Protein Phosphatases−A Phylogenetic Perspective

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Contents

Peter J. Kennelly, born in 1956 near Chicago, IL, received his B.S. degree in Chemistry from the Illinois Institute of Technology in 1978. His interest in Biochemistry stemmed from undergraduate research on bacterial c-type cytochromes in the laboratory of Professor Russell Timkovich. An additional year of work as a laboratory technician followed. In 1985, he received his Ph.D. degree in Biochemistry from Purdue University. Here, he became interested in regulatory protein phosphorylation–dephosphorylation while examining the modulation of 3-hydroxy-3-methylglutaryl-CoA reductase in the laboratory of Professor Victor W. Rodwell. As a postdoctoral fellow in the laboratory of Professor Edwin G. Krebs at the University of Washington, he studied the autoinhibitory mechanism by which calmodulin activates the skeletal muscle isoform of myosin light chain kinase. In 1989, he joined the faculty of the Department of Biochemistry at Virginia Polytechnic Institute and State University, where he currently holds the rank of Professor. His research focuses on the study of eukaryote-like protein kinases and protein phosphatases in prokaryotes.

I. Introduction

Protein phosphorylation−dephosphorylation provides nature with perhaps its most potent and versatile mechanism for the regulation of cellular functions via the modulation of the structural and functional properties of strategically selected proteins.[1,2] Much of its power is derived from the ability of protein phosphatases to restore phosphoproteins to their original, unmodified state. First described as a mechanism by which hormonal/neuronal second messengers such as cAMP and Ca^{2+} exerted their intracellular effects (reviewed in ref 3), early attempts to detect regulatory protein phosphorylation events in microbial organisms proved unconvincing (reviewed in ref 4). Since the 'simple' regulatory requirements of microorganisms appeared to be adequately addressed by allosterism and other ancient mechanisms, early researchers concluded that protein phosphorylation−dephosphorylation repre-

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sented a late evolutionary invention devised to meet the specific needs of higher organisms comprised of multiple, differentiated cells. Thus, for many years the role of protein phosphorylation in prokaryotes (and lower eukaryotes as well) remained little appreciated and largely unexplored.

The first effective challenge to the perception that protein phosphorylation was an exclusively eukaryotic phenomenon appeared in the late 1970s, when Garnak and Reeves[5] reported that the enzyme isocitrate dehydrogenase in *E. coli* was subject to regulatory protein phosphorylation. Phosphorylation resulted in the catalytic inactivation of the enzyme, and significantly, its degree of phosphorylation was sensitive to the nutrient status of the bacterium. At this same point in time, the laboratories of Koshland[6] and Cozzone[7] reported the presence of endogenous protein kinase activity in *Salmonella typhimurium* and *E. coli*, respectively. Together with the subsequent identification of the histidine kinase-driven two-component paradigm as a pervasive mechanism for signal transduction in bacterial organisms (reviewed in refs 8 and 9), these findings established the ubiquitous nature of regulatory protein phosphorylation.

While the provocative observations described above necessitated a revision of the long-standing view that 'simple' unicellular organisms had been excluded from nature's protein phosphorylation club, the basic perception that the protein phosphorylation events taking place in (higher) eukaryotes enjoyed a unique and separate status persisted. Comparison of the first reported sequences for protein kinases of prokaryotic origin—those of the isocitrate dehydrogenase kinase/phosphatase[10,11] and two-component histidine kinases[12−14]—showed them to be completely alien, lacking nearly all of the key sequence features faithfully conserved among the many members of the eukaryotic protein kinase superfamily.[15] Moreover, not only were the polypeptide sequences of the principal macromolecular catalysts of eukaryotic and prokaryotic protein phosphorylation apparently distinct, the chemical strategies employed in each domain exhibited clear contrasts as well. Protein phosphorylation in eukaryotes utilized phosphomonoester chemistry with near 100% frequency, targeting the hydroxyl groups of serine, threonine, and tyrosine. However, while some serine/threonine phosphorylation was detected in bacteria, their numerous two-component histidine kinases phosphorylated the side chain carboxyl group of aspartic acid to form mixed acid anhydrides. Even the catalytic mechanisms of their most prominent protein kinase families differed, as the eukaryotic cAMP-dependent protein kinase and its counterparts facilitated direct phosphoryl transfer between substrates,[16] while the bacterial histidine kinases employed a ping-pong mechanism involving the formation of a phosphohistidine enzyme intermediate.[17] (The name histidine kinase is therefore a misnomer, as the histidine referred to was a phosphoenzyme intermediate produced during the transfer of phosphate to its ultimate destination, an aspartic acid residue on the so-called response regulator.) The nature and magnitude of these differences suggested that the *Bacteria* and the *Eucarya* each had originated independent and exclusive paradigms for harnessing the regulatory potential of the phosphoryl group some time after the two domains had diverged from one another.

The 1990s have witnessed the demolition of this long-standing dichotomy, an event that has been as dramatic in scope as it was unexpected. Aided by the emerging field of genomics, it has been determined that many of the protein kinase and protein phosphatase prototypes previously considered to be exclusively eukaryotic or prokaryotic in origin and residency in fact are shared among the members of the three recognized phylogenetic domains to an extraordinary degree (reviewed in refs 18 and 19). The complex and cosmopolitan distribution of the agents that effect regulatory protein phosphorylation−dephosphorylation have thrown conventional models for its origin and development into a state of flux.

In this article, I survey the current state of knowledge concerning the phylogenetic distribution of the major classes of protein phosphatases identified to date and attempt to glean clues from the phylogenetic record about the evolutionary history of nature's preeminent regulatory mechanism. Sections III, IV, and VI focus upon the protein phosphatases that act upon protein-bound phosphoserine, phosphothreonine, and/or phosphotyrosine; while section V surveys the characteristics of several demonstrated and potential protein phosphatases that target phosphorylated histidine and aspartic acid residues. Since the properties and physiological roles of the protein phosphatases resident in eukaryotic organisms have been reviewed in considerable detail both in this issue and elsewhere,[20−31] herein the primary focus will be upon the enzymes present in the two prokaryotic phylogenetic domains, the *Bacteria* and the *Archaea*.

II. Brief Overview of Phylogeny

The field of phylogeny traces its origins to the publication of the theory of evolution in Darwin's *Origin of the Species* in 1859 (reviewed in ref 32). If all living things evolved from a common ancestor, it stood to reason that by determining the hereditary relationships between contemporary organisms, the order and nature of the evolutionary events that produced them could be reconstructed and the characteristics of their ancient predecessors extrapolated therefrom. The resulting phylogenetic tree would provide the rough equivalent of a biological time machine from which specific evolutionary events and the mechanism(s) underlying them could be discerned. For nearly a century, however, phylogeneticists were hampered by the lack of any direct means for measuring 'heredity' or changes therein. This forced them to rely on inferential phenotypic metrics embodied in the physical anatomy, cellular and subcellular morphology, nutrient and environmental requirements, etc., of living organisms. The model that emerged divided all living organisms into two domains, the eukaryotes and prokaryotes, on the basis of their subcellular morphology/intracellular

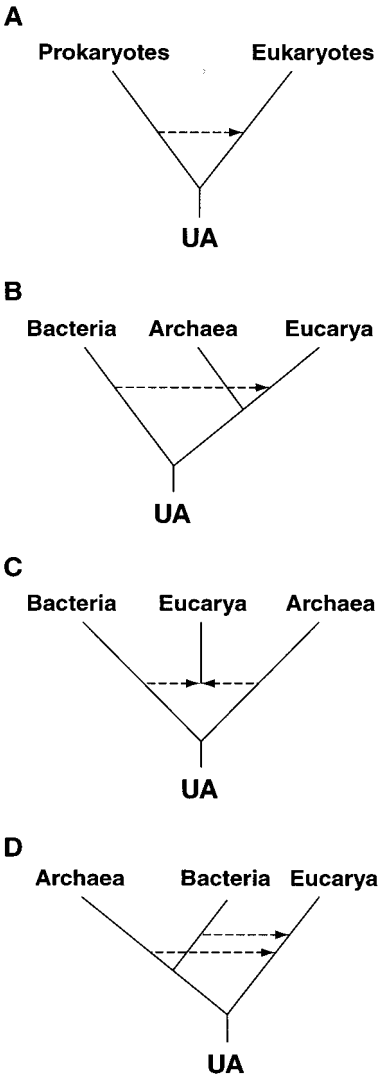

Figure 1. Schematic models for various phylogenetic trees: (A) the classic Prokaryote: Eukaryote tree of Chatton;[33] (B) the Woesian three-domain tree (Olsen, G. J.; Woese, C. R. *FASEB J.* **1993**, *7*, 113); (C) a three-domain tree in which the *Eucarya* emerge from a chimeric fusion of an ancient bacterium with an ancient archaeon;[45] and (D) the three-domain tree of R. F. Doolittle[43] which proposes an alternate rooting of the *Archaea*, *Bacteria*, and *Eucarya* along with two endosymbiotic events that transfer genetic information from each of the prokaryotic domains to the nascent *Eucarya*. Major horizontal gene transfer events, such as endosymbiosis, are indicated by dotted arrows.

organization[33] (Figure 1A). The eukaryotes were distinguished by their possession of a nuclear membrane. In addition, they oftentimes exhibited other forms of internal compartmentalization: mitochondria, chloroplasts, endoplasmic reticulum, Golgi apparatus, etc. The greater degree of development implied by their complex internal organization was also reflected in the fact that the eukaryotic domain encompassed virtually all 'higher' organisms, i.e., those comprised of multiple, differentiated cells. The prokaryotes, on the other hand, in addition to lacking a nuclear membrane or other forms of internal compartmentalization, were small, almost exclusively unicellular, and contained a single circular chromosome.

With the emergence of molecular biology, phylogeneticists acquired the ability to directly measure heredity as embodied in the sequences of the genes that encode it. The results of these analyses proved to be surprising and provocative. While comparison of the sequences of presumed markers of evolutionary change such as 16S ribosomal RNA largely confirmed the order and coherence of the eukaryotic domain, now also called the *Eucarya*, the organisms grouped under the prokaryotic banner bifurcated into two distinct evolutionary domains, the *Bacteria* (initially called the *Eubacteria)* and the *Archaea* (initially called the *Archaebacteria*)[34,35] (Figure 1B). Perhaps even more shocking than the discovery of this third domain, initial attempts to root the new three-domain phylogenetic tree indicated that the *Archaea* were first cousins to the eukaryotes (or *Eucarya*) rather than to the superficially more similar *Bacteria*.[35,36]

Today, genomics have made available the complete hereditary material from a diverse and rapidly expanding array of organisms, freeing scientists from their reliance upon a limited and potentially misleading handful of evolutionary markers. Paradoxically, the possession of a comprehensive, unbiased spectrum of hereditary information has blurred rather than clarified the 'tree of life' (reviewed in refs 37 and 38; Figure 1 C,D). An array of conflicting observations have emerged that have proven difficult to reconcile with the original three-domain tree of Carl Woese and colleagues.[39] New questions have arisen concerning the positions of the three domains relative to one another and to their presumed universal ancestor, the origins of the eukaryotic nucleus, the number and nature of past endosymbiotic events, and whether the *Eucarya* evolved in a gradual, linear fashion or suddenly burst onto the scene as the result of the chimeric fusion of an archaeon with a bacterium (reviewed in refs 40−45). Recently, W. F. Doolittle proposed that evolution is best represented by a criss-crossing web of gene transfer events that precludes the construction of a single, universal phylogenetic tree.[46]

The ultimate resolution of the current foment in phylogeny will require the derivation of more complex models for the evolutionary process itself, ones that will more readily accommodate the unexpectedly high degree of horizontal gene transfer that apparently has taken place.[46,47] However, while their relative positions and prior histories remain the subject of continuing debate, the weight of both evidence and opinion supports the existence and general composition of the three domains *Archaea*, *Bacteria*, and *Eucarya*.[48] Therefore, in this review the phylogenetic distribution of protein phosphatases will be discussed within the framework of the Woesian tree.

