild type cells. Although *prpA* and *pknE* mutants can still form heterocysts, they appear morphologiocally abnormal, and the activity of nitrogenase, a key enzyme for nitrogen fixation inside heterocysts, is greatly reduced in *prpA* and *pknE* mutants. Thus, both PrpA and PknE are involved in heterocyst formation and dinitrogen fixation (50).

3.4. PPPs from other prokaryotes

Three PPP family protein phosphatases from *Archaea* have also been characterized. All are metal-

dependent protein phosphatases with Mn^{2+} giving the greatest stimulation (43-45). PP1-arch1 from *Sulfolobus solfataricus* contains 293 amino acid residues and is about 30% identical to eukaryotic PP1/PP2A/PP2B and 17% identical to PP-lambda. The *Ka* of Mn^{2+} for PP1-arch1 is 50 μM, similar to that for ST-PrpA. PP1-arch1 is a monomer and resistant to microcystin-LR (43, 58). PP1-arch2 from *Methanosarcina thermophila* TM-1, however, is sensitive to microcystin-LR (44). The amino acid sequence of PP1-arch2 is 268 long and possesses a microcystin binding motif in its C-terminal half. The binding affinity of microcystin-LR for PP1-arch2 is about 10 times poorer than that for the eukaryotic PP1/PP2A phosphatases (44, 59). Another archaeal PPP family protein phosphatase, Py-PP1, has been identified from *Pyrodictium abyssi* TAG11. It has 366 amino acid residues and is 40% identical to PP1-arch1 and PP1-arch2 and 31% identical to PP1/PP2A/PP2B. Its enzymatic activity is inhibited by okadaic acid (45). Since okadaic acid and microcystin-LR inhibit eukaryotic PP1/PP2A in a similar way (9), Py-PP1 may be also sensitive to microcystin-LR. The gene for Py-PP1, *pyp1*, is located down-stream of *canB*. The protein product of *canB* is the one of three components that makes up the cannulae, hollow tubules found in extracellular network of *P. abyssi* TAG11 cells. *can*B and *pyp1* are cotranscribed, suggesting that Py-PP1 may be involved in the formation of cannulae (45). The physiological function of PP1-arch1 or PP1-arch2 has not been determined. Unlike most bacterial PPPs, none of the archaeal PPPs shows any protein tyrosine phosphatase activity (43-45, 58-59).

Gram positive bacteria also contain PPP family protein phosphatases. SppA from *Streptomyces coelicolor* A3(2) contains 368 amino acid residues. Recombinant SppA shows low phosphatase activity in the absence of Mn^{2+} and phosphatase activity is greatly enhanced by Mn^{2+}. Ni^{2+} also enhances SppA phosphatase activity, but with less efficiency. The optimal temperature for SppA is 55^{o}C, similar to PrpA from *E. coli* and *S. typhimurium*. SppA is a protein serine/threonine/tyrosine phosphatase whose enzymatic activity is resistant to okadaic acid. Deletion of *sppA* severely impairs vegetative growth of *S. coelicolor* A3(2) and results in much less formation of hyphae and spores (48).

Not all prokaryotic PPP family protein phosphatases are Mn^{2+}-dependent. PrpE from the gram positive bacterium *Bacillus subtilis* is a Ni^{2+}-dependent protein phosphatase. Mn^{2+} or other cations such as Co^{2+} and Ca^{2+} give little or no stimulation. They also inhibit Ni^{2+}-dependent phosphatase activity of PrpE. PrpE has 241 amino acid residues that show 25% identity with symmetric diadenosine tetraphosphatase from *E. coli*, 20% with PP-lambda and 17% with human PP1. PrpE dephosphorylates only phosphotyrosyl residues and shows no phosphatase activity toward phosphoseryl or phosphothreonyl protein substrates (40). Unlike PP1-cyano1 and PP1-cyano2, which hydrolyze diadenosine tetraphosphate symmetrically to produce ADP (46), PrpE hydrolyses diadenosine tetraphosphate asymmetrically and produces ATP and AMP. PrpE also possesses ATPase activity. Despite these varied enzymatic activities, the physiological role of PrpE remains unclear (40).

