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MINIREVIEW

Fancy Meeting You Here! a Fresh Look at "Prokaryotic" Protein Phosphorylation

PETER J. KENNELLY* AND MALCOLM POTTS

Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0308

INTRODUCTION: NEVER THE TWAIN SHALL MEET?

Early attempts to detect protein phosphorylation in microbial organisms by using the techniques that had been so successful with mammals proved negative (8). Thus, for many years protein phosphorylation-dephosphorylation was regarded as a subtle and refined regulatory mechanism that emerged late in evolutionary time to meet the specific needs of organisms composed of multiple, differentiated cells—one for which simple organisms had no need and hence made do without. It eventually became apparent that protein phosphorylation was a universal phenomenon that took place in "lower" eukaryotes and, thanks to the pioneering work of Wang and Koshland (48), Garnak and Reeves (16), and Manai and Cozzone (29) in the late 1970s, in the domain *Bacteria* as well. However, as more was learned about protein phosphorylation in eukaryotic and bacterial organisms during the ensuing decade, the previous "have/have not" dichotomy reemerged but in a new form. Eukaryotes almost exclusively targeted the hydroxyl amino acids serine, threonine, and tyrosine for signal transduction purposes, while bacteria favored the use of histidine and the carboxyl amino acids as phosphoacceptors and were judged to be incapable of phosphorylating tyrosine. The hundreds of protein kinases characterized from eukaryotic organisms fell neatly into a single superfamily, and we will hereafter refer to these enzymes as the Hanks-type protein kinases after the investigator most responsible for defining them (19). These protein kinases proved to be distinct from the first protein kinases sequenced from representatives of the *Bacteria*: the isocitrate dehydrogenase kinase/phosphatase, the so-called protein-histidine kinases of the two-component regulatory system, and the pseudo-protein kinases of the phosphoenolpyruvate-dependent phosphotransferase system. This phylogenetic compartmentation was readily rationalized as the inevitable consequence of the late emergence of protein phosphorylation in evolutionary time—each phylogenetic domain utilized unique, distinct molecular themes which arose and evolved separately.

Recently, many "domain crossover" events have been reported. These reports strongly challenge the notion that genuine "eukaryotic" and "prokaryotic" protein kinases and protein phosphatases exist. Here, we briefly recount these challenges to the conventional viewpoint and discuss the implications of these new findings for understanding the origins and evolution of protein phosphorylation.

* Corresponding author. Phone: (540) 231-4317. Fax: (540) 231-9070. Electronic mail address: pjkennel@vt.edu.

HOLES IN THE PHYLOGENETIC DIKE

The first hint that the phylogenetic compartmentalization of protein kinases and protein phosphatases was not absolute was provided by the discovery of eukaryotic-like protein-serine/threonine phosphatases encoded by bacteriophages λ and φ80 (6, 7). Later, a eukaryotic-like protein-tyrosine phosphatase was discovered to be encoded by the virulence plasmid found in *Yersinia pseudotuberculosis* (17). While these findings were highly provocative, the mobility and malleability of such extrachromosomal elements cast doubt upon the utility of their protein products as evolutionary roadmarks. In the case of *Y. pseudotuberculosis*, it is widely believed that the genes in question were acquired from the eukaryotic hosts infected by this pathogen. There are recent examples, however, of phylogenetically "foreign" protein kinases and protein phosphatases whose presence is firmly cemented in the genomic DNA of the host cells.

(i) Discovery of "eukaryotic" protein kinases and protein kinase homologs in prokaryotes. The first discovery of a "foreign" protein kinase or protein phosphatase homolog encoded by the genomic DNA of an organism was reported in 1991 (32). Using degenerate oligonucleotides modelled after conserved regions found in Hanks-type "eukaryotic" protein kinases, Munoz-Dorado and coworkers cloned a gene, *pkn1*, from the soil bacterium *Myxococcus xanthus*. The predicted gene product showed 27 to 31% identity between its putative catalytic domain and those of representative Hanks-type protein kinases such as the cyclic AMP-dependent protein kinase, protein kinase C, and calmodulin-dependent protein kinase II. When *Escherichia coli* that expressed *pkn1* was grown in the presence of radiolabeled P_i, phosphorylation of Pkn1 on serine and threonine was observed. Phosphorylation did not occur when a mutation known to disable the protein kinase activity of Hanks-type protein kinases was introduced into *pkn1*. This result implied that the phosphate was introduced via an autophosphorylation event. Since the ability to autophosphorylate is typical of most well-characterized protein kinases, this suggested strongly that Pkn1 possessed kinase activity toward exogenous proteins. Northern blot analysis indicated that expression of *pkn1* in *M. xanthus* was developmentally regulated. Deletion of *pkn1* resulted in premature differentiation and spore formation, implying the gene had a role in the organism's developmental cycle.