III. Major Families of Protein–Serine/Threonine/Tyrosine Phosphatases: Prominent Structural and Functional Characteristics

A. Introduction

The side chain functional groups of many of the naturally occurring amino acids possess the chemical

potential to form covalent bonds to phosphate, including the hydroxyl side chains of serine, threonine, and tyrosine; the thiol group of cysteine; the carboxylates of aspartic and glutamic acid; and the nitrogens contained within the imidazole ring of histidine, the epsilon amino group of lysine, and the guanidino group of arginine (reviewed in ref 49). By far the most chemically robust of the resulting phosphoamino acids are the phosphomonoesters of serine, threonine, and tyrosine.[50] Given the great facility with which phosphoesters can be isolated and studied in the laboratory and the vast predominance of the hydroxyl amino acids as the targets for modification by covalent phosphorylation in eukaryotic organisms, it is not surprising that our knowledge of the dephosphorylation of protein-bound phosphomonoesters far outstrips that of protein-bound phosphoramides, acyl phosphates, etc.

To date, five superfamilies of phosphomonoester-specific protein phosphatases have been identified and characterized in molecular detail: the PPP- and PPM-families of protein−serine/threonine phosphatases and three families of protein−tyrosine phosphatases (PTPs), the conventional PTPs, the low molecular weight (LMW) PTPs, and the Cdc25 family (reviewed in refs 20 and 51). In recent years it has become apparent that not all protein phosphatases were absolutely specific for either the aryl phosphoester of tyrosine or the aliphatic phosphoesters of serine and threonine. This was particularly true among the PTPs, where the members of the Cdc25 family[52,53] and several conventional PTPs such as VH1[54] and MKP-1[55,56] dephosphorylated serine and/or threonine residues in addition to tyrosine both in vitro and in vivo. These latter enzymes often are referred to as dual-specific(ity) phosphatases (DSPs).

In this section, the distinguishing features of each of these protein phosphatase families will be introduced by briefly reviewing the key attributes of prototypic representatives drawn from the eukaryotic domain in which they were first discovered and characterized. Next, the physical and functional properties of established family members, i.e., those for which clear and convincing evidence of protein phosphatase activity has been reported, from the *Archaea*, *Bacteria*, and/or viruses will be surveyed and compared to those of their eukaryotic prototypes. Finally, the identities and characteristics of homologues with alternative substrates and/or catalytic functions that expand the sphere of development and action of these protein phosphatase families beyond the realm of protein dephosphorylation will be briefly reviewed.

B. PPP Family

1. Eukaryotic PPPs

In the *Eucarya*, the more prolific of the two superfamilies of protein−serine/threonine phosphatases, both in terms of the total number of family members and their collective contribution to the gross level of protein−serine/threonine phosphatase activity, is the PPP-family (Table 1).[57] The members of this family share a common catalytic core domain ≈280 amino acids in length that is highly conserved, ≥34% amino acid identity, across the *Eucarya*.[58] This high degree of identity ranks the PPPs among the most highly conserved of all eukaryotic enzymes.[59] The sequence signature of the PPPs consists of a trio of short sequence motifs separated by gaps of approximately 25−30 residues: [Gly-Asp-Xaa-His-Gly]-Xaa_{25-30}-[Gly-Asp-Xaa-Xaa-Asp-Arg-Gly]-Xaa_{25-30}-[Gly-Asn-His-Asp/Glu].[58,60]

The earliest, and for many years the only, known members of the PPP-family were protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A), and protein phosphatase 2B (PP2B), also known as calcineurin (reviewed in ref 57). In recent years a number of new PPPs have been identified. Together with their predecessors, they can be grouped by into four subfamilies. These include the PPP1 subfamily, which contains PP1, PPQ, PPY, PPZ, and Glc7; the PPP2A subfamily, which contains PP2A, PP4, PP6, PPH, and Sit4; the PPP2B subfamily, which contains PP2B and CNA; and the PPP5 subfamily, which includes PP5, RdgC, and PP7.[24] Representatives of each of the PPP-subfamilies listed above are present in both man and yeast, implying that each completely permeates the spectrum of eukaryotic organisms.

The eukaryotic members of the PPP-family are metalloenzymes[61,62] that tenaciously bind a pair of closely juxtaposed metal ions, probably Fe^{3+} and either Zn^{2+} or Mn^{2+}. X-ray crystallography has revealed that many of the amino acid residues within the aforementioned trio of conserved sequence motifs participate in metal binding.[63,64] During catalysis, this bimetallic center activates a water molecule that directly attacks and hydrolyzes the phosphoester bond on phosphoprotein substrates.[20,65,66] Although eukaryotic PPPs, in particular calcineurin[67,68] and recombinant forms of PP1,[69] have occasionally been induced to dephosphorylate protein-bound tyrosine residues in the laboratory, this activity is not thought to possess any physiological relevance, i.e., no certified DSPs have been identified within the PPP family. However, despite their strong selectivity for phosphoserine and phosphothreonine, the most extensively studied PPPs, PP1 and PP2A, have displayed little ability to discriminate among potential phosphoprotein substrates in vitro (reviewed in ref 70). For many years this behavior raised serious questions concerning if and how these enzymes might mediate high-fidelity transmission of extracellular signals, leading to speculation that the PPPs served merely as a source of general, constitutive protein dephosphorylating activity that counterbalanced stringently regulated, substrate-specific protein kinases (reviewed in ref 71).

It has since become apparent that variety and specificity in PPP function in the *Eucarya* is achieved primarily through the addition of distinct targeting and regulatory domains, rather than through discrimination at the active site itself (reviewed in refs 72 and 73). In some cases, such as PP5 (reviewed in ref 74), these auxiliary domains are fused directly onto the catalytic polypeptide chain. However, in most cases these auxiliary domains are incorporated in the form of dissociable subunits. For PP1 (reviewed

Table 1. Known and Potential Protein Phosphatases. Listed Below Are the Families of Known or Potential Protein Phosphatases Discussed in This Article[a]

family name/ founding member	pseudonyms/ prominent members	phylogenetic distribution			catalytic mechanism	signature sequence motifs
		A	B	E		
PPP	PP1, PP2A, PP2B	+	+	+	direct hydrolysis	GDXHG, GDXXDRG, GNH(E/D)
PPM	PP2C, SpoIIE	−	+	+	direct hydrolysis	(S/T)DGXX(D/E/N), D(D/N)X(T/S)
conventional PTP	PTP-1B, YopH	?	+	+	ping-pong, Cys-P enzyme intermediate	$D-X_{\approx 30}-HCX_5R(S/T)$
low MW PTP		?	+	+	ping-pong, Cys-P enzyme intermediate	CX_5R-X_{85-105}-DP
Cdc25		−	−	+	ping-pong, Cys-P enzyme intermediate	$D-X_{\approx 45}-CX_5R$
IDH kinase/phosphatase	AceK	−	+	−	phosphotransfer to ADP followed by ATP hydrolysis	n.d.
HPr kinase/phosphatase	HprK, PtsK	−	+	−	unknown, ATP/ADP-independent	GXSGXGKSEXALELIX-RGHXLVADDXVEI, LEIRGLGIIN
HPr phosphatase	HprP, YvoE	−	+	−	unknown	D(L/M)DGTL, KPXP
SixA		−	+	−	unknown	RHG(?)
histidine kinase	NRII/NtrB, KpdD, EnvZ	?	+	?	unknown, ATP/ADP-dependent	$NA-X_{\approx 25}$-DXGXG-$X_{\approx 12}$-F-$X_{\approx 12}$-GXGXG
CheY	response regulator, NRI	?	+	+	unknown, may stimulate response regulator's autophosphatase activity	$(D/E)D-X_{\approx 40}$-$(Hydrophobic)_{2-4}$-D-$X_{\approx 50}-KP$
CheZ		−	+	−	unknown, may activate CheY autophosphatase activity	AQDXQDLTGQXXKR, QDXXDDLLXSLGF
Spo0E		−	+	−	unknown, may stimulate response regulator's autophosphatase activity	n.d.
RAP	Spo0L	−	+	−	unknown, may stimulate response regulator's autophosphatase activity	YXXLXXXR, AE-X_{9-11}-E

[a] For more details, refer to the text. Abbreviations used include the following: A,: *Archaea*; B, *Bacteria*; E, *Eucarya*; and n.d., not determined. A plus sign (+) indicates that one or more examples of this protein from a member of the domain in question has been demonstrated to catalyze or enhance the dephosphorylation of a phosphoprotein or other phosphomonoester. A question mark (?) indicates that an ORF potentially encoding a protein phosphatase from this family has been identified in a member of this domain, but no experimental evidence for function has been reported. A minus sign (−) indicates that no report of an active protein phosphatase has appeared and no ORFs encoding potential protein products displaying homology to the established members of this family can be discerned from the genomic or other DNA sequences currently available from members of this domain.

in ref 29) and PP2A (reviewed in ref 31), the spectrum of auxiliary subunits has attained impressive proportions, thereby allowing a handful of very highly conserved catalytic subunit isoforms to provide the catalytic core for scores of heterooligomeric holoenzymes. This behavior is in direct contrast to that of the protein−serine/threonine kinases, where variety in specificity and function has been achieved via the elaboration of a multiplicity of different catalytic isoforms within each subgroup of protein kinases (reviewed in ref 15), e.g., the calmodulin-regulated protein kinases or protein kinase C, that differ in their active site substrate recognition properties (reviewed in refs 75 and 76). Recent homology searches of the *Caenorhabditis elegans* and *Saccharomyces cerevisiae* genomes indicate that the number of distinct catalytic polypeptides for protein−serine/threonine kinases may outnumber those for PPP- and PPM-family protein−serine/threonine phosphatases by 4:1 to perhaps as high as 7:1.[77]

2. Archaeal PPPs

Three PPP-family protein phosphatases thus far have been identified and characterized from the *Archaea*: PP1-arch1 from *Sulfolobus solfataricus*,[78,79] PP1-arch2 from *Methanosarcina thermophila* TM-1,[80,81] and Py-PP1 from *Pyrodictium abyssi* TAG11[82] (Table 2). The DNA-derived amino acid sequences of the archaeal PPPs exhibit a high degree of sequence similarity with the eukaryotic members of the PPP family, 27−31% over the complete catalytic core domain,[58] with members of the PPP1 subfamily

Table 2. General Characteristics of Prokaryotic PPP-Family Protein Phosphatases

name	organism	domain	size	activating metals	amino acid specificity		
					S/T	Y	H/K
PP1-arch1[78, 79]	*S. solfataricus*	*Archaea*	34 kDa	Mn, Ni, Co	+	−	n.d.
PP1-arch2[80, 81]	*M. thermophila* TM-1	*Archaea*	31 kDa	Mn, Ni, Co	+	−	n.d.
Py-PP1[82]	*P. abyssi* TAG11	*Archaea*	33 kDa	Mn, Ni, Co	+	n.d.	n.d.
PrpA[83]	*E. coli*	*Bacteria*	24 kDa	Mn	+	+	n.d.
PrpB[83]	*E. coli*	*Bacteria*	24 kDa	Mn	+	+	n.d.
SppA[84]	*S. coelicolor* A3(2)	*Bacteria*	40 kDa	Mn, Mg, Ni, Ca	+	+	n.d.
PP1-cyano1[85]	*M. aeruginosa* PCC7820	*Bacteria*	30 kDa	Mn, Mg, Ni, Co	+	+	+
PP1-cyano2[85]	*M. aeruginosa* UTEX2063	*Bacteria*	30 kDa	Mn, Mg, Ni, Co	+	+	+

scoring highest. The degree of sequence conservation between these archaeal and eukaryotic protein phosphatases differed by a surprisingly small degree relative with that observed between the most dissimilar of the eukaryotic PPPs, 34%.[58]

Like their eukaryotic counterparts, all three archaeal PPPs dephosphorylated serine and threonine residues. Moreover, when challenged with phosphotyrosine-containing macromolecules, neither PP1-arch1 nor PP1-arch2 displayed detectable activity, indicating that archaeal PPPs are serine/threonine-specific. However, while eukaryotic PPPs are metalloenzymes, these archaeal PPPs required the addition of an exogenous metal ion such as Mn^{2+}, Ni^{2+}, or Co^{2+} to support activity. In each instance, Mn^{2+} emerged as the preferred metal ion cofactor from among those surveyed in vitro, while Mg^{2+} was either ineffective or marginally effective. Archaeal PPPs do not display the nanomolar sensitivity to microbial toxins such as okadaic acid and tautomycin that is a signature of PP1 and PP2A from eukaryotes.[57] PP1-arch1[78] was completely insensitive, while PP1-arch2[80] and PyPP1[82] were partially inhibited when exposed to high, micromolar concentrations of these compounds. The behavior of the latter two was reminiscent of eukaryotic PP2B, which has an IC_{50} of $\approx$5 μM for okadaic acid.[57]

Insight into the physiological role and the regulation of either the expression or the functional properties of archaeal PPPs currently is lacking. In both sequence and size, 31−34 kDa, the archaeal PPPs resemble the catalytic subunits of PP1 and PP2A. Unlike many eukaryotic PPPs, evidence has yet to emerge for the presence of heterologous auxiliary subunits or inhibitor proteins that might serve to regulate their catalytic capabilities or subcellular location. However, it would be premature to conclude that these phosphatases are unregulated given the limited nature of the investigations carried out to date. In the case of PP1-arch2 and PyPP1, for example, all or nearly all of their functional characterization was carried out upon the recombinant products of their cloned genes, an approach that precludes the formation and detection of any heterooligomeric polypeptide complexes that may exist in vivo. In the case of PP1-arch1, the enzyme as purified from its parent archaeon was monomeric. However, as the protein was tracked during isolation solely on the basis of its catalytic activity, any heterooligomeric species that may have been present in low abundance or which possessed little or no activity toward the exogenous protein substrate used, casein, would have been overlooked and discarded during the isolation process.

