Extremophiles (2009) 13:371–377
DOI 10.1007/s00792-008-0211-5

NOTE

A PPM-family protein phosphatase from the thermoacidophile *Thermoplasma volcanium* hydrolyzes protein-bound phosphotyrosine

Hanan Dahche · AbdulShakur Abdullah · M. Ben Potters · Peter J. Kennelly

Received: 29 September 2008 / Accepted: 10 November 2008 / Published online: 29 November 2008

Abstract The genomes of virtually all free-living archaeons encode one or more deduced protein-serine/threonine/tyrosine kinases belonging to the so-called eukaryotic protein kinase superfamily. However, the distribution of their cognate protein-serine/threonine/phosphatases displays a mosaic pattern. *Thermoplasma volcanium* is unique among the *Archaea* inasmuch as it is the sole archaeon whose complement of deduced phosphoprotein phosphatases includes a member of the PPM-family of protein-serine/threonine phosphatases—a family that originated in the *Eucarya.* A recombinant version of this protein, TvnPPM, exhibited protein-tyrosine phosphatase in addition to its predicted protein-serine/threonine phosphatase activity in vitro. TvnPPM is the fourth member of the PPM-family shown to exhibit such dual-specific capability, suggesting that the ancestral versions of this enzyme exhibited broad substrate specificity. Unlike most other archeaons, the genome of *T. volcanium* lacks open reading frames encoding stereotypical protein-tyrosine phosphatases. Hence, the dual-specificity of TvnPPM may account for its seemingly aberrant presence in an archaeon.

Keywords Protein phosphorylation · Signal transduction · Dual-specific protein phosphatase · *Archaea* · Protein-tyrosine phosphatase · Protein-serine/threonine phosphatase · Zinc

Communicated by L. Huang.

H. Dahche · A. Abdullah · M. Ben Potters · P. J. Kennelly (✉)
Department of Biochemistry,
Virginia Polytechnic Institute and State University,
111C Engel Hall, Blacksburg, VA 24061, USA
e-mail: pjkennel@vt.edu

Introduction

The physicochemical properties of the phosphoryl group render it an exceptionally potent agent for perturbing protein structure (Johnson and Lewis 2001; Kennelly 2008). Consequently, the reversible modification of proteins by covalent phosphorylation and dephosphorylation has evolved into nature's most capable and versatile mechanism for modulating protein function, transmitting molecular signals, and processing sensory information. Critical to the emergence of protein phosphorylation-dephosphorylation as a molecular regulatory mechanism was the development of two classes of specialized signal transmission enzymes: protein kinases to catalyze the addition of phosphoryl groups to proteins and protein phosphatases to restore phosphoproteins to their unmodified starting state. Our laboratory has been investigating the protein kinases, protein phosphatases, and phosphoproteins resident within the *Archaea* in an effort to pinpoint the origins and retrace the development of this important molecular regulatory paradigm.

Open reading frames (ORFS) encoding known or deduced members of the so-called eukaryotic protein kinase (ePK) superfamily are ubiquitous among the *Archaea* (D'Souza et al. 2007). As its name suggests, the members of this superfamily of protein-serine/threonine/tyrosine kinases for a long time were considered to be eucaryal in origin and distribution (Kennelly and Potts 1996). However, the new, global perspective provided by genomics has since revealed that the ePK superfamily appeared long ago—prior to the divergence of the *Eucarya* from the *Archaea* (Leonard et al. 1998; Kannan et al. 2007).

In contrast to the ePKs, the distribution of countervailing protein phosphatases appears strikingly irregular and inconsistent (Kennelly 2003; Bhaduri and Sowdhamini

2005). Surprisingly, the majority of archaeal genomes encode one or more deduced protein-tyrosine phosphatases (PTPs) (Kennelly 2003; Bhaduri and Sowdhamini 2005). In fact, many archeaons contain only deduced PTPs—either conventional PTPs, low molecular weight PTPs, or a combination thereof—in their suite of potential protein-serine/threonine/tyrosine phosphatases. Intriguingly, while many archaeons contain PPP-family protein-serine/threonine phosphatases, only one ORF encoding a deduced PPM-family (Barford 1996) protein-serine/threonine phosphatase has been encountered: *TVN0703* from the acidothermophile *Thermoplasma volcanium* (Kennelly 2003; Bhaduri and Sowdhamini 2005). In this paper we ask whether TvnPPM, the unique protein encoded by ORF *TVN0703*, possesses protein-serine/threonine/tyrosine phosphatase activity.

