Bacterial tyrosine kinases: evolution, biological function and structural insights

Grangeasse, Christophe; Nessler, Sylvie; Mijakovic, Ivan

Published in:
Philosophical Transactions of the Royal Society B: Biological Sciences

Link to article, DOI:
10.1098/rstb.2011.0424

Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Bacterial tyrosine kinases: evolution, biological function and structural insights

Christophe Grangeasse, Sylvie Nessler and Ivan Mijakovic

Phil. Trans. R. Soc. B 2012 **367**, doi: 10.1098/rstb.2011.0424, published 13 August 2012

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

CrossMark click for updates

Phil. Trans. R. Soc. B (2012) **367**, 2640–2655
doi:10.1098/rstb.2011.0424

Review

Bacterial tyrosine kinases: evolution, biological function and structural insights

Christophe Grangeasse[1,*], Sylvie Nessler[2] and Ivan Mijakovic[3]

[1]*Bases Moléculaires et Structurales des Systèmes Infectieux, IBCP, CNRS, Université de Lyon, UMR 5086, 7 passage du Vercors, 69367 Lyon, France*
[2]*Laboratoire d'Enzymologie et Biochimie Structurales, Centre de recherche de Gif, CNRS, 91198 Gif sur Yvette, France*
[3]*Micalis, AgroParisTech/Institute National de la Recherche Agronomique, 78850 Jouy en Josas, France*

Reversible protein phosphorylation is a major mechanism in the regulation of fundamental signalling events in all living organisms. Bacteria have been shown to possess a versatile repertoire of protein kinases, including histidine and aspartic acid kinases, serine/threonine kinases, and more recently tyrosine and arginine kinases. Tyrosine phosphorylation is today recognized as a key regulatory device of bacterial physiology, linked to exopolysaccharide production, virulence, stress response and DNA metabolism. However, bacteria have evolved tyrosine kinases that share no resemblance with their eukaryotic counterparts and are unique in exploiting the ATP/GTP-binding Walker motif to catalyse autophosphorylation and substrate phosphorylation on tyrosine. These enzymes, named BY-kinases (for Bacterial tYrosine kinases), have been identified in a majority of sequenced bacterial genomes, and to date no orthologues have been found in Eukarya. The aim of this review was to present the most recent knowledge about BY-kinases by focusing primarily on their evolutionary origin, structural and functional aspects, and emerging regulatory potential based on recent bacterial phosphoproteomic studies.

Keywords: tyrosine kinase; bacteria; protein phosphorylation; biological role; structure

1. INTRODUCTION

Since its discovery in the mid-1950s, protein phosphorylation in eukaryotes has been shown to be a versatile and sophisticated mechanism for the regulation of cellular processes such as intercellular communication and coordination of complex functions [1,2]. The presence of this post-translational modification was neglected for a long time in bacteria. Indeed, as early attempts to detect its presence were unsuccessful, it was proposed that protein phosphorylation was a regulatory mechanism that emerged late in evolution to meet the specific needs of organisms composed of multiple and differentiated cells. Protein phosphorylation in bacteria was evidenced in the late 1970s through the pioneering work of several groups that showed the presence of phosphorylated amino acids in bacterial proteins and, in some cases, characterized the phosphorylated proteins [3–5]. Considering that bacteria live in environmental conditions that change frequently and rapidly, they needed to develop devices to monitor their surroundings and adjust their physiology and behaviour accordingly. For that, they possess an arsenal of options, such as moving to more favourable conditions, changing the expression of genes involved for instance in detoxification or nutrient use, or implementing developmental processes to survive extreme environmental conditions. It therefore came as no surprise that the studies conducted over the next two decades (until the early 1990s) established protein phosphorylation as a major player in bacterial environmental signalling. However, these early studies essentially showed that bacterial signalling and adaptive responses were mainly dependent on histidine kinases [6]. The only exceptions recognized at the time were the three protein kinases sharing no resemblance with eukaryotic ones (the HPr kinase/phosphorylase, the SpoIIAB from *Bacillus subtilis* and the isocitrate dehydrogenase kinase/phosphatase from *Escherichia coli*) catalysing serine phosphorylation [7–9]. During the second half of the 1990s, the availability of complete genome sequences revealed the widespread presence of eukaryote-like serine/threonine protein kinases (STPK) [10]. Like histidine kinases, they have turned out to participate in key regulatory functions of bacterial physiology (for review, see [11]). Surprisingly, eukaryotic-like tyrosine kinases have not been identified in bacterial genomes. Despite the initial detection of phosphotyrosines in bacterial crude extracts and the characterization of two atypical tyrosine kinases possessing some signatures of the eukaryotic kinase domain (WaaP, MasK) [12,13], it has been widely accepted that tyrosine phosphorylation is confined to Eukarya. However, the characterization in 1997 of a peculiar protein autophosphorylated on tyrosine (Ptk from *Acinetobacter johnsonii*) gave a fresh