3. Bacterial PPPs

Five PPP-family protein phosphatases have thus far been identified and characterized from the *Bacteria*: PrpA and PrpB from *E. coli*,[83] SppA from *Streptomyces coelicolor* A3(2),[84] and PP1-cyano1 and PP1-cyano2 from *Microcystis aeruginosa* PCC7820 and UTEX2063, respectively[85] (Table 2). The degree of sequence identity shared between these bacterial PPPs and either prototypical eukaryotic PPPs or newly discovered archaeal PPPs was much lower than that observed between the latter two (see above), typically 17−19%. As was the case for the archaeal PPPs, all five of these bacterial PPPs required an exogenous metal ion such as Mn^{2+} for activity and were resistant to potent inhibitors of eukaryotic PP1 and PP2A such as okadaic acid or microcystin. This was particularly significant in the case of PP1-cyano1 and PP1-cyano2, as certain strains of *M. aeruginosa* such as PCC7820 produce the latter toxin.[86]

In marked contrast to both their eukaryotic and archaeal counterparts, the bacterial PPPs all displayed significant activity toward tyrosine residues in vitro in addition to their expected protein−serine/threonine phosphatase activity. PP1-cyano1 and PP1-cyano2 also dephosphorylated histidine and lysine residues on synthetic amino acid homopolymers. Missiakis and Raina[83] speculated that PrpA and PrpB might possess either protein−histidine or protein−aspartate phosphatase activity based upon genetic evidence indicating that they participate in a signaling pathway that senses misfolded proteins in extracytoplasmic compartments. Since this pathway also contains two-component histidine kinases, one possible mechanism by which PrpA and PrpB might impact this pathway would be through the dephosphorylation of the former's autophosphorylated histidine residue. Expression of PrpA also was induced by heat shock. In *S. coelicolor* A3(2), disruption of the gene for SppA leads to severe impairment of vegetative growth and markedly diminished formation of aerial hyphae.[84]

4. Viral PPPs

The first indication that 'eukaryotic' protein kinases and/or protein phosphatases might reside outside of the *Eucarya* was provided by the discovery of a PPP-family protein phosphatase, PP-lambda, in

bacteriophage lambda gt10.[87,88] While PP-lambda shared significant sequence similarity with the catalytic subunits of PP1 and PP2A, ≈17−19% amino acid identity, at 221 amino acids in length it was considerably smaller than the conserved catalytic core of eukaryotic PPPs. As also observed to be the case for the archaeal and particularly the bacterial branches of the PPP-family, PP-lambda manifested other 'atypical' properties relative to eukaryotic PPPs such as a requirement for exogenous metal ions[88] and both protein−tyrosine and protein−histidine phosphatase activity.[89]

The physiological role of PP-lambda remains cryptic. Lambda gt11 does not express a functional PPP, presumably because the version coded in this strain was significantly truncated relative to that found in lambda gt10. Yet lambda gt11 was observed to be equally infectious.[88] Phage phi80 also contains an ORF whose predicted product displays 73% identity to PP-lambda;[87] however, no characterization of this potential protein phosphatase has been reported.

5. PPP Homologues

In 1993, Koonin[90] reported the surprising observation, since confirmed elsewhere,[58,91] of sequence homology indicative of an ancestral relationship between the ApaH diadenosine tetraphosphatases from *E. coli* and *Klebsiella aerogenes* and members of the PPP-family of protein phosphatases. Diadenosine tetraphosphate is one of a set of bis(5′-nucleotidyl) tetraphosphates encountered in organisms ranging from bacteria to yeast to humans. In *E. coli*, it serves as an intracellular stimulator cell division (reviewed in ref 92), while in animals, diadenosine polyphosphates have been implicated as intra- and extracellular signaling compounds with potential cardioprotective, tumor suppressive, and acute pharmacological effects (reviewed in ref 93). Diadenosine tetraphosphatases hydrolyze one of the pyrophosphate bonds in this compound to produce either two molecules of ADP (symmetric subgroup) or one molecule each of AMP and ATP (asymmetric subgroup). The regions of homology were centered precisely in those areas conserved within the protein phosphatases of the PPP-family, suggesting that ApaH and the PPPs employ a common hydrolytic mechanism. This supposition was further buttressed by the observation that PP1-cyano1 and PP1-cyano2 harbor weak but detectable symmetric diadenosine tetraphosphatase activity.[85]

Subsequent sequence searches indicated that the PPP-family of protein phosphatases and the ApaH family of diadenosine tetraphosphatases may, in fact, be members of an extensive superfamily of metallophosphoesterases that potentially includes purple acid phosphatase, bacterial exonucleases, as well as some sugar and lipid phosphatases.[65,94] Dixon and coworkers[65] suggested the following five-component signature motif for this metallophosphoesterase superfamily: [Asp-Xaa-His-Gly]-Xaa$_{\approx 25}$-[Gly-Asp-Xaa-Xaa-Asp]-Xaa$_{\approx 25-30}$-[Gln-His]-Xaa$_{40-130}$-[Hydrophobic-Hydrophobic-Xaa-Xaa-His]-Xaa$_{25-70}$-[Hydrophobic-Hydrophobic-Xaa-Gly-His]. The wide range of enzymes in which this motif has been detected suggests that their common progenitor must have been extremely ancient.

6. A PPP-Based Tree

Comparison of the sequences of established bacterial and archaeal PPPs with representatives from the eukaryotes produced a tree (Figure 2) that reprised the basic configuration of the Woesian phylogenetic tree as depicted in Figure 1A.[85] The rooting of this tree indicated that the initial bifurcation segregated the ancestor of the bacterial PPPs from the common ancestor of the archaeal and eukaryotic PPPs. Moreover, it indicated that PPP-lambda was a relatively recent offspring of a bacterial PPP, largely ruling out phage lambda as a vector for transferring the PPPs across domain boundaries. The tree also suggested that the ApaH family of diadenosine tretraphosphatases diverged from an ancient bacterial PPP.

C. PPM Family

1. Eukaryotic PPMs

In eukaryotes, the principal members of the PPM-family (Table 1) are PP2C and pyruvate dehydrogenase phosphatase. Well before their respective amino acid sequences were known, the PPMs were distinguished from the PPPs by the former's requirement for the addition of an exogenous metal ion, usually Mg^{2+}, to support catalytic activity.[95] A recent report indicates that Fe^{2+} was far superior than Mg^{2+} as a cofactor in vitro.[96] However, the physiological significance of this observation is hard to determine as it is difficult to envision that a freely dissociating molecule of Fe^{2+} could be protected against oxidation in the aerobic environment of an actively respiring cell. At the primary sequence level, PPMs possess an ≈290 residue catalytic domain containing 11 conserved motifs in which 8 'absolutely' conserved residues reside.[97,98] The lengthiest individual motifs were numbers 8, Ser/Thr-Asp-Gly-Xaa$_2$-Asp/Glu/Asn, and 11, Asp-Asp/Asn-Xaa-Thr/Ser. The nature and degree of sequence conservation thus appears to be comparable to that observed among the 'eukaryotic' protein kinases, which contain 12 conserved subdomains containing 10 'absolutely' conserved residues.[15] While the PPMs were observed to be completely disparate from the PPPs at the level of their polypeptide sequences,[99,100] X-ray crystallographic analysis has revealed a striking degree of similarity between their active sites, suggestive of convergence upon a common catalytic mechanism.[98]

The PPM-family appears to be ubiquitous in eukaryotes, examples having been isolated and/or cloned from mammals,[99−101] nematodes,[102] insects,[103] plants,[104] fungi,[105] and protozoa.[106,107] With the exception of pyruvate dehydrogenase phosphatase, which is a heterodimer,[100] all other eukaryotic PPMs isolated to date have proven to be monomeric proteins that generally ranged from 31 to 65 kDa in size.[95,107,108] Recently, however, the cloning of a cDNA encoding a deduced PPM > 150 kDa in size has been reported.[103]

No dedicated regulatory or targeting subunits have been identified for the PP2C subfamily of PPMs.