Materials and methods

Materials

Purchased materials included mixed histones type II-AS, a 5% (w/v) solution of partially dephosphorylated casein, myelin basic protein (MBP), the catalytic subunit of the cAMP-dependent protein kinase, and *p*-nitrophenyl phosphate (Sigma, St Louis, MO); *Pfu* Turbo DNA Polymerase, BL21-CodonPlus (DE3)-RIL cells, and a Quik-Change II site-directed mutagenesis kit (Stratagene; LaJolla, CA); QIAquick PCR purification and QIAprep spin miniprep kits (Qiagen; Valencia, CA); expression vector pET-29b, S-protein agarose, and an S Tag Thrombin Purification kit (Novagen; San Diego, CA). All oligonucleotides were from Invitrogen (Carlsbad, CA). Restriction enzymes were from New England Biolabs (Beverly, MA). Genomic DNA from *T. volcanium* was from the American Type Culture Collection (Manassas, VA). [γ-^{32}P]ATP was from Perkin Elmer Life Sciences (Beverly, MA). *Escherichia coli* expressing the Lyn protein-tyrosine kinase as a GST fusion protein was the gift of Profs. Marietta Harrison and Harry Charbonneau of Purdue University (West Lafayette, IN). All other reagents were from Sigma-Aldrich (St Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Routine procedures

Protein concentrations were determined as described by Bradford (1976) using premixed reagent and a standardized solution of bovine serum albumin from Pierce (Rockford, IL). SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli (1970). Gels were stained with Coomassie Brilliant Blue as described by Fairbanks et al. (1971).

Cloning of *TVN0703* and expression of its protein product in *E. coli*

ORF *TVN0703* was amplified by PCR using genomic DNA from *T. acidophilum* as template essentially as described by Li et al. (2005) using the following forward and reverse primers, respectively: 5′-TTGATGGCCATG GTAAA AGTTGAATATTTTTCAGATTCCAG-3′ and 5′-CACAGC GTCGACTTACGCCATTGTAAC-3′. These primers introduced overhanging sequences complementary to the restriction sites generated by *Nco*I or *Sal*I. The resulting PCR product was ligated into expression vector pET-29b and the resulting plasmid used to transform One Shot TOP 10 chemically competent *E. coli*. The orientation and sequence of the cloned gene was verified by sequencing the isolated plasmid. For recombinant protein expression, the plasmid was used to transform *E. coli* BL21-CodonPlus (DE3)-RIL competent cells. The transformed cells were cultured at 37°C in 250 ml of Luria–Bertani medium containing 0.1 mg/ml kanamycin, 34 μg/μl chloramphenicol until the OD_{600} fell within the range of 0.6–1.0. Isopropyl-β-D-thiogalactopyranoside then was added to a final concentration of 1 mM and the culture incubated for another 3 h. Cells were then harvested by centrifugation, lysed by sonic disruption, and cell extracts prepared by standard procedures (Li et al. 2005). Recombinant protein was purified using S-protein agarose and the fusion domain subsequently removed using an S Tag Thrombin Purification kit, both as described by the manufacturer.

Assay of phosphatase activity

Protein phosphatase activity was assayed using a modification of the procedure of McGowan and Cohen (1988). Briefly, 1.0–1.5 μg recombinant TvnPPM was incubated at 55°C in a volume of 50 μl containing 100 mM Tris–HCl, pH 8.5, 1 mM $MnCl_2$, 0.2 mg/ml bovine serum albumin, and, unless indicated otherwise, a phosphoprotein substrate at a concentration 1.2 μM protein-bound [^{32}P]phosphate. Phosphoprotein substrates were phosphorylated on serine and threonine residues using [γ-^{32}P]ATP and the catalytic subunit of cAMP-dependent protein kinase, or on tyrosine residues using [γ-^{32}P]ATP and the lyn protein-tyrosine kinase, as described in (Kennelly et al. 1993). The reaction was terminated, typically after an incubation period of 15 min, by the addition of 150 μl of 20% (w/v) trichloroacetic acid. After agitating briefly using a Vortex mixer, the mixture was centrifuged for 3 min at 12,000×*g*. A portion, 50 μl, of the supernatant liquid was removed and counted for ^{32}P radioactivity in 2.0 ml of Scintisafe 30% liquid scintillation fluid (Fisher Scientific, Pittsburgh, PA). Where indicated, the source of the ^{32}P-labeled material in the TCA supernatant was verified as being inorganic phosphate via

extraction into organic solvents as a molybdate complex by the procedure of Martin and Doty (1949).

Activity toward *p*-nitrophenyl phosphate (pNPP) was assayed under conditions similar to those described above for phosphoprotein substrates with the exception that the volume of the initial incubation was increased to 250 µl and no BSA was added. The concentration of pNPP was 10 mM. The reaction was terminated by the addition of 750 µl of 0.5 M EDTA, pH 10. *p*-Nitrophenolate was then measured spectrophotometrically at a wavelength of 410 nm.