Several reports document the discovery of genes that encode potential Hanks-type protein kinases in other prokaryotes. These genes include *pknA* from *Anabaena* sp. strain PCC 7120 (54); *pkaA*, *pkaB*, and *afsK* from *Streptomyces coelicolor* A3(2) (31, 47); and open reading frames 547, 294, and 114 from the methanogenic archaeons *Methanococcus vannielii*, *Methanococcus voltae*, and *Methanococcus thermolithotrophicus*, respec-

tively (44). Deletion of *pknA* from *Anabaena* sp. strain PCC 7120 led to visible changes in cell morphology and reduced heterocyst frequency, implying a functional role in the normal growth or development of the organism akin to that postulated for *pkn1* in *M. xanthus*. Expression of *pkaA* or *pkaB* from *S. coelicolor* A3(2) in *E. coli* yielded protein products that were phosphorylated mainly on threonine or serine residues, respectively (47). While the latter is consistent with the occurrence of an autophosphorylation event, no experiments were performed to discriminate between the action of the putative protein kinases and enzymes endogenous to the host organism. However, when *afsK* was expressed in *E. coli*, an activity which was capable of phosphorylating a known phosphoprotein from *Streptomyces* species, AfsR, appeared (31). This finding represents the first, and thus far the only, instance in which the ability of a bacterial Hanks-type protein kinase to phosphorylate an exogenous protein, the mark of true protein kinase activity, has been demonstrated.

(ii) Discovery of "bacterial" histidine kinases in eukaryotic organisms. In 1992, Popov and coworkers cloned the gene which encodes branched-chain alpha-ketoacid dehydrogenase kinase (BCKDH kinase) found in rat mitochondria (37). To their surprise, the sequence of this enzyme showed no resemblance to any eukaryotic protein kinase in the Hanks-type superfamily. Rather, the closest homologs to BCKDH kinase were found among the protein-histidine kinases of bacteria. Bacterial protein-histidine kinases become self-phosphorylated on a histidine residue as an intermediate step in the transfer of the phosphoryl group to an aspartic acid residue on a sensor protein or sensor domain fused with the kinase. While a candidate for such a catalytic histidine is present in the sequence of the BCKDH kinase, direct evidence for its participation in catalysis has not been forthcoming. Intriguingly, BCKDH kinase phosphorylates its physiological substrate protein, branched-chain alpha-ketoacid dehydrogenase, on a pair of serine residues (37) and autophosphorylates on serine as well (10).

This first break in the monopoly previously enjoyed by the Hanks-type configuration over the structure of eukaryotic protein kinases subsequently was followed by confirmatory sightings of protein-histidine kinase homologs in a wide range of eukaryotic organisms. Popov and coworkers subsequently cloned and sequenced the gene for the pyruvate dehydrogenase kinase from rats (36), an enzyme that also phosphorylates proteins on hydroxyl amino acids, and observed that, like BCKDH kinase, pyruvate dehydrogenase kinase bore a weak resemblance to protein-histidine kinases. Eucaryal genes whose predicted products exhibited greater structural and functional homology to the bacterial protein-histidine kinases were also uncovered. The predicted product of the *Arabidopsis thaliana* ethylene response gene *ETR-1* (4), and the predicted product of *SLN1* in *Saccharomyces cerevisiae* (28, 33), are sequence homologs of bacterial protein-histidine kinases. Genetic analysis of site-directed mutants indicate that SLN1 acts in the same manner as do its bacterial counterparts, i.e., through formation of a phosphohistidyl enzyme intermediate followed by phosphorylation of an aspartyl residue on its sensor domain (28). This protein kinase forms part of an osmoregulatory signalling cascade in which traditional eukaryotic Hanks-type mitogen-activated protein kinases participate as downstream elements (28).