4. PROKARYOTIC PPM FAMILY PROTEIN PHOSPHATASES

4. 1. Overview

Like prokaryotic PPP family protein phosphatases, the distribution of PPM family protein phosphatases in prokaryotic genomes is also uneven. Only 42% of recently surveyed prokaryotic genomes contain the ORF that encodes potential PPM family protein phosphatase (39). The total numbers of PPM family protein phosphatases possessed by a single prokaryotic genome can be as high as eight (20, 60). Ten prokaryotic PPM family protein phosphatases have been characterized (Table 1). All are from *Bacteria*. Like mammalian PP2C (61), most prokaryotic PPM family protein phosphatases prefer Mn^{2+} over Mg^{2+} to activate their protein phosphatase activity. In some cases, Mg^{2+} has no stimulatory or even inhibitory effects. Their molecular weights vary from 22 to 92 kDa (Table 1). Phylogenetic analysis shows that they can be grouped into two main branches (Figure 3). Branch I include IcfG (Slr1860) from *Synechocystis* sp PCC6803 (48) and SpoIIE (26), RsbP (YvfP) (28), RsbU and RsbX (62) from *B. subtilis*. All lack the signature sequence motifs 5a/5b in their amino acid sequences (19-20) (Figure1) and their cognate protein kinases are the homologs of bacterial two-component histidine kinases that have evolved as phosphoserine protein kinases (48). Branch II includes Stp1 from *Pseudomonas aeruginosa* (PA-Stp1) (63) and *Streptococcus agalactiae* (SA-Stp1) (64), Php1 from Myxococcus *xanthus* (65), PrpC (YloO) from *B. subtilis* (66) and PphA (Sll1771) from *Synechocystis* sp PCC6803 (67). Unlike those found in Branch I, the amino acid sequences of protein phosphatase from Branch II contain the signature sequence motifs 5a/5b (Figure 1). Their cognate protein kinases identified so far are the homologs of eukaryotic protein kinases. In contrast to most prokaryotic PPP family protein phosphatases characterized, most prokaryotic PPM family protein phosphatases have their physiological substrates and biological functions determined (Table 1),

4.2. PPMs from *B. subtilis*.

4.2.1. Phosphatase activity

The genome of *B. subtilis* contains five PPM family protein phosphatase genes (20). All of their protein products have been characterized. Among them, SpoIIE has 827 amino acid residues with a catalytic domain at its C-terminal region (residues 604 to 827). Recombinant cytoplasmic portion (residues 323 to 827) of SpoIIE ($SpoIIE_{323-827}$) shows protein phosphatase activity toward SpoIIAA phosphorylated at serine^{58} (SpoIIAA-PS) and its phosphatase activity is greatly enhanced by the presence of Mn^{2+}. $SpoIIE_{323-827}$ also shows high degree of substrate specificity *in vitro*. It has no phosphatase activity toward either phosphorylated RsbV, a SpoIIAA homolog from *B. subtilis*, or phosphorylated SpoIIAA in which serine^{58} is replaced by a threonine (26). Compared to SpoIIE, RsbX and RsbU are much smaller proteins with 199 and 335 amino acid residues, respectively. Like SpoIIE, RsbX and

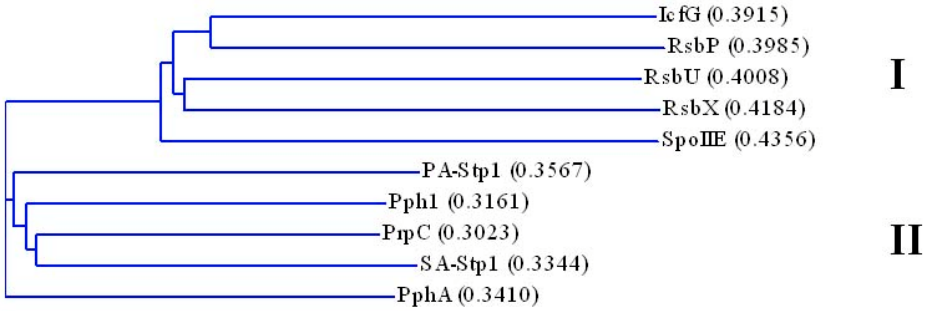

Figure 3. Phylogenetic analysis of characterized PPM family protein phosphatases from prokaryotes. A phylogenetic tree was constructed with AlgnX program of Vector NTI from InforMax (Frederick, MD), based on the amino acid sequences of characterized PPM family protein phosphatases from prokaryotes. The numbers in parenthesis after the phosphatase names were their calculated evolutionary distances. Different branches of these phosphatases were labeled on the right. Abbreviation used included: (Branch I) IcfG, protein serine phosphatase from *Synechocystis* sp PCC 6803 (48); SpoIIE (26) RsbP (28), RsbU and RsbX (62); protein serine phosphatases from *B. subtilis*; (Branch II) PA-Stp1, protein serine/threonine phosphatase 1 from *P. aeruginosa* (63); Pph1, protein phosphatase 1 from *X. xanthus* (65); PrpC, protein phosphatase C from *B. subtilis* (66); SA-Stp1, protein serine/threonine phosphatase 1 from *S. agalactiae* (64); PphA, protein phosphatase A from *Synechocystis* sp PCC 6803 (67).