Results

TvnPPM exhibits divalent metal ion-dependent phosphatase activity

While only 218 residues in length, TvnPPM from *T. volcanium* contains all of the signature motifs conserved among the PPM family of protein phosphatases (Fig. 1). The M in PPM refers to the universal dependence of these enzymes on the presence of divalent metal ions such as Mg^{2+} or Mn^{2+} for catalytic activity, one of the functional characteristics by which they were originally distinguished from the other major family of protein-serine/threonine phosphatases, the PPP family (Barford 1996). TvnPPM was expressed as a recombinant fusion protein in *E. coli*, then incubated with thrombin to remove the fusion domain. When challenged with *p*-nitrophenyl phosphate, a general purpose substrate for phosphohydrolases, TvnPPM exhibited divalent metal ion-dependent phosphatase activity. Of the potential cofactors tested, Mg^{2+}, Ni^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{+2}, only the last named supported catalysis on its own. As the metal centers in PPM-family protein phosphatases are di- (Das et al. 1996; Bellinzoni et al. 2007) or, in some cases, tri- (Pullen et al. 2004; Rantanen et al. 2007; Wehenkel et al. 2007; Schlicker et al. 2008) nuclear, we asked whether adding a second metal ion in addition to Mn^{2+} would yield greater catalytic activity than that obtained using Mn^{2+} alone.

While the inclusion of Mg^{2+}, Ni^{2+}, Ca^{2+}, and Cu^{2+} along with Mn^{2+} had little effect on activity relative to that observed with Mn^{2+} alone, Zn^{2+} inhibited the enzyme in a concentration-dependent manner (Fig. 2). Inhibition by Zn^{2+} was irreversible. The removal of free Zn^{2+} via the addition of EDTA or by extensive dialysis versus EDTA-containing buffers failed to restore any trace of Mn^{2+}-dependent catalytic activity to TvnPPM. Attempts to displace any bound zinc by prolonged dialysis versus Mn^{2+}-containing buffers also had failed to restore activity. While protein phosphatase 2Cα from humans, an eukaryotic PPM, also is inhibited by Zn^{2+}, kinetic analyses revealed that zinc acted via a reversible, competitive mechanism versus metal ion cofactors (Fjeld and Denu 1999). Presumably, the binding of Zn^{2+} to

```
TvnPPM    MIKVEYFSDSSAVPHLHLENEDSYSVTDDLFIVADGVGSYEGSKDASRY--AVNYLSKMA  58
               .   : .:        : . .  ..:.::::.:   :   .     ::... :.
AaPPM          MKWVSGIVYSDRRTYADRFWADGEIFAVADGMGIGKGAAAVAAAEKAVDLIPKF-  53
                              eDxo     ooxooDG
                           Subdomain 1  Subdomain 2

TvnPPM    KEIQSKEQLVEEIIKLSEEI-KSIGIISGRPLMSTTISVLKISTEKYITANVGDSPIVLL 117
          : ..: : . :.. :.  :. : .: .    :  ::.:.: .    ..   :::: :.:.
AaPPM     KPYNSEEKIRENFSKINRELIKELGKLEDSVLSGTTLSLLSFNERNFYIGHVGDSRIYLF 113
                    ox#oox#xox#oo        g*Txoooo     ooo+*oGDsxxoox
                     Subdomain 3          Subdomain 4      Subdomain 5

TvnPPM    RSGKLYKLYIDDSERSSTGNRFALEQAFGWNHVI-VHSFEGKLEKGDLFIICTDGVSDNL 176
          : :.::.:  :.      ::. ..  ..:..    : .:   .: :::::. .::. . :
AaPPM     RKGRLYRLTEDQVK--IKGNKKVV-KVLGLEWNPEVYTFSSHYEEGDLFLLASDGFVEVL 169
          #  #xxxxxosxx#   +xx#o    aoGxoxxo  p+oxxoxo*   ooo+*DGoodoo
              Subdomain 5a  Subdomain 5b Subdomain 6 Subdomain 7   Subdomain 8

TvnPPM    SDEYDLYSILRELDAQTIVRLAL----NKGIKPDDATIIKVYVTMA                218
          : : ..  .:   :..  .  .     .:: . . . .:
AaPPM     S-EGEIEEALSCCDLEKGAERLKNMARQKGGRKEMVFLIVKTD                   211
          *   #oo*oo#   +*+oo#xxo          Dnotooooxo
             Subdomain 9 Subdomain 10         Subdomain 11
```

Fig. 1 DNA-derived amino acid sequence of the protein product of ORF *TVN0703*. Shown is the amino acid sequence of TvnPPM, the protein product of ORF *TVN0703* (GenBank Accession No. NP_111222) aligned against the sequence of AaPPM from *Bacillus subtilis* (GenBank Accession No. NP_213403). Matches are indicated by *colons* and similarities by *periods*. Below the protein sequences are shown the conserved subdomain sequences characteristic of the PPM family of protein phosphatases (Bork et al. 1996) as described using the nomenclature of Hanks and Hunter (1995): *upper case letters* conserved amino acids residues, *lower case letters* generally conserved amino acid residues, *x* any amino acid, *o* positions where non-polar residues are conserved, *plus* positions where small neutral residues are conserved, *asterisk* positions where polar residues are conserved, and *ash* positions where charged residues are conserved