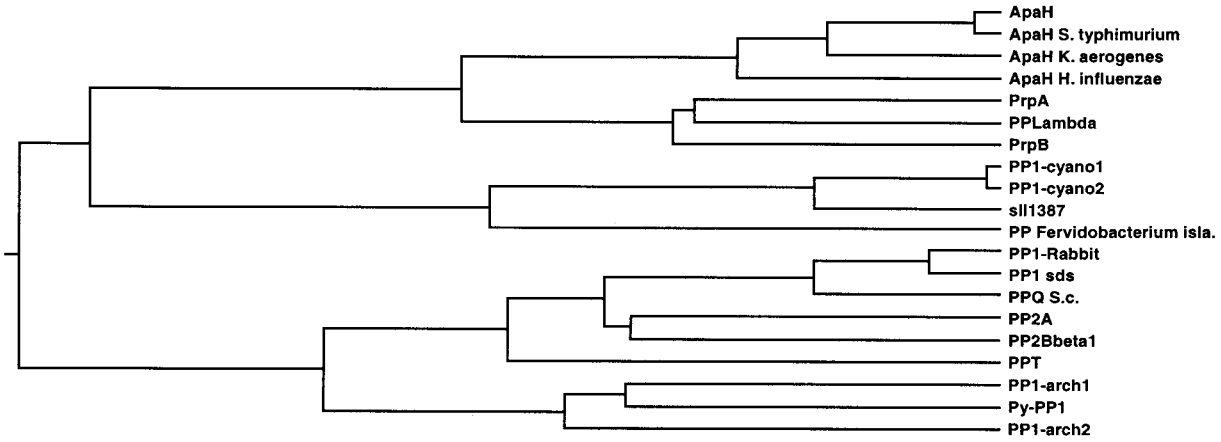

Figure 2. PPP-Family Phylogenetic Tree. Using the sequence regions encompassing their signature sequence triad, a phylogenetic tree was determined using the MegAlign program of Lasergene from DNA* (Madison, WI) using representative members of the PPP-family phosphatases from the *Archaea, Bacteria, Eucarya,* and bacteriophage lambda. The lengths of the horizontal lines indicate relative evolutionary distance. Abbreviations used are as follows: ApaH E. coli, a diadenosine tetraphosphatase from *E. coli* (Blanchin-Roland, S.; Blanquet, S.; Schmitter, J. M.; Fayat, G. *Mol. Gen. Genet.* **1986**, *205*, 515); ApaH S. typhimurium, a diadenosine tetraphosphatase from *Salmonella typhimurium* (GenBank accession number Q56018); ApaH K. aerogenes, a diadenosine tetraphosphatase from *Klebsiella aerogenes* (Azakami, H.; Sugino, H.; Murooka, Y. *J. Bacteriol.* **1992**, *174*, 2344); ApaH H. influenzae, a putative diadenosine tetraphosphatase from *H. influenzae* (Fleischmann R. D.; Adams, M. D.; White, O.; Clayton, R. A.; Kirkness, E. F.; Kerlavage, A. R.; Bult, C. J.; Tomb, J. F.; Dougherty, B. A.; Merrick, J. M.; McKenney, K.; Sutton, G.; FitzHugh, W.; Fields, C.; Gocayne, J. D.; Scott, J.; Shirley, R.; Liu, L.; Glodek, A.; Kelley, J. M.; Weidman, J. F.; Phillips, C. A.; Spriggs, T.; Hedblom, E.; Cotton, M. D.; Utterback, T. R.; Hanna, M. C.; Nguyen, D. T.; Saudek, D. M.; Brandon, R. C.; Fine, L. D.; Fritchman, J. L.; Fuhrmann, J. L.; Geoghagen, N. S. M.; Gnehm, C. L.; McDonald, L. A.; Small, K. V.; Fraser, C. M.; Smith, H. O.; Venter, J. C. *Science* **1995**, *269*, 496); PrpA Anabaena, a potential PPP from *Anabaena* PCC 7120 (Zhang, C.-C.; Friry, A.; Peng, L. *J. Bacteriol.* **1998**, *180*, 2616); PP lambda, a PPP from bacteriophage lambda;[88] PrpA E. coli, a PPP from *E. coli*;[83] PrpB E. coli, a PPP from *E. coli*;[83] PP1-cyano1, a PPP from *Microcystis aeruginosa*;[85] PP1-cyano2, a PPP from *M. aeruginosa* UTEX 2063 (Berndt, N.; Campbell, D. G.; Caudwell, B.; Cohen, P.; da Cruz e Silva, E. F.; da Cruz e Silva, O. B.; Cohen, P. T. W. *FEBS Lett.* **1987**, *223*, 340);[85] PP1-cyano3 (aka sll1387), a potential PPP from *Synechocystis* PCC 6803;[259,260] PP1 Rabbit, a PPP from rabbit (Shi, L.; Carmichael, W. W. *Arch. Microbiol.* **1997**, *168*, 528); PP1 sds, a PPP from *Schizosaccharomyces pombe* (Okhura, H.; Kinoshita, N.; Miyatani, S.; Toda, T.; Yanagida, M. *Cell* **1989**, *57*, 997); PPQ Yeast, a PPP from *Saccharomyces cerevisiae* (Chen, M. X.; Chen, Y. H.; Cohen, P. T. W. *Eur. J. Biochem.* **1993**, *218*, 689); PP2A Rabbit, a PPP from rabbit (Guerini, D.; Klee, C. B. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9183); PP2B beta 1, a PPP from human (da Cruz e Silva, O. B.; Alemany, S.; Campbell, D. G.; Cohen, P. T. W. *FEBS Lett.* **1987**, *221*, 415); PPT Yeast, a PPP from *S. cerevisiae* (Chen, M. X.; McPartlin, A. E.; Brown, L.; Chen, Y. H.; Barker, H. Z.; Cohen, P. T. W. *EMBO J.* **1994**, *13*, 4278); PP1-arch1, a PPP from *Sulfolobus solfataricus*;[79] PPP P abyssi, Py-PP1 from *Pyrodictium abyssi*;[82] PP1-arch2, a PPP from *Methanosarcina thermophila* TM-1.[81] (Reprinted with permission from ref 85. Copyright 1999 American Society for Biochemistry and Molecular Biology).

However, the various N-terminal and C-terminal extensions beyond the catalytic core domain have been observed to include membrane-targeting motifs,[104,107] protein kinase interaction sequences,[104,109] and potential EF hands.[110,111] Recently, a new member of the PP2C family was cloned from *Plasmodium falciparum* that contains two tandem catalytic domains, both of which were active when expressed individually via recombinant methods.[112] In cells, PP2C often acts to terminate or attenuate protein phosphorylation events triggered in response to environmental stresses.[113] Targets of eukaryotic PP2C include progression through cell cycle[114,115] and the heat and osmotic shock signaling cascades in yeast.[105,116]

2. Bacterial PPMs

The PPMs represent the most prolific and best studied of the protein−serine/threonine phosphatases present in bacterial organisms. To date, seven bacterial PPMs have been identified and characterized (Table 3). Five of these were from *Bacillus subtilis*: SpoIIE,[117] RsbP,[118] RsbU and RsbX,[119] and PrpC.[120] The others include Slr1860/IcfG from the cyanobacterium *Synechocystis* PCC6803[121] and Stp1 from *Pseudomonas aeruginosa*.[122] While RsbX, PrpC, and Stp1 rank among the smallest PPMs yet encountered, at 91 kDa SpoIIE ranks as one of the most massive. Its large size is attributable to the presence of a large N-terminal membrane anchor domain containing 10 predicted membrane-spanning segments. The N-terminal portion of RsbP contains a predicted PAS domain.[118] PAS domains, which bind the chromophore 4-hydroxycinnamyl, sense energy-related environmental cues such as oxygen, redox potential, or light.[123] While Slr1860/IcfG from *Synechocystis* PCC6803 also contained considerable 'extra' sequence, its functional role(s) has yet to be determined.[124]

Table 3. General Characteristics of Bacterial PPM-Family Protein Phosphatase

name	organism	size (kDa)	activating metals
SpoIIE[117]	*B. subtilis*	92	Mn, Mg
RsbP[118]	*B. subtilis*	44	Mn
RsbU[119]	*B. subtilis*	39	Mg
RsbX[119]	*B. subtilis*	22	Mg
PrpC[120]	*B. subtilis*	28	Mn
Slr1860/IcfG[121]	*Synechocystis* PCC6803	70	Mn, Mg
Stp1[122]	*P. aeruginosa*	27	Mn

Studies of the enzymatic properties of bacterial PPMs have been limited to the characterization of recombinant forms expressed in *E. coli*. Each bacterial PPM exhibited the divalent metal-ion-dependent protein−serine/threonine phosphatase activity that represents the functional hallmark of their eukaryotic counterparts. Most bacterial PPMs, i.e., SpoIIE,[117] RsbU,[119] RsbX,[119] RsbP,[118] and Slr1860/IcfG,[121] displayed an extraordinarily high degree of substrate specificity in vitro, suggesting that each targets a single protein substrate or some very highly circumscribed set of proteins inside the cell. Even when challenged with a phosphoprotein homologous to that of an efficacious substrate protein, little or no dephosphorylation of the homologue was observed. Perhaps the most striking example of this behavior was reported for SpoIIE. When mutagenic alterations were used to replace the phosphoserine residue normally targeted by the enzyme on its native substrate, SpoIIAA, with phosphothreonine, SpoIIE proved incapable of dephosphorylating the altered protein despite the subtle nature of the change.[117] In the case of Slr1860/IcfG, selectivity proved to be strikingly reciprocal. Not only did Slr1860/IcfG dephosphorylate only one of two highly similar phosphoproteins endogenous to *Synechocystis* PCC6803, i.e., Slr1856 over Slr1859, but both Slr1856 and Slr1859 proved completely resistant to the hydrolytic activities of several prokaryotic PPPs including PP1-arch1, PP1-arch2, and PP1-cyano1.[121] Thus far, the sole exception to this pattern of strict selectivity was Stp1 from *P. aeruginosa*, which displayed significant phosphocasein phosphatase activity in vitro.[122]

Four of the five PPMs in *B. subtilis* modulate specialized programs of gene expression in response to environmental stresses. In contrast to their eukaryotic counterparts, which tend to terminate or attenuate stress responses, these bacterial PPMs supported their initiation and/or propagation. SpoIIE, for example, plays a pivotal role in sporulation. The enzyme is recruited via its membrane-spanning domain to the nascent prespore,[125,126] where it dephosphorylates SpoIIAA.[117] In its phosphorylated state, SpoIIAA binds to and sequesters sigma^F.[127] Once released, sigma^F binds to DNA-dependent RNA polymerase and initiates a program of compartment-specific gene expression that triggers the maturation of the spore. RsbP, RsbU, and RsbX, on the other hand, participate together in a complex pathway that ultimately targets sigma^B. This so-called partner switch cascade possesses two branches, one containing RsbX and RsbU and the other containing RsbP, that converge upon the anti-anti-sigma factor or anti-sigma factor antagonist, RsbV.[118,119] The first branch is activated by general environmental stresses, while the second branch monitors indicators of energy stress via the PAS domain of RsbP. Dephosphorylation of RsbV renders it available to complex with the anti-sigma factor RsbW, thereby releasing sigma^B, which triggers the expression of a broad spectrum of general stress response genes. The odd man out in this *B. subtilis* stress ballet is PrpC, whose expression remains constant during exponential growth and early stationary phase, unaffected by known inducers of major stress response pathways.[120] Intriguingly, the gene for PrpC was directly adjacent to that for a eukaryotic protein kinase, PrkC. PrkC undergoes autophosphorylation in vitro, and phospho-PrkC was dephosphorylated by PrpC in vitro.[120]

The expression of mRNA encoding Slr1860/IcfG required glucose, and genetic evidence implicated this cyanobacterial PPM as a coordinator of single carbon and glucose metabolism in *Synechocystis* PCC6803.[124] Deletion of the gene for this PPM produced cells that could grow on either carbonate or glucose as the sole carbon source but which were unable to grow on low levels of carbonate if either (a) glucose was present or (b) the cells had been preconditioned by growth on glucose in the presence of high carbonate. In vitro, Slr1860/IcfG dephosphorylated Slr1856 with a high degree of specificity.[121] Since Slr1856 displays a high degree of sequence similarity to SpoIIAA, RsbS, and RsbV from *B. subtilis*, it appears likely that the former may modulate the availability of a sigma factor responsible for triggering a nutrient-specific program of gene expression that remodels metabolism for heterotrophic growth.

Adler et al.[128] noted a recurring theme among the PPMs from *B. subtilis* and the mammalian PPM pyruvate dehydrogenase phosphatase, their functional opposition to protein kinases that represent 'atypical' serine/threonine-specific variants of two-component histidine kinase paradigm. Examples of the close physical juxtaposition of the genes encoding such antagonistic protein kinase/protein phosphatase pairs can be found in *Synechocystis* PCC6803[121] as well as *B. subtilis*.[119] These investigators therefore speculated that the phosphorylation−dephosphorylation systems that control bacterial sporulation and the activity of mammalian pyruvate dehydrogenase represent examples of an evolutionarily conserved regulatory module for overseeing stress responses. While this may ultimately prove to be the case, it should be noted that not all genes for bacterial PPMs are closely paired with those for histidine kinase-like protein−serine/threonine kinases. In fact, those for Stp1[122] and PrpC[120] were located adjacent to those for conventional eukaryotic-type protein kinases.

3. PPM Homologues

When Tamura et al.[99] successfully cloned the first PPM, a PP2C from rat, they noted that its deduced amino acid sequence bore a faint but discernible resemblance to that of an adenylate cyclase from yeast. As cDNA and genomic sequences for additional PPMs accumulated, it became apparent that the features conserved among the PPMs overlapped with the set conserved between PPMs and adenylate cyclases,[97,98] suggesting parallels in catalytic mechanism and a common ancestry. Intriguingly, just as the current archaeal genome record was devoid of ORFs encoding potential PPMs, ORFs potentially encoding conventional adenylate cyclases have proved absent as well. While the *Archaea* do harbor adenylate cyclase activity, the only archaeal adenylate cyclases encountered to date belong to a second and completely distinct group of enzymes, the Cya2 family, which were first discovered and characterized in the bacterium *Aeromonas hydrophila*.[129]

D. Conventional PTP Family

1. Conventional PTPs in Eukaryotes

In terms of the sheer number and variety of catalytic polypeptides, the conventional PTPs (Table 1) represent by far the most prolific family of protein phosphatases in the *Eucarya*.[130] Indeed, the number of tyrosine-specific protein phosphatases approaches or may perhaps even exceed that of the countervailing tyrosine-specific protein kinases, implying a much higher overall degree of substrate specificity than would appear to be the case for the more 'promiscuous' catalytic units of the protein−serine/threonine phosphatases (reviewed in refs 22 and 26). The signature sequence motif for the conventional PTPs consists of a catalytic aspartic acid residue located 25−45 residues to the N-terminal side of the active site loop sequence His-Cys-Xaa$_5$-Arg-Ser/Thr, which is located near the center of the ≈230 amino acid catalytic domain.[22,23,51,130] In the *Eucarya*, the catalytic domain unit is encountered in a variety of contexts, including the cytoplasmic domains of a wide variety of transmembrane receptors as well as soluble forms that oftentimes include macromolecular docking motifs such as SH2 domains that assist in targeting to specific subcellular locales, substrate binding, etc. (reviewed in refs 130 and 131).