RsbU are Mn^{2+}-depedent protein phosphatases with high degree of substrate specificity. RsbX only uses RsbS phosphorylated at serine[59] (RsbS-PS) as its substrate, while RsbU only uses RsbV phosphorylated at serine[56] (RsbV-PS) as its substrate (62), despite RsbS and RsbV are 21% identical to each other. RsbP contains 403 amino acid residues. Its catalytic domain (residues 202 to 393) is about 23% identical to the catalytic domain of RsbU (20, 28). Like RsbU, RsbP is a Mn^{2+}-depedent protein phosphatase specific to RsbV-PS (28). PrpC, the fifth member of PPM family protein phosphatases from *B. subtilis*, is 254 amino acid residues long (20, 66). It is a Mn^{2+}-dependent protein phosphatase with an optimal pH of 8.8, and Mg^{2+} inhibits its activity (66). The effects of Mg^{2+} on the phosphatase activity of SpoIIE, RsbX. RsbU and RsbP have not been determined. *In vitro*, PrpC can use PrkC, a eukaryotic like protein kinase which gene is located immediately downstream of *prpC*, phosphorylated at threonine residues (PrkC-PT) and elongation factor-G phosphorylated at threonine residues (EF-G-PT) as its substrates (66, 68).

4.2.2. Biological functions

B. subtilis is a rod-shaped bacterium. When food supply is abundant, *B. subtilis* divides symmetrically to form two equal daughter-cells. During starvation, however, it undergoes an asymmetric division to produce a small prespore and a large mother cell. Eventually, the prespore matures to a spore, and the mother cell lyses. A sophisticated signal transduction system regulates the formation of the spore (sporulation). Protein phosphatase SpoIIE is a key member of this system (69-71). Roles of SpoIIE in sporulation are two fold: it helps to form the asymmetrically positioned septum that separates the mother cell from the prespore during the early stage of sporulation and then subsequently activates sigma-F in the prespore after the septum is formed (26, 72-74). Sigma-F is the first sporulation-specific transcription factor that is activated only in the prespore. In turn, activated sigma-F controls activation and expression other sporulation-specific transcription factors (69-71). Sigma-F is produced before sporulation. However, it remains inactive by binding to an anti-sigma factor SpoIIAB throughout cells. SpoIIAB is also a protein serine kinase that phosphorylates its antagonist, anti-anti-sigma factor SpoIIAA at serine[58]. Phosphorylated SpoIIAA (SpoIIAA-PS) is inactive and loses its ability to bind to SpoIIAB. The SpoIIE phosphatase activates sigma-F indirectly by removing the phosphate group from SpoIIAA-PS. Dephosphorylated SpoIIAA binds to SpoIIAB, releasing sigma-F from SpoIIAB. Free sigma-F then regulates other sporulation specific transcription factors in the prespore (26, 72-74).

The SpoIIE phosphatase is a membrane protein localized at the septum (26). Localization of SpoIIE to the septum is FtsZ-dependent (25). Several hypotheses suggest that the concentration of functional SpoIIE is higher in the prespore than in mother cell (26, 76-77). Protein phosphatase activity of SpoIIE toward SpoIIAA-PS measured *in vitro* is 100 time higher than the protein kinase activity of SpoIIAB toward SpoIIAA, although the concentrations of SpoIIAB and SpoIIE are nearly the same inside *B. subtilis* cells (78). A higher concentration of SpoIIE in the prespore and the higher ratio of protein phosphatase activity of SpoIIE to protein kinase activity of SpoIIAB would result in rapid activation of sigma-F in the prespore (26, 75-78). Protein phosphatase activity of SpoIIE is controlled *in vivo* by a regulatory site located at the N-terminal end of its protein-protein interaction domain. Mutations at this site render the cells to activate sigma-F without formation of the septum. However, it is still unclear how this site regulates the phosphatase activity of SpoIIE (79). Mutations of conserved aspartic acid residues at the signature sequence motifs 8 and 11 of SpoIIE (Figure 1) abolish its ability to activate sigma-F *in vivo* but have no effect on the formation of an asymmetrically positioned septum, suggesting that phosphatase activity is not required for SpoIIE-assisted formation of the septum (80).