Protein-histidine kinases have also been found in *Neurospora crassa* (1) and *Dictyostelium discoideum* (42), as well as the archaeons *Pyrococcus furiosus* (43) and *Halobacterium salinarium* (40). In *H. salinarium*, a protein-histidine kinase has been implicated as a participant in both the phototactic and chemotactic sensory-response pathways. Not only does the function of this archaeal protein-histidine kinase closely parallel those of its bacterial counterparts, but the sensor molecule it apparently targets for phosphorylation also strongly resembles those of the two-component systems of bacteria (53).

(iii) Discovery of "eukaryotic" protein phosphatases in the domains *Bacteria* and *Archaea*. The gene for the phosphomonoesterase IphP was isolated via expression cloning from the cyanobacterium *Nostoc commune* UTEX 584. Surprisingly, the predicted gene product contained the sequence (His-Cys-Xaa_5-Arg [Xaa is any amino acid]) characteristic of the active site of the eukaryotic enzymes known to possess physiologically relevant protein-tyrosine phosphatase activity: the protein-tyrosine phosphatases and the dual-specificity protein phosphatases (38). In these enzymes, the cysteine residue functions as the active-site nucleophile, with catalysis proceeding through formation of a cysteinyl-phosphate intermediate (18). Assays of IphP with a variety of exogenous substrates showed that it is capable of dephosphorylating phosphotyrosine, phosphoserine, and phosphothreonine on a range of phosphoprotein and phosphopeptides in vitro, placing it in the class of the dual-specificity protein phosphatases (20).

It has been proposed that a set of low-molecular-weight acid phosphatases that contain an active-site motif (Phe-Ile/Val-Cys-Xaa_5-Arg) strikingly similar to that of IphP compose a second family of protein-tyrosine phosphatases in eukaryotes (52). Recently, PtpA, a phosphatase sharing strong sequence homology with these so-called small, acidic protein-tyrosine phosphatases was identified and its gene was cloned from *S. coelicolor* A3(2) (26). PtpA dephosphorylated both a phosphotyrosine-containing peptide and free phosphotyrosine, but not free phosphoserine or phosphothreonine, in vitro. Genes that encode potential sequence homologs of PtpA are present in a number of other bacteria, including *Bacillus subtilis*, *Pseudomonas solanacearum*, *Erwinia amylovora*, and *Klebsiella pneumoniae* (26). However, it is unknown whether the predicted products of these genes possess phosphatase activity of any kind.

The physiological roles of PtpA and IphP remain uncertain. Like all previously characterized dual-specificity and protein-tyrosine phosphatases, these proteins display phosphohydrolase activity toward both phosphoproteins and low-molecular-weight compounds such as *p*-nitrophenyl phosphate in vitro. Thus, they have the catalytic potential to function either as dedicated protein phosphatases or as nonspecific phosphate scavengers in the mold of the acid and alkaline phosphatases. IphP contains a 24-residue N-terminal signal sequence that is removed upon secretion of the recombinant protein from *E. coli*. Therefore, it is likely that IphP is targeted to or beyond the cell membrane and may function as an ectoenzyme. IphP displays a strong preference for phosphoproteins and phosphopeptides in vitro. On average, k_{cat}/K_m ratios for macromolecular substrates exceed those for low-molecular-weight phosphomonoesters by nearly eightfold (20). There is no evidence implicating that PtpA is secreted, but it has yet to be resolved whether its eukaryotic homologs function as protein-tyrosine phosphatases in vivo. However, at present IphP and PtpA represent the only bacterial enzymes known with the potential to dephosphorylate the phosphotyrosyl proteins that have been uncovered in increasing numbers in cyanobacteria and other bacteria (see below).