The conventional PTPs employ a two-step catalytic mechanism that is conserved among all three of the PTP families that have converged upon the Cys-Xaa$_5$-Arg catalytic motif (reviewed in refs 22 and 26). In this mechanism, the active site cysteine carries out a nucleophilic attack on the protein-bound phosphoester, leading to formation of a phosphocysteinyl enzyme intermediate. In the conventional PTPs, the conserved histidine abstracts the thiol proton to increase the nucleophilicity of the adjacent cysteine. The arginine residue participates in binding the phosphoryl group in the active site. The conserved aspartic acid residue serves as a proton donor to the alkoxide leaving group. After the dephosphorylated protein dissociates, water enters the active site and hydrolyzes the phosphocysteinyl intermediate to regenerate the free enzyme. Hydrolysis is accomplished with the assistance of the now deprotonated aspartic acid residue, which abstracts a proton to enhance the nucleophilicity of the attacking water molecule.

While the majority of conventional PTPs display a high degree of selectivity, $\geq 10^4$:1, for protein-bound tyrosine over serine or threonine residues,[132] a number of these enzymes will efficiently hydrolyze both. These have been dubbed dual-specific(ity) phosphatases (DSPs). The underlying determinant for the respective phosphoamino acid specificities of DSPs versus strict PTPs would appear to be the relative depth of their active site pockets, with deep pockets restricting hydrolytic action to the long side chain of tyrosine (reviewed in refs 51 and 133).

2. Conventional PTPs in Bacteria

Thus far, four members of the conventional PTP family have been characterized in detail from the *Bacteria*: YopH from pathogenic strains of *Yersinia*,[134] SptP from *Salmonella typhimurium*,[135] MPtpB from *Mycobacterium tuberculosis*,[136] and IphP from *Nostoc commune* UTEX584[137] (Table 4). While few in number, these enzymes embody an intriguing set of properties.

Table 4. General Characteristics of Bacterial Protein-Tyrosine Phosphatases

name	family	organism	size (kDa)	amino acid specificity S/T	amino acid specificity Y
YopH[134]	conventional	*Yersinia*	50	−	+
SptP[135]	conventional	*S. typhimurium*	60	n.d.	+
MPtpB[136]	conventional	*M. tuberculosis*	30	−	+
IphP[137]	conventional	*N. commune* UTEX584	30	+	+
PtpA[175]	LMW	*S. coelicolor* A3(2)	18	n.d.	+
Ptp[176]	LMW	*A. johnsonii*	16	-	+
Wzb[177]	LMW	*E. coli*	16	n.d.	+
MPtpA[136]	LMW	*M. tuberculosis*	18	−	+

YopH constitutes an essential virulence determinant for various pathogenic strains of *Yersinia*, including that which was responsible for the Black Death in medieval Europe.[134] This PTP functions as a molecular missile that is secreted from the bacterium to assault its eukaryotic host. Once inside, it attacks tyrosine-phosphorylated proteins localized to focal adhesions, among them p130^{Cas} and paxillin, thereby disabling host defenses.[138−140] YopH is tyrosine-specific.[134] Deletion of the gene that encodes it did not detectably perturb growth of *Yersinia* in culture, suggesting that this PTP does not act on endogenous bacterial proteins.[141] The catalytic domain occupies the C-terminal half of a 50 kDa polypeptide whose N-terminal region contains the elements required for secretion from the bacterium and translocation into mammalian cells[142] as well as a novel domain that contributes to high-affinity binding of substrates.[139] YopH is encoded on a virulence plasmid that also contains the gene for a eukaryotic protein kinase.[143] It therefore appears to have been acquired from a eukaryotic host via a horizontal gene transfer event.[134]

Like YopH, Stp from the pathogen *S. typhimurium* targets proteins within a eukaryotic host cell, where it disrupts the actin cytoskeleton.[144] Purified StpP dephosphorylated a pair of phosphotyrosine-containing peptides in vitro, while mutagenic alteration of the presumed catalytic cysteine ablated PTP activity as predicted.[135] Its deduced sequence resembles a fusion product of a YopH-like PTP with the YopE cytotoxin of *Yersinia*.[135] A DNA fragment potentially encoding a similar protein has been cloned and sequenced for *S. typhi*;[145] however, no information regarding its potential catalytic properties has been forthcoming. In contrast to YopH, which was encoded on a potentially mobile plasmid, the gene for Stp resides within the chromosomal DNA of *S. typhimurium*.[135]

MPtpB may represent yet another example of a secreted bacterial smart bomb, as it was observed to be present in both whole cell lysates and culture filtrates of *M. tuberculosis* along with its companion LMW PTP, MPtpA.[136] MPtpB exhibited tyrosine-specific protein phosphatase activity. Mutagenic al-

teration of the conserved cysteine residue eliminated catalytic function.

Unlike YopH, Stp, and MPtpB, IphP is associated with a free-living cyanobacterium, *N. commune*, rather than a pathogen.[137] IphP also displays dual-specific rather than tyrosine-specific protein phosphatase activity in vitro, another point of difference with its pathogenic counterparts.[132,146] In common with other characterized PTPs of bacterial origin, IphP also appears to be a secreted protein that presumably resides within an extracytoplasmic region such as the periplasm.[137]

3. Virally Encoded Conventional PTPs

Some viruses have been demonstrated to contain conventional PTPs, prominent among them vaccinia virus,[147] myxoma virus,[148] and shope virus,[148] while others contain ORFs potentially encoding such enzymes. The latter include several orthopoxviruses[149] and chlorella virus.[150] All of the viral enzymes examined to date behave as DSPs in vitro and bear a noticeable family resemblance to VH1 from vaccinia, a DSP which is a homologue of VHR from mammals.[151] They thus appear to be descendants of a single virally acquired PTP. Artificially imposed repression of VH1 led to a drop in viral infectivity that correlated with a defect in the ability to direct transcription of vaccinia early genes.[152]

4. Homologues of the Conventional PTPs

Recently, several eukaryotic and viral proteins have been discovered that contain the requisite catalytic signature elements of conventional PTPs but which direct their hydrolytic capabilities toward nonprotein substrates. The first of these was the CEL-1 triphosphatase from *Caenorhabditis elegans*.[153] CEL-1 was first identified on the basis of its homology to the C-terminal domain of the guanylyltransferases that work in conjunction with RNA 5′-triphosphatases and methyltransferases to carry out mRNA capping. It was subsequently determined that RNA 5′-triphosphatase activity resided within its N-terminal domain, which contained the signature sequence elements characteristic of a conventional PTP. Mutational alteration of the presumed active site cysteine eliminated all RNA hydrolytic activity. Intriguingly, CEL-1 exhibited no signs of vestigial PTPase activity, even when challenged with the general phosphomonoesterase substrate *p*-nitrophenyl phosphate.[153]

The closest known homologue of the N-terminal catalytic domain of CEL-1 was BVP from the baculovirus *Autographica californica*, which originally had been classified as a DSP.[154] However, its protein phosphatase activity was quite modest relative to other well-characterized PTPs, and subsequent investigation revealed that BVP was in fact an RNA 5′-di- and triphosphatase.[155] Deletion of BVP produces a viable but functionally defective baculovirus whose production by host cells was both cell line and cell cycle sensitive.[156] Recently, a human protein, PIR1, has been added to this growing family of PTP-like RNA 5′-triphosphatases.[157]

The second offshoot of the conventional PTP superfamily is comprised of a set of phospholipid phosphatases. The patriarch of this family is the tumor suppressor PTEN. First identified as a very inefficient DSP,[158] subsequent work revealed that its physiological substrate was in fact phosphatidylinositol 3,4,5-trisphosphate, which it specifically hydrolyzes at the 3 position on the inositol ring.[159] The hydrolytic activity of PTEN required the presence of the conserved 'PTP' cysteine residue and proved critical for tumor suppressor activity.[160] X-ray crystallography revealed that the catalytic domain of the enzyme generally resembled that of a conventional PTP in which the active site was enlarged to accommodate the lipid substrate.[161] More recently, a second lipid phosphatase that acts on the phosphoryl group of phosphatidylinositol 3-phosphate, myotubularin, has been identified and characterized.[162] Mutations in the gene for myotubularin that are associated with human myotubular myopathy have been demonstrated to perturb the hydrolytic efficiency of this phosphatase.

E. Low Molecular Weight PTPs

1. LMW PTPs in Eukaryotes

The LMW PTPs (Table 1) were first characterized as a set of small, ≈18 kDa, atypical acid phosphatases that were active against free phosphotyrosine and tyrosine-phosphorylated macromolecules in vitro (reviewed in ref 25). The conserved sequence signature of the LMW PTPs is a Cys-Xaa$_5$-Arg-Ser/Thr catalytic loop located near, usually within 5−10 residues, the amino terminus of an ≈150 residue catalytic domain. In contrast to the conventional PTPs, the active site cysteine is generally preceded by an aliphatic hydrophobic residue such as valine or isoleucine rather than histidine. Approximately 85−105 residues to the C-terminal side of this sequence is a catalytically important aspartic acid residue that is usually located immediately N-terminal to a proline residue.[23,25] Although the LMW PTPs shared no significant sequence homology with other PTPs beyond the Cys-Xaa$_5$-Arg motif, the roles of the conserved Cys, Arg, and Asp residues were comparable to those first defined through the study of conventional PTPs.[51,163−165] X-ray crystallography[166,167] and NMR spectroscopy[168] revealed that, while disparate in primary sequence, both the active site configuration and the inventory and order of secondary structural elements in the conventional and LMW PTPs were remarkably similar. When considered at the level of gross secondary structure, it could be imagined that each type of PTP had been generated by differential processing of a common, circular precursor polypeptide.[23,168] Thus, the conventional and LMW PTPs appear to have converged from wholly separate origins upon a common active site configuration and catalytic mechanism.[25] No dual-specific variants of the LMW PTP family have been reported.

LMW PTPs appear to be ubiquitous in the *Eucarya*, with examples having been characterized from mammals,[163] plants,[169] and yeast.[170,171] However, the LMW

PTPs constitute only a minor proportion of the total PTP population in higher eukaryotes. Their small size seems barely sufficient to support catalytic activity, offering few clues to their physiological function of the type provided by the PEST, SH2, and transmembrane domains so often found in conventional PTPs. In yeast, neither the overexpression nor the elimination of a gene encoding a LMW PTP produced a detectable phenotype.[170,171] However, evidence recently has been reported from mammalian tissue culture cells indicative of both the dynamic regulation of LMW PTPs and the potential physiological consequences of such regulatory modulation. In NIH-3T3 cells, a LMW PTP was observed to be partitioned between constitutive cytoplasmic and cytoskeletal pools, each of which targeted different substrates and responded differently when cells were stimulated by PDGF.[172] The cytoplasmic pool targeted the autophosphorylated PDGF receptor,[173] while the cytoskeletal pool was phosphorylated by Src-family protein kinases in response to PDGF, an event which stimulated their catalytic activity 20-fold.[174] When the sites phosphorylated by Src were eliminated by site-directed mutagenesis, the influence of PDGF on cell adhesion and migration was markedly attenuated.[174]

2. LMW PTPs in Bacteria

To date, four LMW PTPs have been characterized from bacterial organisms: PtpA from *Streptomyces coelicolor* A3(2),[175] Ptp from *Acinetobacter johnsonii*,[176] Wzb from *E. coli*,[177] and MPtpA from *M. tuberculosis*[136] (Table 4). Each of them was small, 15−18 kDa, and hydrolyzed typical, albeit nonphysiological, PTP substrates such as *p*-nitrophenyl phosphate and free phosphotyrosine in vitro. In addition to these small organophosphates, both MPtpA and PtpA have been demonstrated to dephosphorylate phosphotyrosine-containing protein or peptide substrates, respectively, while both Ptp and Wzb have been shown to dephosphorylate the autophosphorylated forms of their presumed cognate bacterial protein−tyrosine kinases, Ptk and Wzc. None of the PTPs hydrolyzed free phosphoserine or free phosphothreonine. It should be noted, however, that this behavior represents a dubious indicator of absolute fidelity for protein-bound phosphotyrosine, as bona fide DSPs typically exhibit little if any activity toward free phosphoserine or phosphothreonine.[132] Only MPtpA has been challenged with a phosphoserine-containing protein substrate, which it proved unable to dephosphorylate.[136]

Mutagenic alteration of the cysteine residue within the Cys-Xaa$_5$-Arg sequences of Ptp or MPtpA, or of the conserved arginine in Ptp, produced proteins possessing negligible catalytic activity, suggesting that bacterial LMW PTPs employ the same catalytic mechanism used by their eukaryotic counterparts.[136,176] Overexpression of the gene for PtpA in *S. coelicolor* A3(2) led to overproduction of secondary metabolites such as undecylprodigiosin and A-factor.[178] MPtpA appears to be a secreted protein, as it could be detected in both whole cell lysates and culture filtrates of *M. tuberculosis*.[136] These investigators therefore speculated that, by analogy to the conventional PTPs YopH and Stp (see above), MPtpA and its companion conventional PTP, MPtpB, represent virulence determinants for this bacterial pathogen.