Activity of the general stress transcription factor sigma-B is regulated similarly to sigma-F but more elaborately involved in three protein serine phosphatases:

RsbX, RsbU and RsbP. They help to convey two different types of stress signals to sigma-B. Sigma-B is a stable protein, and its activity is controlled by protein-protein interaction through protein serine phosphorylation/dephosphorylation (28, 62, 81). In addition to RsbX, RsbU and RsbP, two protein serine kinases, RsbW and RsbT, and two phosphoserine proteins, RsbV and RsbS are directly involved in regulating sigma-B activity. In this system, RsbU along with RsbX mediate a signal transduction pathway that senses environmental stress signals such as acid, ethanol, heat or salt, while RsbP regulates a pathway that senses energy stress such as limitation of carbon, phosphorus and oxygen. Under stress-free condition, sigma-B binds to anti-sigma factor RsbW and remains inactive. In addition to its protein kinase activity, RsbW is a switch protein that changes its binding partners, sigma-B and the antagonist RsbV, under different conditions. The phosphorylation state of RsbV determines which partner RsbW binds to. RsbW specifically phosphorylates RsbV at serine[56] rendering it unable to bind to RsbW. Environmental and energy stresses activate RsbU and RsbP, respectively, which, in turn, activate RsbV-PS by removing the phosphate group from serine[56]. Dephosphorylated RsbV binds to RsbW. Binding of RsbV to RsbW results in release of sigma-B from RsbW and activation of sigma-B. Activated sigma-B then regulates various genes that mediate responses to the stresses (28, 62, 81).

The protein phosphatase activity of RsbU is in turn controlled by another bi-functional protein, RsbT, through protein-protein interaction. Like RsbW, RsbT switches between its binding partners, RsbU and its own antagonist RsbS, under different conditions. RsbS is phosphorylated specifically at serine[59] by RsbT. When it is phosphorylated, RsbS-PS is unable to bind to RsbT. The latter then interacts with and activates RsbU. Protein phosphatase RsbX, however, inactivates RsbU and consequently sigma-B by specifically dephosphorylating RsbS-PS. Similar to RsbV, dephosphorylated RsbS binds to RsbT, and RsbU is subsequently released from RsbT/RsbS complex becoming inactive (62). Protein phosphatase activity of RbsX is not regulated by environmental stress signals. Instead, it serves a negative regulator to limit magnitude of the stress response (82-83). Environmental stress signals enter the RbsU-mediated signal transduction cascade via RsbT and, probably, RsbS (84-87). It is still unknown how RsbP is regulated by energy stress signals. Since RsbP contains a PAS domain (28), which senses redox potential and regulates enzymatic activity of other enzymes (27), the phosphatase activity of RsbP could be regulated by its own PAS domain (28). RsbP also interacts with RsbQ, which amino acid sequence shows similarity to the members of alpha/beta hydrolase superfamily such as bromoperoxidase from *Streptomyces aureofaciens* and epoxide hydrolase from *Agrobacterium radiobacter*. Although its hydrolase activity has not been biochemically demonstrated, RsbQ is involved in activation of sigma-B in the response to energy stress. Interaction with RsbQ may serve a mean to control protein phosphatase activity of RsbP (88).

Mutations of *prpC* or *prk*C have no effects on (A) cell growth in minimal medium, (B) sigma-B activity under salt stress or entry into stationary phase, or (C) sporulation in double strength Schaeffer's medium. However, they have opposite effects on cell density during stationary-phase. Mutation of *prp*C increases cell density, while mutation of *prkC* decreases it, indicating that both PrpC and PrkC have important roles in stationary-phase physiology (68).