"Eukaryotic" protein-serine/threonine phosphatases have been discovered in the *Archaea*. We have cloned and sequenced the gene encoding PP1-arch, a divalent metal ion-stimulated protein-serine/threonine phosphatase (24) first

TABLE 1. Bacterial tyrosine-phosphorylated proteins

Organism[a]	Tyrosine-phosphorylated protein(s)	Reference
Cyanobacteria		
Nostoc commune UTEX 584	85 kDa	38
Prochlorothrix hollandica	88 kDa	49
Synechococcus sp. strain PCC 7942	88 kDa	49
Bacteria		
Acinetobacter calcoaceticus	81, 61, 46, and 17 kDa	9
Escherichia coli	80 kDa	14
Pseudomonas solanacearum	85 kDa	2
Pseudomonas syringae	66 kDa	39
Streptomyces coelicolor A3(2)	Numerous polypeptides	50
Streptomyces griseus	Numerous polypeptides	50
Streptomyces hygroscopicus	Numerous polypeptides	50
Streptomyces lavendulae	Numerous polypeptides	50
Streptomyces lividans	Numerous polypeptides	50
Streptomycesrimosus	Numerous polypeptides	50

[a] Listed are those bacteria in which the phosphorylation of proteins on tyrosine has been demonstrated by using antiphosphotyrosine antibodies and/or differential labelling techniques.

characterized in the extreme acidothermophilic archaeon *Sulfolobus solfataricus* (21). Protein phosphatase activities displaying similar chromatographic behavior, divalent metal ion activation, and substrate preferences have been detected in both a halophilic archaeon, *Haloferax volcanii* (34), and a methanogenic archaeon, *Methanosarcina thermophila* TM-1 (35). The gene encoding the *M. thermophila* protein phosphatase has been cloned, and its DNA-derived amino acid sequence was determined (45). The predicted product exhibits clear sequence similarity to the enzyme from *S. solfataricus*, indicating that PP1-arch is the first representative of a widely distributed family of archaeal protein phosphatases. PP1-arch is a monomer of 293 amino acids that exhibits 28 to 30% sequence identity over nearly its entire length to the catalytic subunits or domains of the major family of protein-serine/threonine phosphatases from the eukaryotes, the PP1/2A/2B superfamily. This degree of identity approaches the lower limit of that observed between the eukaryotic members of the superfamily alone, about 35% (3). Thus, it would appear not only that the archaeal and eukaryotic members of this protein phosphatase superfamily share a common progenitor but that this progenitor may have closely resembled currently extant protein phosphatases in its structure and functional capabilities.

(iv) Discovery of protein-tyrosine phosphorylation in the *Bacteria*. One of the most persistent tenets of the prokaryote/eukaryote protein phosphorylation dichotomy has been the special status ascribed to the phosphorylation of proteins on tyrosine. Today, many still regard protein-tyrosine phosphorylation as a phylogenetically compartmentalized process confined to higher eukaryotes (25). Several articles appeared during the 1980s reporting the detection of phosphotyrosine in acid hydrolysates of bacterial proteins. However, the existence of high levels of nucleotidylated tyrosine in these organisms, which breaks down to form phosphotyrosine during the acid hydrolysis procedures employed in these studies, raised serious questions concerning the interpretation of these reports (12).

Today, by using differential labeling techniques ([γ-versus [α-^{32}P]ATP) and antibodies to phosphotyrosine, it has proven possible to detect the presence of tyrosine-phosphorylated proteins—free from interference by nucleotidylated tyrosine—in a broad range of bacteria (Table 1). Intriguingly, in nearly every instance a protein was detected with a molecular mass of between 80 and 88 kDa. In *E. coli*, this phosphotyrosyl protein was identified as the product of the o591 open reading frame, whose predicted product showed significant sequence similarity to elongation factor G (14). Although possibly coincidental, this correlation raises the possibility that a phosphotyrosine-containing protein that is ubiquitous in distribution and function among bacteria exists. It is also noteworthy that in the cyanobacterium *Prochlorothrix hollandica*, the degree of phosphorylation of the 88-kDa phosphotyrosine-containing protein was observed to be light dependent (49). In *N. commune* UTEX 584, the appearance of the 85-kDa tyrosine-phosphorylated protein was dependent upon the presence of fixed nitrogen (38). In *Streptomyces lividans*, *Streptomyces hygroscopicus*, and *Streptomyces lavendulae*, changes in the gross quantity and pattern of tyrosine-phosphorylated proteins were observed when cells were transferred from minimal to rich medium as well as during the transition from logarithmic growth to stationary phase (50). Two strains, *N. commune* UTEX 584 and *S. coelicolor* A3(2), also contain potential protein phosphatases (IphP and PtpA, respectively) which dephosphorylate tyrosine-phosphorylated peptides and proteins in vitro (see above). Such behaviors are consistent with a dynamic, regulatory role for protein-tyrosine phosphorylation events in bacteria.