3. Homologues of the LMW PTPs

Most well-characterized arsenate reductases from bacteria contain the [Cys-Xaa$_5$-Arg]-Xaa$_{85-105}$-[Asp-Pro] sequence characteristic of the LMW PTPs.[179−181] The sole exception to date was found in *E. coli*, where the spacing between the cysteine and arginine residues was reduced from five to three amino acids.[182] At first glance, the reaction catalyzed by these enzymes, the reduction of arsenate to arsenite, would appear to be fundamentally different from the hydrolysis of phosphoesters. However, arsenic lies directly under phosphorus in the periodic table and the catalytic mechanism of bacterial arsenate reductases involves formation of an arsenyl−cysteine enzyme intermediate analogous to the phosphocysteinyl intermediate of the LMW PTPs.[183] It also may be noteworthy that, for some PTPs including at least one LMW PTP,[184] oxidation−reduction of the active site cysteine reportedly can be modulated in vitro. Since oxidation of the cysteine renders the enzyme catalytically inactive, this may provide a mechanism for regulating PTP activity inside cells.

F. Cdc25 Family

1. Cdc25 in Eukaryotes

The Cdc25 family (Table 1) encompasses a set of dual-specific protein phosphatases that participate in the regulation of the cell division cycle in the *Eucarya*. The enzyme is rigorously specific. Each isoform of Cdc25 targets a set of adjacent threonine and tyrosine residues on a particular cyclin-dependent protein kinase but only when the latter is bound to its appropriate cyclin (reviewed in ref 30). The active site consensus sequence of Cdc25 closely resembles that of the conventional PTPs, Asp-Xaa$_{\approx 46}$-Cys-Xaa$_5$-Arg (reviewed in refs 23 and 51), and the enzyme employs a virtually identical catalytic mechanism involving the formation of a phosphocysteinyl enzyme intermediate.[185] However, X-ray crystallography revealed that the Cdc25 was topologically distinct from both the conventional and LMW PTPs and, in fact, most closely resembled the sulfur-metabolizing enzyme rhodanese in its three-dimensional structure.[186] Evidence to date indicates that the Cdc25 family of protein phosphatases are found solely in eukaryotes, as examination of the genomes from numerous bacterial and archaeal organisms has yet to reveal the presence of any obvious prokaryotic homologues. However, given the limitations of differentiating specific PTP types based upon primary sequence considerations alone and the presence of the Cdc25 homologue (and potential precursor?) rhodanese in the *Bacteria*, present indications of exclusivity to eukaryotes should be considered preliminary in nature.

2. Cdc25 Homologues

In an intriguing parallel to the LMW PTPs (see above), the extended Cdc25 family also encompasses

a homologous arsenate reductase—ACR2 from yeast.[187,188] Thus, on at least two occasions involving two distinct families of PTPs, nature has adapted the phosphocysteinyl chemistry used for the hydrolysis of phosphoester bonds to create arsenocysteinyl intermediates for the purpose of chemical reduction. Each of these transformations apparently took place after the divergence of the eukaryotic and bacterial phylogenetic domains from one another, as the known arsenate reductases of bacterial origin resemble LMW PTPs, while the sole eukaryotic example resembles Cdc25.

Comparison of the three-dimensional structure of rhodanese with Cdc25 revealed striking similarities in topology, including the active site loops containing their catalytic cysteine residues, indicating that these two functionally disparate enzymes share a common evolutionary ancestor.[186,189] In vitro, rhodanese catalyzes the transfer of sulfur from compounds such as thiosulfate to acceptors such as cyanide using a mechanism that involves formation of an S−S bond to the conserved cysteine residue (reviewed in ref 190). Its physiological role remains cryptic, although some investigators have suggested that the enzyme may play a role in cyanide detoxification.[191] Rhodanese differs from Cdc25 at the gross level inasmuch as the former contains duplicate catalytic domains of ≈15 kDa each, while Cdc25 contains only a single catalytic domain within its polypeptide sequence. The existence of a 12 kDa rhodanese containing only a single catalytic domain recently was reported in *E. coli*; however, this enzyme existed as a homodimer in solution.[192] Intriguingly, while Cdc25 thus far only has been detected in eukaryotes, rhodanese has been found in both the *Bacteria* and *Eucarya*. In the latter, its activity is normally found associated with mitochondria, although it is nuclearly encoded. Rhodanese activity has recently been detected in the archaeon *Acidianis ambivalens*.[193] However, no sequence information for an archaeal rhodanese has been reported, leaving open the question of whether the protein embodying this catalytic activity is a homologue of previously characterized rhodaneses from the other phylogenetic domains.

IV. Bifunctional Kinase/Phosphatases: The Fossil Remains of an Evolutionary Dead End?

The first 'endogenous' bacterial phosphoprotein, i.e., one that was phosphorylated by a protein kinase indigenous to the bacterium rather than one introduced via viral infection, was the enzyme isocitrate dehydrogenase (IDH).[5] Through a combination of biochemical[194] and genetic[195] means it became apparent that a single polypeptide was the source of both the protein kinase and the protein phosphatase activities responsible for modulating its state of phosphorylation. The two catalytic activities contained within this polypeptide were regulated in a complex manner by a spectrum of allosteric regulators that included 5′-AMP, pyruvate, 3-phosphoglycerate, isocitrate, and NADPH.[196]

While a wide array of bifunctional enzymes exist in nature, the isocitrate dehydrogenase kinase/phosphatase (IDH kinase/phosphatase) proved unusual inasmuch as both catalytic activities emanate from the same active site.[197,198] This has been achieved through an unusual mechanism in which the net hydrolysis of a protein-bound phosphoester is accomplished in two steps, a 'reverse' protein kinase reaction in which the phosphoryl group undergoes 'retrotransfer' to ADP to form ATP, followed by the subsequent hydrolysis of ATP to ADP and P_i.[198] The coupling of ATP hydrolysis produces an overall process whose net stoichiometry and thermodynamics are identical to those of the conventional protein phosphatases that catalyze the direct hydrolysis of phosphoesters by water (although ATP is required as a cofactor[194]). Modulation of the relative levels of protein kinase and protein phosphatase activity has been achieved through allosteric effector-induced alterations in active site conformation that favor one or the other activity.[198,199] Simplistically, this is accomplished by enabling or disabling the ATPase activity that provides the thermodynamic driving force for phosphoester hydrolysis.

Recently, it has been reported in *B. subtilis*[200] and *Enterococcus faecalis*[201] that the protein−serine kinase that phosphorylated the histidine-containing protein (HPr) of the bacterial phosphotransfer system also possessed endogenous protein phosphatase activity. Phosphorylation of HPr on the affected serine inhibits the transport of sugar during catabolite repression. Although the HPr kinase/phosphatase shares the same unusual bifunctional capabilities of the IDH kinase/phosphatase, it is a novel protein unrelated in sequence to either previously characterized protein kinases or protein phosphatases.[200,202] It has yet to be determined whether protein phosphorylation and dephosphorylation take place at separate or overlapping active sites in the HPr kinase/phosphatase. Hydrolytic activity did not require the presence of adenine nucleotides,[201] indicating that dephosphorylation proceeds by a mechanism distinct from that of the IDH kinase/phosphatase.

As was the case for the IDH kinase/phosphatase, the relative levels of protein kinase and protein phosphatase activity in the HPr kinase/phosphatase are regulated by the allosteric effects of cellular metabolites. Fructose-1,6-bisphosphate stimulates the protein kinase activity of the HPr kinase/phosphatase, while P_i concomitantly inhibits its protein kinase activity and stimulates its protein phosphatase activity.[200,202,203] In *E. faecalis*, an additional layer of regulation is provided by ATP.[201] At high levels of ATP, i.e., 2 mM, protein kinase activity is favored while protein phosphatase activity predominates at low levels of ATP, i.e., ≤0.2 mM. Thus, when the levels of glycolytic intermediates and ATP fall, with an accompanying rise in P_i, the HPr kinase/phosphatase will tend to act as a protein phosphatase, stimulating sugar uptake by dephosphorylating HPr.

An autonomous phosphatase capable of dephosphorylating HPr independent of its bifunctional kinase/phosphatase also has been identified.[200] The DNA-derived amino acid sequence of the former, HprP, indicates that it represents a potentially unique protein phosphatase resident within a diverse

phosphohydrolase family. This family includes glycerol-3-phosphatase and 2-deoxyglucose-6-phosphate phosphatase from yeast and the phosphoglycolate phosphatases from bacteria.[200] The physiological role of HprP remains to be determined. Side-by-side comparisons against the HPr kinase/phosphatase revealed HprP to be a very inefficient HPr phosphatase in vitro, implying that HPr does not constitute its physiological substrate.[201]

The bifunctional IDH kinase/phosphatase appears to be a highly specialized, 'one of a kind' enzyme with few imitators. Not only has it thus far proved restricted to enteric bacteria such as *E. coli* and *Salmonella enterica*,[204] but in the 20 years since its discovery, no evidence has emerged for either additional family members or alternative substrates. Although it is premature to draw equally firm conclusions concerning the HPr kinase/phosphatase, thus far it too has proven to be restricted to a few members of the *Bacteria* and to the regulation of a single protein substrate, HPr. Thus, the IDH kinase/phosphatase and the HPr kinase/phosphatase apparently represent the remnants of evolutionary dead ends in the development of enzymatic protein phosphorylation−dephosphorylation.

One might argue that the difficulty in developing a practical bifunctional enzyme accounts for the greater proliferation of dedicated protein kinases and protein phosphatases over bifunctional protein kinases/phosphatases. However, a much more important factor may reside in the restrictive nature of the bifunctional mechanism. Inherent in the rigid physical linkage of the sources of protein kinase and protein phosphatase activity is the almost inevitable obligation that they directly oppose one another, depriving them of the opportunity to target additional proteins independent of their partner. Dedicated, specialized protein kinases and protein phosphatases, on the other hand, generally exhibit overlapping but distinct selectivity for protein targets. The resulting flexibility offers far greater scope for creating interlinked networks of protein phosphorylation processes capable of integrating a broad spectrum of environmental signals (reviewed in refs 1, 2, and 205−208). While the IDH kinase/phosphatase (reviewed in ref 196) and, to a lesser degree, the HPr kinase/phosphatase[201] have overcome this basic limitation by virtue of their ability to respond to a multiplicity of allosteric effectors, these represent remarkable feats of protein engineering that seemingly would prove difficult to replicate on a mass scale.

V. Protein–Histidine and Protein–Aspartate Phosphatases

A. Protein–Histidine Phosphatases

The modification of proteins by phosphorylation of the so-called N-amino acids, histidine, lysine, and arginine, was first reported in the 1960s.[209,210] Early studies indicated that histones and other chromatin proteins become N-phosphorylated in eukaryotes, with the phosphorylation of one or more histidine residues on histone H4 serving as the major focus of these investigations (reviewed in ref 211). In *Bacteria* the most abundant source of protein-bound phosphohistidine are the histidine kinases and histidine phosphotransfer proteins (HPt) of the two-component regulatory paradigm, which also is operative in some members of the *Archaea* and *Eucarya* (reviewed in refs 212−216).

The modules from which the two-component system derives its name consist of a histidine kinase, whose activity is generally regulated by an associated receptor protein or covalently linked receptor domain, and the response regulator domain that serves as its cognate substrate. The term histidine kinase is derived from the catalytic mechanism of these enzymes, the first step of which is to phosphorylate themselves on a conserved histidine residue using ATP as phosphodonor substrate. In the most basic form of the two-component signaling cascade, the phosphoryl group is then transferred to a conserved aspartate residue located on the response regulator. Phosphorylation of the latter modulates the activity of an associated 'output' domain, oftentimes a transcriptional activating factor, to effect a physiological response. Numerous variations on this basic theme exist in which histidine kinase domains and response regulator domains are fused to form composite proteins or in which the phosphate group attached to a response regulator domain is ultimately transferred to a second response regulator protein using a third component module, a histidine-phosphorylated HPt domain, as a shuttle to create a His-Asp-His-Asp phosphorelay. Despite the modular construction of these cascades, individual histidine kinases exhibit high selectivity for particular response regulators, thus ensuring the fidelity of signal transmission.