4.3. IcfG and PhpA from *Synechocystis* sp PCC 6803

4.3.1. Phosphatase activity

The genome of cyanobacterium *Synechocystis* sp PCC 6803 contains eight homologs of PPM family protein phosphatase genes (20, 60). Two of their protein products, IcfG and PhpA, have been characterized (48, 67, 89). IcfG possesses 634 amino acid residues with its catalytic domain at the C-terminal region (residues 408 to 634) (19-20). Whether its non-catalytic region has any physiological role has yet to be determined. Phylogenetic analysis shows that IcfG is closely related to SpoII/Rsb-like protein phosphatases (Figure 3). Indeed, genes surrounding *icfG* also encode a SpoII/Rsb-like protein kinase, Slr1861, and two SpoII/Rsb-like phosphoproteins, Slr1856 and Slr1859. Slr1861 is an ATP-dependent protein kinase that phosphorylates Slr1856 and, albeit with lower efficiency, Slr1859 *in vitro* on serine[54] and serine[57], respectively. Despite Slr1856 and Slr1859 share 31% identity, IcfG catalyzes the dephosphorylation of Slr1856-PS but not of Slr1859-PS *in vitro* (48), demonstrating similar substrate specificity showed by SpoII/Rsb-like protein phosphatases (26, 28, 62). The protein phosphatase activity of IcfG is Mn^{2+}or Mg^{2+}-dependent. It has not been determined if $Mn^{2+}$is more effective than Mg^{2+} to activate IcfG (48).

PhpA contains 254 amino acid residues. The physiological substrate for PphA is the signaling protein P_{II} phosphorylated at serine[59] (P_{II}-PS). In addition to P_{II}-PS, PhpA can also use phosphorylated casein and phosphoserine/threonine/tyrosine peptides as its substrates. Both $Mn^{2+}$and Mg^{2} activate the protein phosphatase activity of PphA , however, Mn^{2+} is at least five times more effective. The protein phosphatase activity of PphA toward P_{II}-PS is inhibited by ATP, GTP and ADP. Although 2-ketoglutarate alone has no effects on the phosphatase activity of PhpA toward P_{II}-PS, it enhances the inhibitory effects of ATP and, with much less extent, of ADP. These inhibitors exert their inhibitory effects on P_{II}-PS rather than PphA because they have no effects on the protein phosphatase activity of PphA toward its artificial substrates such phosphorylated casein (89). The P_{II} proteins are homotrimers with binding sites for ATP and 2-ketoglutarate, and the biding of ATP and 2-ketoglutarate to P_{II} is also synergic (90-91). Their bindings to P_{II}-PS could cause conformation change of P_{II}-PS, which may block the access of PphA to the phosphorylation sites and result in the inhibition of dephosphorylation of P_{II}-PS by PphA (89). Thus, the binding of ATP and 2-ketoglutarate to P_{II}-PS provides means for these low molecular weight regulators to regulate the dephosphorylation process. GTP and ADP are also thought to bind at the ATP binding sites in P_{II}-PS to exert their inhibitory effects on the PphA protein phosphatase activity. However, their bindings to P_{II} are not enhanced by the binding of 2-ketoglutarate, consistent with

the observation that their inhibitory effects on the PphA's protein phosphatase activity toward P_{II}-PS are not increased or increased slightly by 2-ketoglutarate, respectively (89).

4.3.2. Biological functions

IcfG regulates utilization of different carbon sources. *Synechocystis* sp PCC 6803 can grow autotrophically by fixing CO_2 or heterotrophically on a fixed source of carbon, such as glucose. Expression of *icfG* requires glucose. Inactivation of *icfG* severely impairs, in a glucose-dependent fashion, the ability of *Synechocystis* sp PCC 6803 to successfully shift from growth on a high level of inorganic carbon to growth on low, limiting levels. While *icfG* mutant cells cultured in high levels of inorganic carbon as a sole carbon source grow normally following a step down to low inorganic carbon, they fail to grow if glucose is added concomitant with this shift. The presence of glucose in the growth medium prior to the step down to low inorganic carbon also blocks growth of *icfG* mutant cells, regardless of whether glucose is present following the shift (92). IcfG may regulate carbon metabolism in a way similar to what SpoII/Rsb-like protein phosphatases do in *B. subtilis*. Since IcfG and Slr1861 can effectively use Slr1856 as their substrate *in vitro*, they most likely form a three-partner regulatory module to control the activities of another protein, possibly an sigma factor, through protein-protein interactions (48).