IMPLICATIONS, EXTRAPOLATIONS, AND SPECULATIONS

In the face of recent events, the apparently sharp and fundamental distinctions between the molecular architecture of eukaryotic and prokaryotic protein phosphorylation networks have blurred together like a "watercolor in the rain". It is apparent that most currently recognized protein kinases and protein phosphatases, regardless of the organism(s) in which they were first discovered, are derived from a set of common prototypes. However, the demise of this eukaryote/prokaryote dichotomy raises a whole new set of questions. Are any types of protein kinases or protein phosphatases found exclusively within one domain? How did similar protein kinases and protein phosphatases arise in the different domains—through gene transfer or via direct inheritance from the universal ancestor? And perhaps most intriguing of all, when were these progenitors first employed as agents for the regulation of protein function?

(i) Is commonality absolute? As outlined above, many protein kinases and protein phosphatases that long were considered to be "eukaryotic" or "prokaryotic" in origin and residency turn out to be of general distribution. With regard to protein kinases, universality appears to be the norm. To date, two major families of true protein kinases have been identified, the protein-histidine kinases and the Hanks-type protein kinases, and they are ubiquitous. Well-characterized examples from each family, or genes encoding proteins which are potential candidates, have been observed in members of the *Bacteria*, *Eucarya*, and *Archaea*. The very dramatic nature of these revelations naturally raises the question of where the pendulum will halt in its current swing. In other words, are all phosphorylation-based signalling systems assembled out of the same basic suite of protein kinase and protein phosphatase prototypes? Yet one exception also exists, the isocitrate dehydrogenase kinase/phosphatase, which has been found solely in bacteria and whose protein kinase domain reflects neither of the aforementioned paradigms (22).

The situation with regard to the protein phosphatases is much less clear, partly because of the current lack of information as to how prokaryotes catalyze protein dephosphorylation events. Two major families of protein-serine/threonine phos-