While sporadic reports have appeared concerning the detection of 'novel' histidine and lysine phosphatase activities in eukaryotic cells,[217−220] the identities and precise characteristics of the enzymes that were the source(s) of these activities have proven elusive. Several protein−serine/threonine phosphatases have been demonstrated to dephosphorylate histidine and even lysine in vivo, such as mammalian PP1, PP2A, and PP2C,[221] PP1-cyano1 and PP1 cyano2 from *M. aeruginosa*,[85] and PP-lambda.[91] However, clear and compelling evidence establishing the physiological relevance, if any, of this activity has not been forthcoming.

Recently, the first sequence of a specialized protein−histidine phosphatase from *E. coli*, SixA[222] (Table 1), was reported. This protein−histidine phosphatase dephosphorylated a histidine residue within the ArcB protein in vitro. ArcB contains four distinct functional domains: a receptor domain, a histidine kinase domain, a response regulator domain, and an HPt domain. In ArcB, receptor-mediated phosphorylation of the conserved histidine residue within the histidine kinase domain is followed by transfer to the conserved aspartate within the response regulator domain. This aspartate, in turn, transfers its phosphoryl group to a histidine residue contained within the HPt domain of ArcB. Ultimately, the phosphoryl group is transferred from the histidine of the HPt domain to an aspartic acid residue within the response regulator domain of a second protein, the

ArcA transcriptional activator.[223] Only the second histidine in this His-Asp-His-Asp phosphorelay was targeted by SixA in vitro. The Arc two-component signaling cascade oversees the adaptation of *E. coli* to growth under anaerobic conditions. Mutants of *E. coli* that were deficient in SixA[224] or that grossly overexpressed the enzyme[222] displayed impaired responsiveness to activators of the Arc signaling pathway, behavior consistent with SixA's predicted role as an ArcB-directed protein−histidine phosphatase in vivo.

B. Protein-Aspartate Phosphatases

1. Autodephosphorylation of Response Regulator Proteins

The first reports of protein−aspartate phosphatase activity appeared shortly after the discovery of the two-component signaling paradigm.[225,226] Measurements of the lifetime of the phosphoaspartate moiety on the isolated nitrogen response regulator protein, NRI, indicated that it was so short relative to that observed for the denatured phosphoprotein, $t_{1/2} \approx 3.5$ min versus ≈5.5 h, that some form of catalytic enhancement must be taking place.[225] Similarly short half-times were observed for the chemotaxis regulator proteins CheB and CheY[227] and PhoB,[228] the response regulator protein of the sensor cascade that controls phosphate assimilation. Aspartate dephosphorylation required the presence of divalent metals such as Mg^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+}, or Co^{2+}, which bind directly to the response regulator.[229] In CheY, the substitution of Lys-109 with Arg via site-directed mutagenesis produced a 'stably' phosphorylated protein, further reinforcing the notion that dephosphorylation was autocatalytic in nature.[230] Not all bacterial response regulator proteins exhibit detectable autophosphatase activity. Half-lives of an hour or more have been reported for the phosphorylated forms of OmpR, FrzE, Spo0A, Spo0F, and VirG (reviewed in ref 231).

Kinetic measurements indicate that the autodephosphorylation of response regulator proteins such as CheY occurs intramolecularly. While no evidence for the dephosphorylation of one response regulator protein by another has been reported, the discovery that phosphoryl groups sometimes can be shuttled between heterologous response regulator domains suggests an indirect mode by which net intermolecular dephosphorylation might take place.[223,232] Shuttling involves an adenine nucleotide-independent retrotransfer of phosphate from aspartate to a histidine residue on an HPt domain or histidine kinase, followed by transfer from histidine to the conserved aspartate on a different response regulator. The phosphoryl groups attached to downstream response regulator domains thus can be 'drained' into various phosphate 'sinks' upon termination of the activating stimulus. Some sinks store the high-energy phosphate groups in readiness to react to future stimuli, while in others phosphotransfer to the response regulator proteins within the sink may activate alternate branches of the signaling cascade. If the phosphate acceptor happens to be a response regulator possessing high autophosphatase activity, such as ArcB in *E. coli* [233] or BvgS in *Bordetella pertussis*,[234] the resulting 'leakage' from the sink could drive the complete removal and eventual hydrolysis of the phosphoryl groups from a response regulator protein that may exhibit little or no autophosphatase activity.

Recently, it has been reported that the phosphoaspartyl moiety on a response regulator protein from a eukaryotes, SSK1 from *Saccharomyces cerevisiae*, displays an unusually short half-life when incubated in the presence of Mg^{2+}.[235] Intriguingly, the presence of the HPt protein YPD1 inhibits this apparent autophosphatase activity nearly 200-fold.[236] This reviewer is not aware of any reports of potential autophosphatase activity in a response regulator protein resident in a member of the *Archaea*.

2. Bifunctional Histidine Kinases

Several two-component histidine kinases have been reported to possess bifunctional protein kinase/phosphatase activity including NRII,[226,237] KpdD,[238] and EnvZ[239] (Table 1). The behavior of these proteins in many ways resembled that of the IDH kinase/phosphatase, inasmuch as (a) both enzymatic functions appear to reside within a single structural domain,[233,239−241] (b) dephosphorylation required ATP[226,242] (although unlike the IDH kinase/phosphatase, it did not require hydrolysis of the ATP), and (c) the ratios of protein kinase to protein phosphatase activity could be modulated by effector molecules known to alter protein conformation.[243,244] Several observations indicate that dephosphorylation proceeds via a mechanism distinct from that by which histidine kinases phosphorylate response regulator proteins. First, the product of the reaction was inorganic phosphate and not the expected product of a 'reverse' kinase reaction, ATP.[245] Second, when the conserved histidine residues essential for the protein kinase activity of NRII[240] or EnvZ[241] were altered by site-directed mutagenesis, both enzymes retained significant protein−aspartate phosphatase activity. Third, the ratio of protein kinase to protein phosphatase activity in NRII can be modulated by the binding of a second protein, PII.[226, 237]

Truncation of the EnvZ histidine kinase/phosphatase reportedly produced a 67-residue 'phosphatase domain' whose activity, in contrast to full-length EnvZ, was dependent upon the presence of the conserved histidine essential for protein kinase activity but independent of adenine nucleotides.[245] The small size of this phosphatase domain raises questions as to whether it acted catalytically or simply stimulated autophosphatase activity latent within its substrate, the OmpR response regulator protein. Similar questions persist concerning the full-length form of EnvZ and other bifunctional histidine kinases as well, since virtually every assay of their hydrolytic activity utilized a cognate response regulator as substrate, proteins imbued with known or potentially latent autophosphatase activity. Not all histidine kinases, e.g., CheA, exhibited bifunctional capabilities. To date, only bacterial histidine kinases have been reported to catalyze or facilitate protein dephosphorylation.

3. Other Potential Protein–Aspartate Phosphatases

In certain of the *Bacteria*, a third potential mechanism for promoting the dephosphorylation of aspartate residues on two-component response regulator proteins is provided by specialized protein−aspartate phosphatases. Three distinct types of putative protein−asparate phosphatases have been discovered thus far: CheZ, Spo0E, and the response regulator aspartyl−phosphate phosphatases or Raps (Table 1; reviewed in ref 215). The sequences of these proteins bear no discernible homology either to one another or to other protein phosphatases. CheZ functions in the control of chemotaxis in *E. coli*, while Spo0E and the Raps intervene at different stages in the His-Asp-His-Asp phosphorelay that modulates sporulation in *B. subtilis* (reviewed in ref 246). Each of the three displayed a high degree of selectivity for a particular response regulator: CheZ for CheY,[227] RapA and RapB for Spo0F,[247] and Spo0E for Spo0A.[248] All three exhibited a requirement for divalent metal ions mirroring that observed for the intrinsic autophosphatase activity of CheY and other response regulator proteins. The phylogenetic distribution of each appears to be limited to a few members of the *Bacteria*.[249,250] The action of the Raps can be modulated via the localization and processing of a set of peptide inhibitors dubbed Phrs.[251,252] These inhibitors appear to be selective for individual Rap isozymes, conferring additional potential for specificity in signaling.[253]

As with the bifunctional histidine kinases, the only known substrates for these enzymes are response regulator proteins that harbor known or potential autophosphatase activity. Hence, it remains to be determined whether any or all of them function as autonomous catalytic units rather than as allosteric activators dependent upon the intrinsic hydrolytic capabilities of their substrate proteins. The latter role would be analogous to that of the GTPase activating proteins, or GAPs, that stimulate the otherwise listless catalytic activity of their target G proteins (reviewed in ref 254). In the case of CheZ, two observations would appear to favor the interpretation that it functions as an activator. First, CheZ must be present at stoichiometric levels to ensure efficient dephosphorylation of the CheY response regulator.[255] Second, mutationally produced alterations that abolished the intrinsic autophosphatase activity of CheY inevitably have led to the concomitant abolition of CheZ-mediated dephosphorylation.[230] The interactions between CheY and CheZ appear to be quite complex, involving conformational changes in each accompanied by the oligomerization of CheY-bound CheZ.[256,257] In the case of Spo0E, it should be noted that its size, ≈10 kDa,[258] lies at or below the lower limit that experience suggests is necessary to support catalytic activity.

4. Summary

Signal termination in two-component His-Asp and His-Asp-His-Asp phosphorelays can be accomplished by the dephosphorylation of aspartate residues on response regulator proteins via one or more of the following mechanisms: (a) self-dephosphorylation via intrinsic autophosphatase activity, (b) dephosphorylation by a bifunctional histidine kinase, (c) dephosphorylation (or enhancement of autodephosphorylation?) by a specialized protein−aspartate phosphatase such as CheZ, Spo0E, or one of the Raps, (d) shuttling of phosphoryl groups via HPt domains or histidine kinases to response regulator proteins possessing high autophosphatase activity, (e) shuttling of phosphoryl groups to response regulator proteins that are targeted by exogenous protein−aspartate phosphatases, or (f) retrotransfer of phosphoryl groups to histidine residues on HPt domains that are targeted by protein−histidine phosphatases such as SixA. The possibility that all response regulator proteins may possess intrinsic autodephosphorylating activity has greatly complicated the definitive determination of the mechanisms of action for several putative protein−aspartate phosphatases, particularly CheZ, Spo0E, and the Raps. It thus remains to be determined if any or all of these proteins represent catalytically competent protein phosphatases or whether they serve as allosteric effectors of the intrinsic autophosphatase activity within response regulator proteins.

This variety of potential mechanisms permits a handful of basic modules to be assembled into regulatory networks of impressive sophistication. However, none of these mechanisms appears to be universal, suggesting that a fairly long interval passed between the development of the phosphotransferase activities of the two-component system and the emergence of means for catalytically dephosphorylating aspartate (or histidine) residues. By providing a source of constitutive signal termination, the relatively high rate at which the chemical hydrolysis of phosphoasparate proceeds at neutral pH vis-à-vis phosphoesters presumably moderated the selective pressure to develop enzymatic mechanisms for accomplishing this.

While two-component histidine kinases and response regulator proteins are found in members of all three phylogenetic domains, SixA and the CheZ, Spo0E, and Raps appear to be confined to relatively limited subsets of the *Bacteria*. The clear inference of their restricted phylogenetic distributions is that each represents a quite recent enhancement to a specific two-component signaling cascade. Response regulator autodephosphorylation represents a more general and considerably more ancient means for dephosphorylating aspartate residues operative in response regulators resident in both the *Bacteria* and the *Eucarya*. The emergence of phosphatase activity in certain of the histidine kinases presumably took place before the emergence of the specialized protein−aspartate phosphatases as well. However, until the potential protein−aspartate phosphatase activity of histidine kinases from the *Archaea* and *Eucarya* is investigated in greater depth, one can only guess as to the relative order in which response regulators and histidine kinases acquired their respective protein−aspartate phosphatase activities.