Signal transduction protein P_{II} plays a critical role in nitrogen assimilation in *Bacteria*, *Archaea* and possible higher plants. It is involved in the signal transduction cascades that regulate one of the two major ammonium assimilation pathways: the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. GOGAT catalyzes the first reaction of this pathway by using 2-ketoglutarate, an intermediate from tricarboxylic acid (TCA) cycle, and glutamine as substrates to form two glutamates. GS then adds ammonium moiety to glutamate to form glutamine, which reaction requires ATP. In *E. coli*, P_{II} participates in at least two separate signal transduction cascades that control the transcription of *glnA*, the gene for GS and enzymatic activity of GS, respectively. P_{II} functions as a sensor protein that detects intracellular carbon, nitrogen and possible energy levels. Intracellular 2-ketoglutarate, glutamine and ATP serve as the carbon, nitrogen and energy signals for P_{II}, respectively. P_{II} detects 2-ketoglutarate and ATP by direct binding and indirectly by reversible post-translational modification in that 2-ketoglutarate and ATP activate the enzyme that catalyzes the modification of P_{II}. P_{II} senses glutamine, however, only by post-translational modification. In contrast to 2-ketoglutarate and ATP, glutamine inhibits the activity of the enzyme catalyzing the modification. Most bacterial P_{II} proteins, including the two from *E. coli,* are modified by uridylation at a conserved tyrosine residue (90-91). In cyanobacteria, however, P_{II} is modified by reversible phosphorylation at a conserved serine residue (93). Its phosphorylation state varies depending on the specific nitrogen and carbon supplies. In the cyanobacterium *Synechococcus* sp PCC 7942 cells, none of the P_{II} subunits are phosphorylated when ammonium is abundant. Under limited nitrogen, however, most or all of its subunits become phosphorylated. When supplied with nitrate, the phosphorylation level of P_{II} is intermediate and is secondarily regulated by CO_2. The more CO_2, the more subunits are phosphorylated (94). Two evidences show that the role of PhpA is to dephosphorylate P_{II}-PS in *Synechocystis* sp PCC 6803 cells. First of all, PphA displays phosphatase activity toward purified P_{II}-PS *in vitro* and this phosphatase activity is inhibited by ATP and 2-ketoglutarate (89). Secondly, in cells lacking PhpA, dephosphorylation of P_{II}-PS is blocked when ammonium is added to medium (67). Unlike in *E. coli cells*, 2-ketoglutarate is proposed to serve as both carbon and nitrogen signals for P_{II} proteins in cyanobacterial cells. When grown autotrophically, the intracellular concentration of 2-ketoglutarate in cyanobacterial cells is regulated only by two metabolic pathways: reductive TCA cycle to produce 2-ketoglutarate, and GS/GOGAT-mediated ammonium assimilation to convert 2-ketoglutarate to glutamate by GOGAT. Since the concentration of 2-ketoglutarate in cyanobacterial cells is directly affected by the changes of carbon and nitrogen metabolisms, cyanobacterial P_{II} may not need another molecule, such as glutamine in *E. coli*, as an indicator for intracellular nitrogen status (95). To support this hypothesis, glutamine was found to have no effect on the activity of protein kinase that phosphorylated P_{II} in *Synechococcus* sp PCC 7942, while ATP and 2-ketoglutarate stimulated the kinase activity (96). It has not been determined whether ATP and 2-ketoglutarate bind to the protein kinase or glutamine affects the activity of PphA on P_{II}-PS in cyanobacterial cells. Nevertheless, intracellular concentrations of ATP and 2-ketoglutarate directly affect the phosphorylation levels of P_{II}-PS in cyanobacterial cells, most likely by binding to P_{II}, the higher concentrations; the more P_{II} subunits are phosphorylated. It is still unclear how P_{II} regulates ammonium assimilation in *Synechocystis* sp PCC 6803 cells. Its target protein(s) has not been identified. Unlike GS from *E. coli* which activity is regulated by P_{II} proteins through reversible post-translational modification, the enzymatic activity of GS in *Synechocystis* sp PCC 6803 is inhibited by two small proteins through protein-protein interaction (97). Thus, the signal transduction pathway that regulates the activity of GS in *Synechocystis* sp PCC 6803 should be expected to be different from that in *E. coli.* It is also still unknown which protein kinase works with PhpA to control the phosphorylation state of P_{II} in *Synechocystis* sp PCC 6803 cells. PphA is a PPM family protein phosphatase with signature sequence motifs 5a/5b (20). It is closely related to the bacterial PPMs whose identified cognate protein kinases are eukaryotic-type protein kinases (Figure 3). The genome of *Synechocystis* sp PCC 6803 contains at least nine eukaryotic-like protein kinase genes (20, 60). One of these putative kinase genes, *sll1770*, is located immediately upstream of *pphA*, the gene for PphA. However, mutation of *sll1770* has no effects on phosphorylation state of P_{II}(67), so either it is not the relevant kinase or more than one kinase is associated.