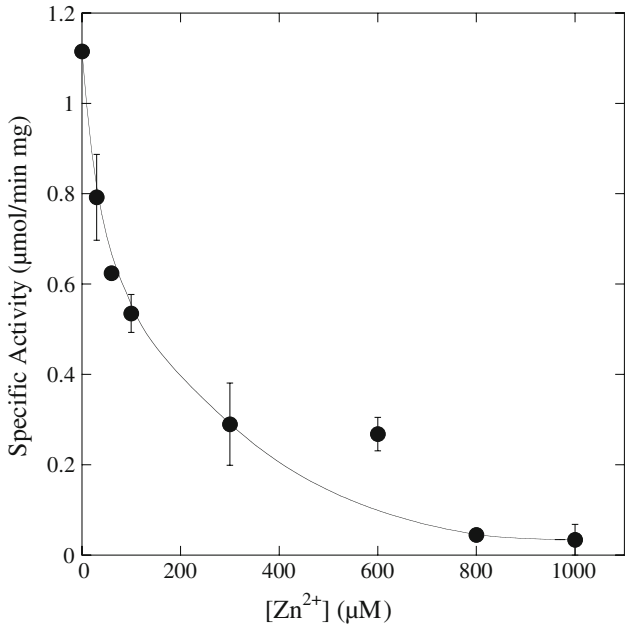

Fig. 2 Zn^{2+} inhibits the activity of TvnPPM. The catalytic activity of TvnPPM, 0.5 μg, toward pNPP was assayed as described in "Materials and methods" with the exception that, where indicated, $ZnCl_2$ was added to the listed final concentration. Shown is a plot of specific activity versus Zn^{2+} concentration ± SE ($n = 3$). The curve shown was fitted using all points but one, that for 600 μM Zn^{2+}

TvnPPM triggers a conformational transition analogous to that by which this metal ion irreversibly inhibits alkaline phosphatase from the green crab *Scylla serrata* (Zhang et al. 2001).

TvnPPM exhibits dual-specific protein phosphatase activity in vitro

TvnPPM was challenged with a series of radiolabeled phosphoproteins. As is typical of other members of the PPM family, TvnPPM dephosphorylated casein and reduced, carboxyamidomethylated and maleylated (RCM) lysozyme that had been phosphorylated on serine and threonine residues using the catalytic subunit of the cAMP-dependent protein kinase (P-Ser/Thr casein and P-Ser/Thr RCM lysozyme) (Table 1). The reaction product could be extracted into organic solvents as a molybdate complex, unambiguously establishing its identity as inorganic phosphate (Data not shown). TvnPPM is not completely promiscuous, however, as it displayed little activity toward P-Ser/Thr histones.

The limited number and heterogeneous distribution of deduced protein-serine/threonine/tyrosine phosphatases in the *Archaea* (Kennelly 2003) suggests that these enzymes may—of necessity—be more versatile than their eucaryal counterparts. For example, in those archaeons that possess only canonical PTPs, it is quite likely that these enzymes

Table 1 TvnPPM exhibits dual-specific protein phosphatase activity

Substrate	Specific activity		
	pmol/min mg	P-Ser/Thr casein (%)	P-Ser/Thr RCM (%)
Lysozyme			
P-Ser/Thr casein	160 ± 20	100	269
P-Ser/Thr histones	3 ± 1	2	5
P-Ser/Thr RCM lysozyme	59 ± 3	37	100
P-Tyr casein	15 ± 4	9	25
P-Tyr RCM lysozyme	8 ± 2	5	14
P-Tyr MBP	18 ± 2	11	31

The catalytic activity of TvnPPM, 1.5 μg, was assayed toward the listed phosphoprotein substrates as described in "Materials and methods". Shown is the protein phosphatase activity toward each phosphoprotein ± SE ($n = 2$) as relative activity versus selected substrates

function as dual-specific, i.e. protein-serine/threonine as well as protein-tyrosine, phosphatases. The converse apparently does not, however, hold for archaeal PPP-family protein phosphatases. All archaeal PPPs characterized to date are strictly serine/threonine-specific (Kennelly et al. 1993; Leng et al. 1995; Solow et al. 1997; Mai et al. 1998), consistent with their high sequence identity to eucaryal PPPs, which are serine/threonine-specific. *T. volcanium* is unusual inasmuch as it does not possess any deduced PTPs, only TvnPPM and a deduced PPP (Kawashima et al. 2000). We therefore asked whether, in *T. volcanium,* TvnPPM fulfills the catalytic function(s) normally supplied in other archaeons by either conventional or low molecular weight PTPs. If so, we would expect that TvnPPM would exhibit dual-specific protein phosphatase activity.

TvnPPM was challenged with several phosphotyrosine-containing [P-Tyr] proteins that had been prepared using [γ-^{32}P]ATP and the lyn protein-tyrosine kinase in vitro: P-Tyr casein, P-Tyr RCM lysozyme, and P-Tyr MBP. As can be seen in Table 1, TvnPPM dephosphorylated all three phosphoproteins at rates comparable in magnitude to that at which it dephosphorylated P-Ser/Thr casein and P-Ser/Thr RCM lysozyme, and ≈3–6 fold greater than P-Ser/Thr histones.