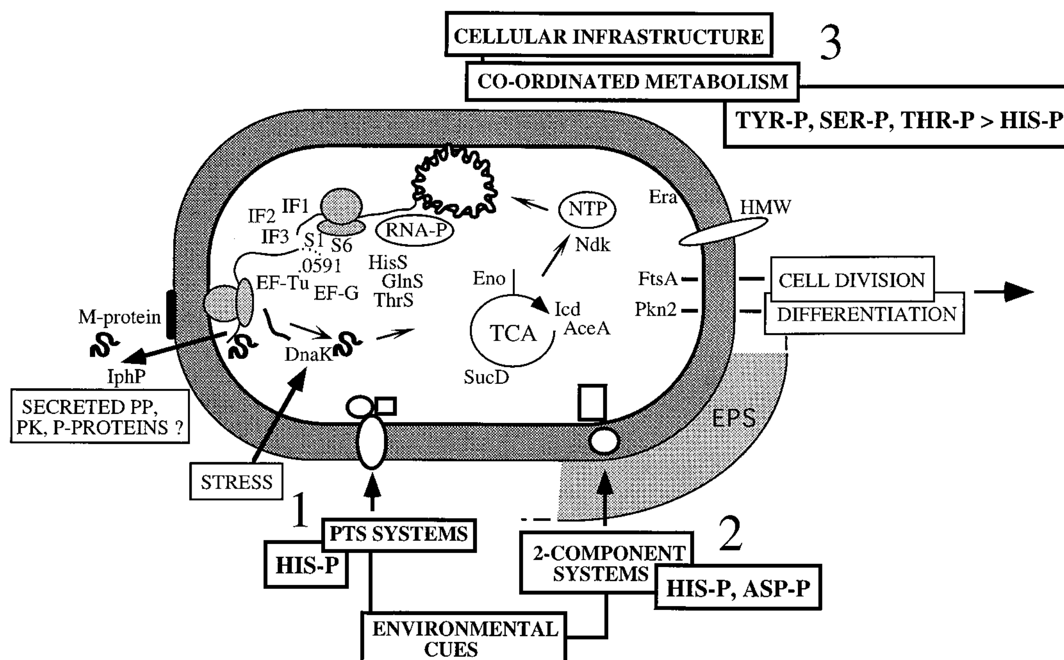

FIG. 1. Protein phosphorylation provides a cellular infrastructure for sensing extracellular signals and coordinating intracellular events. Shown is a schematic representation of a "typical" bacterial cell briefly summarizing the major protein phosphorylation network components discovered so far, protein kinases (PK), protein phosphatases (PP), and phosphoproteins (P-proteins). For further information, refer to the text of this minireview and to the reviews by Cozzone (8), Freestone et al. (13), Mann (30), Saier (41), and Swanson et al. (46). Abbreviations: TYR-P, phosphorylated tyrosine; Icd, isocitrate dehydrogenase; AceA, isocitrate lyase; Eno, enolase; SucD, succinyl-coenzyme A synthetase; Ndk, nucleoside diphosphate kinase; RNA-P, RNA polymerase; S1 and S6, ribosomal proteins S1 and S6; IF, initiation factor; 0591, an 80-kDa tyrosine-phosphorylated protein that copurifies with RNA-P; EF, elongation factor; HisS, GlnS, and ThrS, the histidyl-, glutaminyl-, and threonyl-tRNA synthetases, respectively; DnaK, an autophosphorylated chaperone; M-protein, a cell surface phosphoprotein from *Streptococcus pyogenes*; Era, a membrane-bound GTPase; FtsA, a phosphoprotein required for cell division; HMW, unidentified high-molecular-weight phosphoproteins of *Mycoplasma pneumoniae*; TCA, tricarboxylic acid cycle; EPS, extracellular polysaccharide sheath. The numbers 1, 2, and 3 refer to the three major protein phosphorylation systems in bacteria, the phosphoenolpyruvate-dependent phosphotransferase (PTS) system, two-component signalling system, and eukaryote-like phosphomonoester-based systems, respectively.

phatases are found in the eukaryotes, the PP1/2A/2B and the PP2C superfamilies. The former (PP1/2A/2B) ranks among the most highly conserved enzyme families yet encountered (5). However, while a PP1/2A/2B homolog exists in the archaeons *S. solfataricus* (24) and *M. thermophila* (45), no evidence for a genome-encoded protein phosphatase from this superfamily has been found in bacteria, despite intensive study. Bacteria contain the necessary template to assemble such an enzyme, as evidenced by the significant sequence similarity between diadenosine tetraphosphatase from *E. coli* and the consensus sequences for the PP1/2A/2B superfamily (23). Intriguingly, at least two bacteriophages, λ and φ80, encode potential PP1/2A/2B homologs (7). One of these, λ gt11, was shown to produce a functional protein-serine/threonine phosphatase, open reading frame 221 (6). This curious state of affairs suggests that bacteria may have chosen alternative molecular paradigms to those employed by the archaeons and eukaryotes, e.g., the isocitrate dehydrogenase kinase/phosphatase, in order to construct their protein-serine/threonine phosphatases. One possible rationale for this may be as a defence mechanism against microbial toxins, such as microcystin-LR, which act as potent inhibitors of PP1/2A/2B family enzymes (27).

Protein phosphatases in another major family employ the His-Cys-Xaa_5-Arg catalytic motif: the protein-tyrosine phosphatases and dual-specificity protein phosphatases. While numerous examples of such enzymes occur in representatives of the *Eucarya*, the only bacterial examples identified to date are IphP from *N. commune* UTEX 584, which displays dual-specificity protein phosphatase activity in vitro (38), and YopH from *Y. pseudotuberculosis*, which is a protein-tyrosine phosphatase (17). However, only the former would seem to be of genuine bacterial ancestry, while there is no evidence for the existence of such enzymes in representatives of the *Archaea*. Likewise, members of the small, acidic protein-tyrosine phosphatase family appear in eucaryal and bacterial organisms, but not in any archaeon. At this juncture it would appear that commonality, although widespread, probably will not prove to be absolute.