4.4. PPMs from other bacteria.

Pph1 from gram-negative bacterium *M. xanthus* possesses 254 amino acid residues. Its phosphatase activity toward different artificial substrates is activated by Mn^{2+} or

Mg^{2+}, with Mn^{2+} giving a two-fold greater stimulation than Mg^{2+}. *pph1* is expressed during the stages of both vegetative growth and development of fruiting bodies, but which expression is controlled by different promoters. Expression of *pph*1 is at the highest level during the early stage of development of fruiting bodies. Compared to wild type cells, *pph1* mutant cells show slower growth rate in liquid culture and reduced motility on soft agar. During the stage of development of fruiting bodies, *pph1* mutant cells aggregate, but at a much slower pace, and they fail to form fruiting bodies (65). Thus, Pph1 has functional roles in the stages of both vegetative growth and development of fruiting bodies. Like PphA from *Synechocystis* sp PCC 6803, Pph1 is grouped with PPM family protein phosphatases containing signature sequence motifs 5a/5b (Figure 3). Several *M. xanthus* eukaryotic-like protein serine/threonine kinases have been shown to be involved in the vegetative growth and development of fruiting bodies. (98-102). Among them, Pph1 interacts with Pkn5 in the yeast two-hybrid system, suggesting they may work as a pair *in vivo* (65). The physiological substrate(s) of Pph1 has yet to be identified.

PA-Stp1 from *P. aeruginosa* and SA-Stp1 from *S. agalactiae* contain 242 and 245 amino acid residues, respectively. Both of them require Mn^{2+} for their phosphatase activity and neither Mg^{2+} nor Ca^{2+} can substitute (63-64). They are 78% similar to each other. In each species, the phosphatase gene, *stp1,* overlaps by one nucleotide with a eukaryotic-like protein kinase gene named *stk1*, and these two genes are cotranscribed (63-64). Stk1 from *P. aeruginosa* (PA-Stk1) undergoes autophosphorylation at threonine residues and, with lower efficiency, at serine residues (63), while Stk1 from S. *agalactiae* (SA-Stk1) undergoes autophosphorylation only at serine residues. In addition, SA-Stk1 can phosphorylate casein and myelin basic protein at threonine residues (64). In contrast to PA-Stp1 and PA-Stk1, whose functions and substrates remain to be determined (63), the functions and substrate of SA-Stp1 and SA-Stk1 have been identified. Both are required for normal cell segregation. S. *agalactiae* strains deficient in Stp1 or Stk1 form abnormally long chains. Mutation of the *stk1* kinase gene in S. *agalactiae* also decreases bacterial growth rate and attenuates bacterial virulence in a neonatal rat sepsis model. Mn^{2+}-dependent inorganic pyrophosphatase is identified as one of the endogenous substrates for both SA-Stp1 and SA-Stk1 where phosphorylation occurs at serine residues (64).

Most archaeal genomes lack possible PPM family protein phosphatase genes (20, 39). A recent genomic survey shows that homologs of PPM family protein phosphatase gene could be identified in only one of the eight archaeal genomes (39). No information is available about their potential biochemical properties and physiological functions.

5. PROKARYOTIC PHP FAMILY PROTEIN PHOSPHATASE

To date, only two PHP family phosphatases have displayed their predicted phosphatase activity and CpsB from gram positive bacterium *S. pneumonia* is the only member showing protein phosphatase activity. CpsB, containing 243 amino acid residues, shows phosphatase activity toward CpsD phosphorylated at tyrosine residues (CpsD-PY) as well as synthetic phosphotyrosyl peptides. Its phosphatase activity is activated by Mn^{2+} and, albeit with much lower efficiency, Co^{2+}. Other metal ions such as Mg^{2+}, Ca^{2+} and Ni^{2+} has no effects on its phosphatase activity. Mutations of conserved aspartic acid and histidine residues in its signature sequence motif 4 (Figure 1) greatly impair the phosphatase activity of CpsB and formation of cell capsule, demonstrating that (A) the motif 4 is most likely involved in the catalysis and (B) the tyrosine phosphatase activity of CpsB is required for *S. pneumonia* cells to produce capsular polysaccharide (CPS) (30).