Phylogenetic position of TvnPPM

Phylogenetic trees show TvnPPM to be very deeply rooted (Fig. 3), suggesting that *T. volcanium* acquired the gene encoding this protein phosphatase at a relatively early stage in the evolution of the PPM family. This supposition is reinforced by the close association between TvnPPM with AaPPM, a PPM-family protein phosphatase from the bacterium *Aquifex aeolicus* (Purcarea et al. 2008). *A. aeolicus* is a thermophile that occupies the lowest branch within the

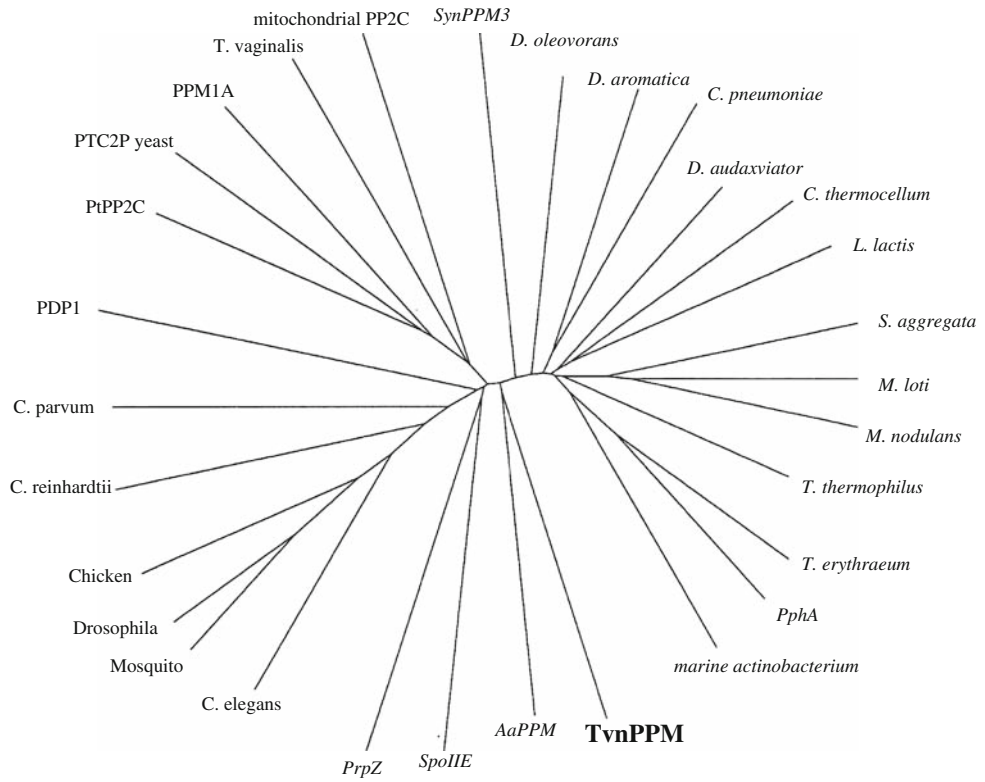

Fig. 3 Phylogenetic analysis of TvnPPM. Shown is an unrooted tree generated using CLUSTALW available through the Kyoto University Bioinformatics Center (http://align.genome.jp/). TvnPPM is located near the 5:30 position in *large bold font*. The eucaryal members of the PPM family of protein phosphatases represented include mitochondrial PP2C, a mitochondrial PP2C from *Homo sapiens* (AAX77016); *T. vaginalis*, a putative PPM from the protozoan *Trichomonas vaginalis* G3 (XP_001324774); PPM1A, PPM1A isoform 2 from *H. sapiens* (NP_808820); PTC2P yeast, protein phosphatase type IIC from *Saccharomyces cerevisiae* (AAB64644); PtPP2C, a PP2C protein phosphatase from *Paramecium tetraraurelia* (CAA85448); PDP1, catalytic subunit of pyruvate dehydrogenase phosphatase from cow (P35816); *C. parvum*, a deduced PPM from *Cryptosporidium parvum* (XP_628612); *C. reinhardtii*, a deduced type 2C protein serine/threonine phosphatase from *Chlamydomonas reinhardtii* (XP_001696623); Chicken, TA-PP2C from *Gallus gallus* (XP_415161); Drosophila, a deduced PPM from fruit fly *Drosophila pseudoobscura* (XP_001354256); Mosquito, a putative PP2C from *Aedes aegypti* (XP_001652369); and *C. elegans*, a putative PPM from nematode *Caenorhabditis elegans* (NP_499362). The bacterial members of the PPM family of protein phosphatases represented include PrpZ, the PrpZ protein phosphatase from *Salmonella enterica* serovar Typhi (NP_458902); SpoIIE, the SpoIIE protein phosphatase from *Bacillus subtilis* (AAB58073); AaPPM, the AaPPM protein phosphatase from *Aquifex aeolicus* (NP_213403); marine actinobacterium, a deduced PPM from marine actinobacterium PHSC20C1 (ZP_01129185); PphA, a PPM from cyanobacterium *Synechocystis* sp. PCC 6803 (NP_440991); *T. erythraeum* protein-serine/threonine phosphatase from *Trichodesmium erythraeum* (YP_722878); *T. thermophilus*, protein phosphatase 2C from *Thermus thermophilus* (YP_005768); *M. nodulans*, a deduced PPM phosphatase from *Methylobacterium nodulans* (ZP_02116970); *M. loti*, a probable PPM protein phosphatase from *Mesorhizobium loti* (NP_103727); *S. aggregata*, a probable PPM protein phosphatase from *Stappia aggregata* (ZP_01546339); *L. lactis*, deduced PPM protein phosphatase from *Lactococcus lactis* (YP_811673); *C. thermocellum*, PPM protein phosphatase from *Clostridium thermocellum* (YP_001037002); *D. audaxviator*, deduced PPM from *Candidatus Desulforudis audaxviator* (YP_001717723); *C. pneumoniae*, deduced PPM from *Chlamydophila pneumoniae* (NP_224597); *D. aromatica*, PP2C-like protein from *Dechloromonas aromatica* (YP_283664); *D. oleovorans*, PPM from *Desulfococcus oleovorans* (YP_001529247); and SynPPM3, SynPPM3 protein phosphatase from *Synechocystis* sp. strain PCC 6803 (NP_440091). GenBank accession numbers are given in parentheses