* Author for correspondence (c.grangeasse@ibcp.fr).

One contribution of 13 to a Theme Issue 'The evolution of protein phosphorylation'.

impetus in the field. Ptk shares no sequence homology with eukaryotic tyrosine kinases and possesses the Walker A and B ATP/GTP-binding motifs [14,15]. Ptk homologues have since been biochemically and physiologically characterized in several bacterial species, and the presence of homologous genes has been detected in a majority of sequenced bacterial genomes, but not in Eukarya [16]. Therefore, these enzymes idiosyncratic to bacteria have consequently been unified in the same family named bacterial tyrosine kinases (BY-kinases) [17]. BY-kinases account for the vast majority of tyrosine phosphorylation events detected in bacteria, but there are some other kinases that contribute. These include the protein PtkA of *Mycobacterium tuberculosis* that does not possess any signature or pattern related to BY-kinases and eukaryotic tyrosine kinases [18], a histidine-kinase-like protein autophosphorylating on tyrosine (DivL from *Caulobacter crescentus*) and a kinase using a guanidino-tranferase domain that catalyses both arginine and tyrosine phosphorylation (McsB from *B. subtilis* and *Bacillus anthracis*) [19–22].

2. CHARACTERIZATION OF FIRST BY-KINASES

The first indication of a protein-tyrosine kinase activity in bacteria was reported in the case of *E. coli* in which phosphotyrosine was detected in hydrolysates of proteins [23]. This observation was rapidly confirmed in a variety of bacterial species, and in 1996 tyrosine phosphorylation was detected in 22 different bacterial species [24]. However, protein-kinase activities catalysing this reaction were not identified in the initial studies, and proteins phosphorylated on tyrosine were most often characterized only by their molecular mass and isoelectric point. Nevertheless, in some cases, a clear-cut phenotype was associated to tyrosine phosphorylation. For example, it was suggested that tyrosine phosphorylation could be involved, like in eukaryotic cells, in signal transduction, allowing the bacterial cell to adapt to its environment. Indeed, tyrosine phosphorylation was observed to vary during bacterial adaptation to cold and light in *Pseudomonas syringae* and the cyanobacterium *Prochlorothrix hollantica*, respectively [25,26]. Phosphotyrosines were detected in pathogenic mycobacteria, but not in non-pathogenic, thus suggesting a possible function in virulence [27]. The identification of certain phosphotyrosine-proteins also suggested that tyrosine phosphorylation could serve other functions involved in metabolism or motility [28,29]. Unfortunately, all these initial studies were not followed up. From the knowledge that we now have about bacterial tyrosine phosphorylation, it would certainly be justified to go back to some of those studies with new methods of the 'omic' era.