S. pneumonia is an important human pathogen that causes pneumonia, meningitis, bacteremia, otitis media or sinusitis. One of the characteristics of virulent *S. pneumonia* strains is their capability to produce CPS. All virulent strains are encapsulated and their colonies give smooth appearance on blood agar plates. When a virulent strain spontaneously loses the ability to produce CPS, it becomes avirulent and appears rough on blood agar plates. CPS may serve as a shield to prevent (A) the activation host cell alternative complement pathways by bacterial cell wall component teichoic acid or (B) the interaction of bacterial surface proteins with the receptors on host phagocytic cells. Ninety different serotypes of pneumococcal CPS have been identified. Their synthesis is regulated by the genes of the *cps* locus (103). The first four genes in the 5' region of the *cps* locus, *cpsA*, *cpsB*, *cpsC* and *cpsD*, are conserved in all serotypes except type 3. Mutation of *cpsA* has no major effects on CPS production, but in-frame deletion of *cpsB*, *cpsC* or *cpsD* impairs CPS synthesis. CpsC shows high degree of sequence similarity to the N-terminal region of Wzc from *E. coli*, while CpsD shows high degree of sequence similarity to the C-terminal region of Wzc (104). Wzc is a protein kinase that autophosphorylates at tyrosine residues in its own C-terminal tail (Wzc-PY). Wzb, a low molecular weight protein tyrosine phosphatase from PTP family, dephosphorylates Wzc-PY. Inactivation of autokinase activity of Wzc or phosphatase activity of Wzb impair cells' ability to produce CPS, demonstrating that enzymatic activity of Wzc and Wzb are required to regulate production of CPS. Wzc is a membrane protein with two distinct structural domains. Its N-terminal domain, bordered by two transmenbrane helices, is located in the periplasm, while its C-terminal domain is in the cytoplasm and contains all phosphorylation sites (105-106). Consistent with these homologies, CpsC is a membrane protein with two transmembrane helices, while CpsD is a cytoplasmic protein. CpsD exhibits functional protein tyrosine kinase activity that autophosphorylates at tyrosine residues located in its own C-terminal tail. Mutation of the ATP-binding motif of CpsD results in loss of ability of CpsD to autophosphorylate itself and reduction of CPS biosynthesis to a basal level, while mutation of the tyrosine residues at phosphorylated sites increases encapsulation, demonstrating that tyrosine phosphorylation of CpsD negatively regulates CPS biosynthesis.

Autophosphorylation by CpsD also requires the presence of CpsC, implicating possible protein-protein interaction between CpsD and CpsC. It is hypothesized that CpsC and CpsD form protein complex to regulate CPS biosynthesis. Binding of ATP to CpsD might cause conformation change in CpsD and, subsequently, CpsC. This conformation change in CpsC could cause the increase of CPS biosynthesis. Autophosphorylation of CpsD may cause dissociation of CpsD-PY from CpsC, and the conformation of CpsC could change back to its inactive form, resulting in reduction of CPS biosynthesis to a basal level. How CpsC controls CPS biosynthesis has not been determined (104). CpsB regulates CPS production by dephosphorylating CpsD-PY. The dephosphorylated CpsD are then ready to interact with CpsC to increase CPS production. Inactivation of CpsB results in accumulation of CpsD-PY and reduction of CPS biosynthesis (30).

6. CONCLUSIONS

Reversible protein phosphorylation/dephosphorylation is a universal mechanism to regulate protein functions. Contrary to the early belief that protein phosphorylation occurred only at histidine and aspartic acid residues in prokaryotic cells, results over the last ten years unequivocally demonstrate that protein O-phosphorylation at serine, threonine and tyrosine residues is also a common regulatory mechanism for prokaryotes. Protein phosphatases, working in concert with protein kinases, directly control the phosphorylation state of O-phosphoproteins. Some of these phosphatases found in prokaryotes require Mn^{2+} ion as a cofactor to activate their protein phosphatase activity. Although only limited number of Mn^{2+}-dependenct protein O-phosphatases from prokaryotes have their physiological substrates and functions determined, the overall picture obtained from the studies so far clearly shows that they are functionally diverse enzymes that regulate different signal transduction pathways important to prokaryotic cells. Continued study of the functions of these prokaryotic protein O-phosphatases and of their Mn^{2+}-dependence will undoubtedly contribute substantially to new insight into Mn^{2+} homeostasis and to the many roles that Mn^{2+} and protein O-phosphorylation play in the physiological function of prokaryotic cells. Such studies have clear implications for eukaryotic cells given the extensive homologies of the phosphatases and suggest that Mn^{2+} also play an important and currently underappreciated role in eukaryotic signal transduction.

7. ACKNOWLEDGEMENTS

I would like to thank Dr. Michael E. Maguire from Case Western Reserve University for his critical reading of this manuscript. Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the U. S. Department of Energy through contract DE-AC06-76RLO 1830.

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Key Words: Protein Phosphorylation, Protein Serine/Threonine Phosphatase, Protein Tyrosine Phosphatase, Review

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