prokaryotic portion of the phylogenetic tree (Coenye and Vandamme 2004).

Discussion

TvnPPM ranks as one of the most distinctive members of the PPM family of protein phosphatases. While the PPM paradigm has long been considered to be serine/threonine-specific (Barford 1996; Jackson and Denu 2001), TvnPPM exhibited significant protein-tyrosine phosphatase activity in vitro in addition to its predicted protein-serine/threonine phosphatase activity. Moreover, TvnPPM remains the sole archaeal representative of the PPM family (Bhaduri and Sowdhamini 2005). Since TvnPPM constitutes the only apparent source of protein-tyrosine phosphatase activity—or perhaps some other phosphatase activity for which tyrosine phosphatase activity is a manifestation—in *T. volcanium,* we suggest that its dual-specific activity and unique phylogenetic status are linked.

Is it reasonable to expect that *T. volcanium* requires a source of protein-tyrosine phosphatase activity? The fact that the majority of archaeal genomes encode one or more canonical PTPs, and that some possess only conventional and/or low molecular weight PTPs certainly suggests as much (Kennelly 2003; Bhaduri and Sowdhamini 2005). More importantly, proteins recognized by antibodies against phosphotyrosine have been detected in halophilic, methanogenic, as well as thermophilic archaeons (Smith et al. 1997; Jeon et al. 2002).

Is TvnPPM a *bona fide* dual-specific protein phosphatase? While the range of phosphoprotein substrates tested was constrained by the difficulties of generating them in reagent quantities, the enzyme's activity toward all three phosphotyrosine-containing proteins tested approached that exhibited toward P-Ser/Thr casein and P-Ser/Thr RCM lysozyme, and exceeded by several-fold its protein-serine/threonine phosphatase activity toward P-Ser/Thr histones. The magnitude of TvnPPM's protein-tyrosine phosphatase activity thus exceeds that attributable to non-specific "background" hydrolysis. Moreover, TvnPPM is not the first PPM to exhibit dual-specific capabilities in vitro. PphA (Ruppert et al. 2002) and SynPPM3 (Li et al. 2005) from the cyanobacterium *Synechocystis* sp. strain PCC6803 as well as PrpZ from *Salmonella enterica* serovar Typhi (Lai and Le Moual 2005) all reportedly possess significant protein-tyrosine phosphatase activity. In addition, PP2C from rabbit exhibits protein-histidine phosphatase activity in vitro (Kim et al. 1993). Taken together, it appears likely many PPMs, including TvnPPM, function as dual-specific protein phosphatases.

How did *T. volcanium* acquire TvnPPM? Phylogenetic trees constructed by our laboratory and others (Zhang and Shi 2004) indicate that TvnPPM is deeply rooted, suggesting that *T. volcanium* acquired the gene encoding this protein phosphatase at a relatively early stage in evolution. This supposition is reinforced by close association between TvnPPM and AaPPM from *A. aeolicus* (Purcarea et al. 2008), which resides near the root of the bacterial phylogenetic tree (Coenye and Vandamme 2004). While it is difficult to discern whether the *Archaea* acquired the gene for TvnPPM directly from the *Eucarya* or through a bacterial intermediary, the respective genetic contexts of TvnPPM and AaPPM favor the latter route. The gene for AaPPM overlaps that of its cognate protein kinase, AaSTPK, by one base pair, while the region surrounding the gene for TvnPPM is devoid of coding sequences for potential protein kinases. As the typical subfamily of ePKs to which AaSTPK belongs apparently arose in the *Eucarya* (Leonard et al. 1998) it is likely that both kinase and phosphatase were acquired together (Ponting et al. 1999). Subsequently, the gene for an AaPPM-like protein phosphatase found its way to a neighboring archaeon within an ancient community of thermophiles.