The first characterizations of BY-kinases illustrate perfectly that point. In 1993, the group of A. J. Cozzone used massive amounts of radioactive phosphate for *in vivo* labelling and treatment of proteins with bases and acids to detect a tyrosine phosphorylation signal of an 80 kDa protein from *A. johnsonii*. Subsequent purification of a few micrograms of this phospho-protein required litres of cell culture [14,30]. After screening of an *A. johnsonii* genomic library with a degenerate oligonucleotide probe, they successfully identified the gene sequence and named it *ptk*. The *ptk* gene encodes a membrane protein capable of autophosphorylating on several tyrosine residues but sharing no resemblance with eukaryotic tyrosine-kinase receptors [15]. Instead of the canonical Hanks-motifs of eukaryotic kinase domains [31], Ptk displays homology with other membrane proteins with Walker A and B ATP/GTP-binding motifs, encoded by genes located in exopolysaccharide synthesis loci. This similarity prompted several groups to test Ptk homologues for their tyrosine-autokinase activity. Thus, proteins Wzc and Etk from *E. coli*, AmsA from *Erwinia amylovora*, Orf6 (now renamed Wzc) from *Klebsiella pneumoniae*, ExoP from *Sinorhizobium meliloti* and CpsD from *Streptococcus pneumoniae* were also characterized as possessing tyrosine-autokinase activity [32–35]. However, when analysing the phosphorylation patterns of some of these strains and, for example, *E. coli* K12, no tyrosine autophosphorylation of Wzc and Etk was detected. *etk* and *wzc* genes are located in two distinct capsular polysaccharide loci, expressed either only in pathogenic *E. coli* strains (for Etk) or dependent upon transcriptional regulators of two-component systems (for Wzc) [33]. Further analyses showed unambiguously that the two-component system RcsABC must be activated to allow Wzc expression and the same effect could be achieved by overproduction of the response regulator RcsA [36]. For Etk, such a clear-cut demonstration has not been made, but its expression requires specific growth conditions, including low pH and low magnesium concentration, that are also required for activation of two other two-component systems, the PmrAB and PhoPQ systems [37].

One now understands better why it was so difficult to characterize the first BY-kinase: it was simply not expressed in standard laboratory growth conditions. Once this expression obstacle had been overcome, BY-kinases-mediated tyrosine phosphorylation was extensively studied *in vivo* over the last decade and shown to be a key regulatory device of bacterial physiology [38]. However, as demonstrated by recent analyses of bacterial phosphoproteomes (discussed below), there are still many uncharacterized substrates of BY-kinases and what we know about their role in bacterial physiology probably corresponds to the tip of an iceberg.

3. EVOLUTION OF BY-KINASES: WHENCE DO THEY HAIL?

BY-kinases clearly share no significant sequence homology with Hanks-type kinases that phosphorylate serines, threonines and tyrosines in Eukarya. However, this does not mean that Hanks kinases evolved in Eukarya after the separation from the bacterial kingdom. Genes encoding Hanks-type kinases are also present in almost all sequenced bacterial genomes, and it is therefore reasonable to assume that they were present before the separation from Eukarya [11] or there was lateral transfer of these genes from a eukaryote. Nevertheless, the bacterial Hanks-type kinases catalyse almost exclusively the phosphorylation