(ii) How did commonality arise? Universal ancestors versus gene transfer. The shared molecular architecture of many prokaryotic and eukaryotic protein kinases and protein phosphatases must have arisen via one or more of the following mechanisms: direct inheritance from a common ancestor, gene transfer between established taxa, or convergent evolution from distinct precursors. Although numerous examples of mechanistic or functional convergence in proteins exist, convergence at the primary sequence level remains an exceedingly remote and currently unprecedented phenomenon (11). This suggests that few, if any, of the observed commonalities could have arisen in this manner. On the other hand, lateral gene transfer represents a plausible and probable mechanism for the propagation of protein kinases and protein phosphatases. Genes for protein kinases and protein phosphatases are carried by a range of viral vectors, while in the past, endosymbiotic events provided a route for the mass transfer of genetic information between primitive bacteria and nascent eukaryotes. To date, at least one instance of a probable lateral gene transfer event has been documented, the acquisition of a eukaryotic protein-ty-

rosine phosphatase (17) and a Hanks-type protein kinase (15) by the virulence plasmid of *Y. pseudotuberculosis*.

A strong case can also be made for a significant contribution of direct inheritance from a "universal ancestor." For example, the clear sequence similarities between the eucaryal and archaeal members of the PP1/2A/2B superfamily and the diadenosine tetraphosphatase from *E. coli* strongly suggests the presence of an ancient progenitor which provided the basic template for a number of phosphohydrolases. The presence of protein-histidine kinases in representatives of the *Eucarya*, *Archaea*, and *Bacteria* is more readily explained by a shared ancestor than by some extremely pervasive viral transfer phenomenon. Moreover, given the prominent role played by phosphoester and phosphoramide chemistry in the first biological macromolecules (51), it would be only natural for phosphotransferases and phosphohydrolases to be numbered among the earliest proteinaceous catalysts.

(iii) When did phosphorylation and dephosphorylation emerge as a regulatory phenomenon? It seems probable that the prototypes for at least some of today's protein kinases and protein phosphatases date back to the epoch of the universal ancestor. When did the first descendants who utilized their phosphotransferase and phosphohydrolase ability to modulate protein structure and function emerge? When did these enzymes stop being part of the machinery used to construct and recycle biological macromolecules and begin to regulate metabolic processes and help transmit signals? The latter is a difficult question to answer, but the available clues indicate that it was much earlier than previously envisioned. The major evidence for this view is provided by the protein-histidine kinases. In bacteria, it is well-known that these protein kinases are coupled with a receptor protein, which they phosphorylate on aspartate, to form two-component signalling modules. When one examines lower representatives of the *Eucarya*, such as *S. cerevisiae*, or the archaeon *H. salinarium*, one finds that not only is the core structure of the protein-histidine kinase conserved but that homologs of its receptor protein also are present. In other words, the molecular elements of this signalling module are found in representatives of all three phylogenetic kingdoms, arguing strongly that what was inherited from the common ancestor was not simply a phosphotransferase protein but an emerging signal transduction system.

Another benchmark in the emergence of modulatory protein phosphorylation is provided by PP1-arch, the archaeal homolog of the eucaryal PP1/2A/2B family of protein-serine/threonine phosphatases. Sequence comparisons between PP1-arch and its eucaryal counterparts may suggest that the prototype phosphatase was present in the common predecessor of archaeal and eucaryal organisms as a dedicated, highly refined protein-serine/threonine phosphatase. Since the presence of a dedicated protein phosphatase implies the prior action of dedicated protein kinases, one may conclude that the phosphorylation-dephosphorylation of serine and threonine residues emerged as a dynamic modulatory process prior to the divergence of the *Eucarya* from the *Archaea.*

SUMMARY

Bacteria play host to a wide range of protein phosphorylation-dephosphorylation systems (Fig. 1). As little as five years ago the known systems were thought to be late-emerging and absolutely prokaryote specific. Today we know that most protein kinases and protein phosphatases are descended from a set of common, and possibly quite ancient, prototypes. Prokaryote- and eukaryote-specific protein kinases and protein phosphatases are rare and represent exceptions, not the rule as previously thought. Commonality suggests that a dynamic and versatile regulatory mechanism was first adapted to the modulation of protein function as early if not earlier than more "basic" mechanisms such as allosterism, etc. The existence of common molecular themes confirms that the microbial world offers a unique, largely untapped library and a powerful set of tools for the understanding of a regulatory mechanism which is crucial to all organisms, tools whose diversity and experimental malleability will provide new avenues for exploring and understanding key modes of cellular regulation.

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