The early acquisition of TvnPPM by the domain *Archaea* begs the question, why didn't this protein phosphatase family spread to other members of the domain? It certainly was not for lack of opportunity. The environments favored by *T. volcanium* are inhabited by a variety of acidothermophiles, including numerous archaeons (Segerer et al. 1988; Simmons and Norris 2002; Wagner and Wiegel 2008), which engaged in an extensive exchange of genetic material (Boucher et al. 2003; Choi and Kim 2007). One possible explanation derives from the hypothesis that broad segments of the archaeal population have undergone significant reductive evolution (Kurland et al. 2007; Wang et al. 2007). As the latecomer amongst the protein phosphatases in the *Archaea,* the forces tending to support the dissemination of this new signal transmission module likely could not withstand the countervailing tide of genomic downsizing. However, in the case of *T. volcanium,* circumstances conspired to cause its endogenous PTP(s) to be lost, leading to the retention of TvnPPM as substitute. If this conclusion is correct, when additional archaeal PPMs are eventually encountered, then the host archaeon will prove lacking genes encoding deduced PTPs.

Acknowledgments This work was supported by grant MCB-0315122 from the National Science Foundation.

References

Barford D (1996) Molecular mechanisms of the protein serine/threonine phosphatases. Trends Biochem Sci 21:407–412

Bellinzoni M, Wehenkel A, Shepard W, Alzari PM (2007) Insights into the catalytic mechanism of PPM ser/thr phosphatases from the atomic resolution structures of a mycobacterial enzyme. Structure 15:863–872

Bhaduri A, Sowdhamini R (2005) Genome-wide survey of prokaryotic O-protein phosphatases. J Mol Biol 352:736–752

Bork P, Brown NP, Hegyi H, Schultz J (1996) The protein phosphatase 2C (PP2C) superfamily. Protein Sci 5:1421–1425

Boucher Y, Douady CJ, Papke RT, Walsh DA, Boudreau ME, Nesbo CL, Case RJ, Doolittle WF (2003) Lateral gene transfer and the origins of prokaryotic groups. Annu Rev Genet 37:283–328

Bradford MM (1976) A rapid and simple method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Choi IG, Kim SH (2007) Global extent of horizontal gene transfer. Proc Natl Acad Sci USA 104:4489–4494

Coenye T, Vandamme P (2004) A genomic perspective on the relationship between the aquificales and the epsilon-proteobacteria. Syst Appl Microbiol 27:313–322

Das AK, Helps NR, Cohen PT, Barford D (1996) Crystal structure of the protein serine/threonine phosphatase 2C at 2.0 A resolution. EMBO J 15:6798–6809

D'Souza M, Glass MD, Syed MH, Zhang Y, Rodriguez A, Maltsev N, Galperin MY (2007) Sentra: a database of signal transduction proteins for comparative genome analysis. Nucleic Acids Res 35:D271–D273

Fairbanks G, Steck TL, Wallace DFH (1971) Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606–2617

Fjeld CC, Denu JM (1999) Kinetic analysis of human serine/threonine protein phosphatase 2Cα. J Biol Chem 274:20336–20343

Hanks SK, Hunter T (1995) The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J 9:576–596

Jackson MD, Denu JM (2001) Molecular reactions of protein phosphatases—insights from structure and chemistry. Chem Rev 101:2313–2340

Jeon SJ, Fujiwara S, Takagi M, Tanaka T, Imanaka T (2002) *Tk*-PTP, protein-tyrosine/serine phosphatase from hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1: enzymatic characteristics and identification of its substrate proteins. Biochem Biophys Res Commun 295:508–514

Johnson LN, Lewis RJ (2001) Structural basis for control by phosphorylation. Chem Rev 101:2209–2242

Kannan N, Taylor SS, Zhai Y, Ventner JC, Manning G (2007) Structural and functional diversity of the microbial kinome. PLoS Biol 5:0467–0478

Kawashima T, Amano N, Koike H, Makino SI, Higuchi S, Kawashima-Ohya Y, Watanabe K, Yamazaki M, Kanehori K, Kawamoto T, Nunoshiba T, Yamamoto Y, Aramaki H, Makino M, Suzuki M (2000) Archaeal adaptation to higher temperatures revealed by genomic sequence of *Thermoplasma volcanium*. Proc Natl Acad Sci USA 97:14257–14262

Kennelly PJ (2003) Archaeal protein kinases and protein phosphatases—insights from genomics and biochemistry. Biochem J 370:373–389

Kennelly PJ (2008) Protein phosphorylation at 80°C and beyond. In: Robb F, Antranikian G, Grogan D, Dreissen A (eds) Thermophiles: biology and biotechnology at high temperatures. CRC Press, Boca Raton

Kennelly PJ, Potts M (1996) Fancy meeting you here! A fresh look at 'prokaryotic' protein phosphorylation. J Bacteriol 178:4759–4764

Kennelly PJ, Oxenrider KA, Leng J, Cantwell JS, Zhao N (1993) Identification of a serine/threonine-specific protein phosphatase from the archaebacterium *Sulfolobus solfataricus*. J Biol Chem 268:6505–6510

Kim Y, Huang J, Cohen P, Matthews HR (1993) Protein phosphatases 1, 2A, and 2C are protein histidine phosphatases. J Biol Chem 268:18513–18518