VI. Phylogenetic Distribution of the Major Families Protein–Serine/Threonine/Tyrosine Phosphatases: A Genomic Perspective

In the preceding pages, this review has focused primarily upon the characteristics and distribution of bacterial and archaeal proteins that exhibit, in either native or recombinant form, demonstrable protein phosphatase activity in vitro and/or in vivo. While this approach offers a high degree of assurance that the proteins discussed represent bona fide protein phosphatases, it suffers from the limited and fragmentary nature of the current record. Only a small handful of prokaryotic protein−serine/threonine/tyrosine phosphatases have been characterized to date, and these are scattered over a wide range of different organisms. These factors render it difficult to discern either the pattern of distribution of any one protein phosphatase family or the composition of the protein phosphatase population within representative prokaryotes.

The growing library of complete genome sequences for a phylogenetically, morphologically, and phenotypically diverse array of prokaryotes provides the means for a systematic and comprehensive examination of the complete complement of potential serine-, threonine-, and/or tyrosine-specific phosphatases within the *Archaea* and *Bacteria*. However, as can be seen from the recent discoveries of 'functionally aberrant' offshoots of the various protein phosphatase families such as the diadenosine tetraphosphatases for the PPPs or the arsenate reductases for the LMW PTPs and Cdc25, the inference of catalytic function and/or physiological role for the predicted products of any ORF must be undertaken with some degree of caution. Nor can it be taken as a given that the existence of a particular ORF must necessarily lead to the expression of the protein encoded therein.

Table 5 summarizes the outcome of searches of the genome sequences from 26 prokaryotic organisms, 5 archaeons and 21 bacteria, for the presence of ORFs encoding potential 'eukaryotic' protein phosphatases. Ten of these were subject to in-depth analysis specifically for the presence of eukaryote-like protein kinases and protein phosphatases.[259,260] The remainder were identified by the genome project teams themselves.[261−276] It should be noted that, by their very nature, such mass annotation efforts cannot be considered exhaustive with regard to identifying any and all of the potential members of a particular protein family.

At first glance it can be seen that the distribution of potential protein phosphatase ORFs in the prokaryotes appears extremely and provocatively heterogeneous, in marked contrast to the *Eucarya*, where a representative of each of the major protein−serine/threonine/tyrosine phosphatase families—PPP, PPM, conventional PTP, LMW PTP, and Cdc25—can be found in every organism examined to date. No single protein phosphatase was found in every prokaryote, every archaeon, or every bacterium. In fact, three bacterial organisms reportedly were devoid of obvious ORFs for any of the aforementioned protein phosphatases: *Buchnera, Rickettsia prowazackii,* and *Treponema palladium*. Upon closer examination, it was apparent that none of the established molecular paradigms for protein phosphorylation, including the eukaryotic protein kinase paradigm[277] or the two-component histidine kinase paradigm,[278] appear to be universally present in the members of either the *Archaea* or the *Bacteria*. For example, *Treponema palladium* contains ORFs potentially encoding two-component histidine kinases but lacks the eukaryotic protein kinase paradigm, while *Mycoplasma genitalium*, *M. pneumoniae*, and *M. jannaschii* contain only potential eukaryotic protein kinases.[278] *R. prowazackii* and *Buchnera* appear to be completely devoid of any recognizable protein kinases or protein phosphatases at all.

One interpretation of this heterogeneous pattern of distribution of protein phosphorylation−dephosphorylation enzymes is that no bona fide bacterial or archaeal versions of these enzymes ever existed. All such enzymes originated in the *Eucarya* and were acquired ad hoc by prokaryotic organisms via horizontal gene transfer, accounting for the contrast between the seemingly random pattern of distribution in the *Archaea* and the *Bacteria* and the consistent and complete protein kinase and protein phosphatase populations of the *Eucarya*.

Several factors argue against an exclusively eukaryotic origin for all protein kinases, protein phosphatases, and regulatory protein phosphorylation−dephosphorylation itself. For example, although the two-component system may not be universally present in all members of the *Archaea* or the *Bacteria*, few would argue that this regulatory paradigm originated in an ancient prokaryote if not even the universal ancestor.[279] Perhaps the most compelling support for this comes from the recent discovery that both bacterial and plant phytochromes were descended from protein−histidine kinases, indicating that both form and function have been conserved across phylogeny (reviewed in ref 280). An analysis of the eukaryotic protein kinase family[277] indicated that while horizontal gene transfer between domains had occurred on more than one occasion, an ancestral protein kinase founder probably emerged before the divergence of the three domains. Similarly, the progenitor of the PPP family of protein phosphatases appears to predate the first bifurcation of the universal phylogenetic tree.[85]

The mosaic pattern of protein phosphatase distribution across the prokaryotes presumably reflects a combination of four processes: inheritance of a set of common precursors from the universal ancestor, emergence of new protein phosphatases following bifurcations in the evolutionary tree, horizontal gene transfer between and within established domain boundaries, and the elimination of redundant or unneeded protein phosphatases in prokaryotes resident in monotonic environmental niches. The first three factors would account for the variety of protein phosphatases found in prokaryotes, while the last-named phenomenon in particular would help account for the heterogeneity of this pattern.

It should be noted that the current genomic record is heavily weighted toward organisms subject to niche evolution with a concomitant reduction in genome

Table 5. Summary of ORFs Encoding Known* or Potential 'Eukaryotic' Protein Serine, Threonine, and/or Tyrosine Phosphatases from the Genomes of 10 Prokaryotes. Listed Below Are ORFs from 23 Prokaryotic Organisms That Exhibit Discernible Homology to the PPP, PPM, Conventional PTP, and LMW PTP Families of Protein Phosphatases[a]

organism	PPP	PPM	conv. PTP	LMW PTP
		Archaea		
Aeropyrum pernix K1[261]	APE0777			
Archeaoglobus fulgidus [259]	AF1822			AF1361
Methanobacterium thermoautotrophicum[259]			mt1586	mt1355
Methanococcus jannaschii[259]			MJ0215	
			MJ1098	
			MJECL20	
Pyrococcus hirokoshii OT3[262]			PH1732	
		Bacteria		
Aquifex aeolicus[263]		AA0483		
Bacillus subtlis[259]	YjpP	RsbP*	YtrC	YfkJ
		RsbU*	YvcJ	YwlE
		RsbX*		
		SpoIIE*		
		PrpC*		
Borrelia burgdorferi [259]		BB0836		
Buchnera[264]				
Campylobacter jejuni[265]	Cj0184			
Chlamydia pneumoniae[266]		CPn0397		
Chlamydia trachomatis[267]		TC0530		
Deinococcus radiodurans[268]	DR0295		DR1912	
	DR0935		DR2161	
	DR2249			
Escherichia coli K-12[259]	PtpA*	f729	o430	f147
	PtpB*			f152
Haemophilus influenzae[259]	HI0551			
Helicobacter pylori[259]		HP0431		
Mycobacterium tuberculosis[269]		Rv0018c	MPtpB*	MPtpA*
Mycoplasma genitalium[259]	MG108			
Mycoplasma pneumoniae[270]		orf259		
Neisseria meningitidis[271]				NMA1472
Pseudomonas aeruginosa[272]		Stp1*		PA2978
		PA0075		
Rickettsia prowazekii[273]				
Synechocystis PCC6803[259, 260]	sll1387	sll0602		slr0328
		sll1033		slr0946
		sll1365		slr1617
		sll1771		
		slr0114		
		slr1860/IcfG*		
		slr1983		
		slr2031		
Thermotoga maritima[274]	Tm0742			
Treponema palladium[275]				
Vibrio cholerae[276]			VC1070	VC0916
				VC1041

[a] Asterisks (*) indicate those ORFs whose protein products have been determined to possess protein phosphatase activity. Assignments for *Archeaoglobus fulgidus*, *Bacillus subtlis*, *Borrelia burgdorferi*, *Escherichia coli* K-12, *Haemophilus influenzae*, *Helicobacter pylori*, *Methanobacterium thermoautotrophicum*, *Methanococcus jannaschii*, and *Mycoplasma genitalium* were by Shi et al.[259] Those for *Synechocystis* PCC6803 were by Shi et al.[259] and Zhang et al.[260] All others were by the individual genome project teams.

size. For example, the majority of bacterial genomes sequenced to date are derived from obligate pathogens. Such pathogens are prone to lose genetic material as the presence of parallel metabolic and other systems in the host blunts the selective pressure to maintain 'duplicate' proteins and their genes. Once dependence upon the host for one vital factor becomes established, accelerated functional erosion occurs leading to a mass 'meltdown' in which the ≥4 Mbp genome typical of free living prokaryotes shrinks to 0.5−2 Mbp.[281] A similar argument can be made for many of the *Archaea*, which have become specialized to 'infecting' extreme, but highly consistent, environmental niches. A natural target for 'downsizing' in an organism that becomes restricted to a stable, monotonic environmental niche would be the machinery responsible for sensing, transducing, and responding to environmental variables. Thus, the protein phosphatase complements of free living organisms subject to a broader range of selective pressures, such as *Synechocystis* PCC6803 or *B. subtilis*, may therefore be more reflective of the protein phosphatase populations of early *Bacteria* than are those for obligate pathogens such as *M. genitalium* or *R. prowazackii*.

Given the lack of a firm, generally accepted tree as a framework for analyzing the phylogenetic distribution of protein phosphatases, it is difficult to

confidently provide definitive answers to the question of which protein phosphatases were inherited in a linear fashion and which were acquired by horizontal gene transfer. However, this does not mean that any and all speculation is unwarranted, although much of what is to follow undoubtedly will be subject to revision as more information comes to light.

Sequence and functional comparisons would suggest that the PPP-family ranks as a very ancient family of phosphohydrolases, one that may trace its origins back to the universal ancestor. The number of homologues that act on nonprotein phosphoesters also is suggestive, at least superficially, of age. At the other extreme, the Cdc25 family would appear to be the youngest of the five major protein−serine/threonine/tyrosine phosphatase families. It has been found only in the *Eucarya*, where it apparently developed from rhodanese or a rhodanese-like protein. The relatively small number of family members and their dedication to a single cellular process, the eukaryotic cell cycle, also suggests a short evolutionary history.

Intermediate in age between Cdc25 and the PPPs are the PPMs, which probably emerged in the *Eucarya* some time after their segregation from the *Archaea* and *Bacteria*, or perhaps vice versa.[282] Several factors suggest this. First and foremost, no archaeal PPMs have been reported in the literature and none of the five archaeal genomes published to date contain a recognizable ORF for either a PPM or a homologous adenylate cyclase. Comparison of the sequences of bacterial and eukaryotic PPMs also failed to detect the type of deep division between the two groups indicative of direct inheritance from a common ancestor.[97]

The conventional PTPs are harder to date given that no comprehensive phylogenetic analysis covering all three domains has been published. The number and variety of conventional PTPs in the eukaryotes would appear to be indicative of a long period of development and diversification. However, it also has been argued that the PTPs and tyrosine phosphorylation itself were late-emerging cellular processes characteristic of higher eukaryotes.[283] On balance, their widespread distribution, which permeates members of all three domains, suggests that they are older than the PPMs—although it is difficult to judge whether they are as old as the PPPs. The LMW PTPs are equally problematic. Their structural simplicity and limited numbers suggest either relative youth or, alternatively, confinement to a narrow functional niche resulting from their failure to successfully compete with other protein phosphatase paradigms for a predominant role in cell regulation. The phylogenetic analysis of Li and Strohl[175] suggests that while the bacterial LMW PTPs are quite diverse, their eukaryotic counterparts tend to closely cluster, as if the latter were derived from a single founder acquired from either the common ancestor or a very early horizontal gene transfer event.

VII. Conclusion

The unexpectedly widespread and provocatively varied distribution of what were formerly considered eukaryotic and bacterial protein phosphatases hints at an extremely rich phylogenetic history. Clearly, protein phosphorylation emerged as an important regulatory mechanism much earlier in evolutionary time than was imagined just a few short years ago. Reconstructing both the history underlying the current phylogenetic distribution and tracing the point that protein phosphorylation−dephosphorylation crossed over from being a passive structural event to a dynamic regulatory process represents a challenging task for molecular archaeologists. While comparison of the primary sequences of the protein kinases and protein phosphatases that serve as the vehicle for effecting regulatory protein phosphorylation−dephosphorylation has and will continue to provide clues to the order and nature of these events, insights into the functional role of these enzymes in prokaryotes and the degree that they these roles have been conserved across phylogeny represents the essential Rosetta Stone for unraveling these events.

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IX. References

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