Kurland CG, Canback B, Berg OG (2007) The origins of modern proteomes. Biochimie 89:1454–1463

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lai SM, Le Moual H (2005) PrpZ, a *Salmonella enterica* serovar Typhi serine/threonine protein phosphatase 2C with dual substrate specificity. Microbiology 151:1159–1167

Leng J, Cameron AJ, Buckel S, Kennelly PJ (1995) Isolation and cloning of a protein-serine/threonine phosphatase from an archaeon. J Bacteriol 177:6510–6517

Leonard CJ, Aravind L, Koonin EV (1998) Novel families of putative protein kinases in bacteria and archaea: evolution of the "eukaryotic" protein kinase superfamily. Genome Res 8:1038–1047

Li R, Potters MB, Shi L, Kennelly PJ (2005) The protein phosphatases of *Synechocystis* sp. Strain PCC6803: open reading frames sll1033 and sll1387 encode enzymes that exhibit both protein-serine and protein-tyrosine phosphatase activity in vitro. J Bacteriol 187:5877–5884

Mai B, Frey G, Swanson RV, Mathur EJ, Stetter KO (1998) Molecular cloning and functional expression of a protein-serine/threonine phosphatase from the archaeon *Pyrodictium abyssi* TAG11. J Bacteriol 180:4030–4035

Martin JB, Doty DM (1949) Determination of inorganic phosphate. Modification of isobutyl alcohol procedure. Anal Chem 21:965–967

McGowan CH, Cohen P (1988) Protein phosphatase-2C from rabbit skeletal muscle and liver: an Mg^{2+}-dependent enzyme. Meth Enzymol 159:416–426

Ponting CP, Aravind L, Schultz J, Bork P, Koonin EV (1999) Eukaryotic signaling domain homologues in *archaea* and *bacteria* ancient ancestry and evidence for horizontal gene transfer. J Mol Biol 289:729–745

Pullen KE, Ng HL, Sung PY, Good MC, Smith SM, Alber T (2004) An alternate conformation and a third metal in PstP/Ppp, the *M. tuberculosis* PP2C-family ser/thre protein phosphatase. Structure 12:1947–1954

Purcarea C, Fernando R, Evans HG, Evans DR (2008) The sole serine/threonine protein kinase and its cognate phosphatase from *Aquifex aeolicus* targets pyrimidine biosynthesis. Mol Cell Biochem 311:199–213

Rantanen MK, Lehtio L, Rajagopal L, Rubens CE, Goldman A (2007) Structure of *Streptococcus agalactiae* serine/threonine phosphatase The subdomain conformation is coupled to the binding of a third metal ion. FEBS J 274:3128–3137

Ruppert U, Irmler A, Kloft N, Forchhammer K (2002) The novel protein phosphatase PphA from *Synechocystis* PCC 6803 controls dephosphorylation of the signalling protein PII. Mol Microbiol 44:855–864

Schlicker C, Fokina O, Kloft N, Grune T, Becker S, Sheldrick GM, Forchhammer K (2008) Structural analysis of the PP2C phosphatase tPphA from *Thermosynechococcus elongatus:* a flexible flap subdomain controls access to the catalytic site. J Mol Biol 376:570–581

Segerer A, Langworthy TA, Stetter KO (1988) *Thermoplasma acidophilum* and *Thermoplasma volcanium* sp. nov. from soltara fields. Syst Appl Microbiol 10:161–171

Simmons S, Norris PR (2002) Acidophiles of saline water at thermal vents of Vulcano, Italy. Extremophiles 6:201–207

Smith SC, Kennelly PJ, Potts M (1997) Protein-tyrosine phosphorylation in the *archaea*. J Bacteriol 179:2418–2420

Solow B, Young JC, Kennelly PJ (1997) Gene cloning and expression and characterization of a toxin-sensitive protein phosphatase from the methanogenic archaeon *Methanosarcina thermophila* TM-1. J Bacteriol 179:5072–5075

Wagner ID, Wiegel J (2008) Diversity of thermophilic anaerobes. Ann NY Acad Sci 1125:1–43

Wang M, Yafremava LS, Caetano-Anoles D, Mittenhall JE, Caetano-Anolles G (2007) Reductive evolution of architectural repertoires in proteomes and the birth of the third world. Genome Res 17:1572–1585

Wehenkel A, Bellinzoni M, Schaeffer F, Villarino A, Alzari PM (2007) Structural and binding studies of the three-metal center in two mycobacterial PPM ser/thr protein phosphatases. J Mol Biol 374:890–898

Zhang W, Shi L (2004) Evolution of the PPM-family protein phosphatases in *Streptomyces*: duplication of catalytic domain and lateral recruitment of additional sensory domains. Microbiology 150:4189–4197

Zhang RQ, Chen QX, Xiao R, Xie LP, Zeng XG, Zhou HM (2001) Inhibition kinetics of green crab *(Scylla serrata)* alkaline phosphatase by zinc ions: a new type of complexing inhibition. Biochim Biophys Acta 1545:6–12