of serine and threonine residues, and there are very rare occurrences when they phosphorylate tyrosine [12,13]. One might therefore argue that bacterial Hanks kinases have somehow lost the feature of phosphorylating tyrosines after the separation from Eukarya. But there seems to be no obvious evolutionary benefit gained from such loss of function. A more plausible hypothesis seems to be that ancestral Hanks-type kinases, present in the common ancestor before branching off of Bacteria and Eukarya, must have possessed the capacity to phosphorylate serines and threonines, but not tyrosines. After the separation of Eukarya and Bacteria, tyrosine phosphorylation mechanisms appeared in both kingdoms, which effectively means that tyrosine phosphorylation evolved twice, completely independently. In Eukarya, the evolution of tyrosine kinases took the path of modifying the existing Hanks kinases and creating a slightly altered active site that accommodates tyrosines. So what happened in Bacteria? Active sites of contemporary BY-kinases share signatures known as the Walker motifs with a number of ubiquitous ATP/GTPases [17,39]. Walker motifs found in BY-kinases however differ from the canonical sequences found in ATP/GTP-binding proteins. Indeed, only the GKS/T motif is conserved in the (A/G)xxxxGK(S/T) Walker A motif (phosphate-binding loop or P-loop), whereas the hhhhD canonical Walker B motif is extended to hhhDxxP. A third motif, named A′ and located between motifs A and B, is nearly identical to the Walker B motif (hhhhDXDXR). These three motifs have been shown to be essential for the activity of BY-kinases and substitution of either the Walker A lysine, or any aspartic acid of Walker A′ and Walker B motifs induced a strong decrease of the autokinase activity of BY-kinases [40,41]. Interestingly, the simultaneous presence of these three Walker motifs is found in certain P-loop kinases of small molecules like the dephosphocoenzyme A, but is absent in the majority of them [42]. Still, they are also found in some ATPases closely related in sequence with BY-kinases and belonging to the SIMIBI class [39]. More precisely, BY-kinases exhibit significant sequence similarity with arsenite ATPases (ArsA) and MinD proteins. ArsA proteins are involved in expulsion of arsenite and antimonite ions [43], whereas MinD is involved in determining the position of the new septum during cell division [44]. The extent of sequence similarity with BY-kinases suggests common evolutionary ancestry and it seems plausible to propose that BY-kinases, MinDs and ArsAs evolved by splitting off from an ancestral bacterial ATPase [39]. How soon did this happen, after the separation of Bacteria from Eukarya? To answer this question, we selected a number of bacterial ArsA proteins, MinD proteins and BY-kinases (catalytic domains) and attempted to construct evolutionary trees using different methods. The neighbour joining method produced an evolutionary tree that exhibits three clearly distinct clades corresponding to ArsA, MinD and BY-kinases, and is in more or less perfect accord with the contemporary view on the evolution of bacterial phyla (figure 1*a*). The BY-kinase and MinD ATPase branches are more closely related, whereas ArsA proteins have clearly split off from the rest at an earlier point. The maximum-likelihood tree (figure 1*b*) is in agreement with the neighbour joining tree with respect to positioning of ArsA proteins, but some controversy emerges concerning the separation between MinDs and BY-kinases. Most MinD proteins and BY-kinases are grouped in two separate clades. However, some MinD sequences (from Actinobacteria and alpha Proteobacteria) and BY-kinase sequences (from Cyanobacteria, Firmicutes and Actinobacteria) form a third mixed clade (figure 1*b*, highlighted in orange). It is not uncommon that neighbour joining and maximum-likelihood methods provide evolutionary trees of slightly different topology. Neighbour joining is a simple and robust method, but in a recent benchmark study, maximum-likelihood algorithm was clearly tipped to outperform the competitors with small datasets of under 100 species [46]. Be that as it may, one conclusion that clearly emerges from this analysis is that among the three protein families, ArsA ATPases branched off first, and this node is rooted deeply in the bacterial family tree. By contrast, MinD and BY-kinase families separated more recently and, as suggested by the maximum-likelihood tree, may have still not been fully separated before the branching off of the main bacterial families as we know them today. This observation is in accord with the report by Leipe and co-workers [42] who found that the Walker A′ motif (DXD; see §§4 and 5) is present in MinD ATPases but is not fully conserved in ArsA ATPases. BY-kinases contain the canonical Walker A′, which again puts their evolutionary origin closer to MinDs rather than to ArsAs. When considering the evolution of such closely related protein families, one is well advised to keep an open mind concerning the specific protein functions. A one-gene–one-function paradigm may well prove to be too simplistic to account for these events. BY-kinases are even today still described as polysaccharide co-polymerases by the researchers interested in polysaccharide biosynthesis [47], and are called BY-kinases by those interested in bacterial signalling and tyrosine phosphorylation of protein substrates [17]. Moreover, BY-kinases have phenotypes related to the cell cycle [48] and it would not be utterly unexpected if one day a direct mechanical interplay between BY-kinases and closely related MinD proteins were to emerge in the context of bacterial cell cycle control. This notion is strengthened by the fact that in Firmicutes, where BY-kinases have evolved with an option to dissociate from their trans-membrane modulators, BY-kinases were in some growth conditions found to co-localize with cytosolic substrates rather than with the trans-membrane modulators [49].

4. DESCRIPTION OF BY-KINASES

As indicated above, BY-kinases possess Walker A and B motifs [50], which are characteristics of nucleotide-binding proteins (NTPases) called P-loop proteins [39,42,51]. Because of that, they were initially thought to function as ATPases in the extracellular polysaccharide synthesis pathway and it is likely that this idea would have persisted for a long time without the characterization of the autokinase activity of Ptk

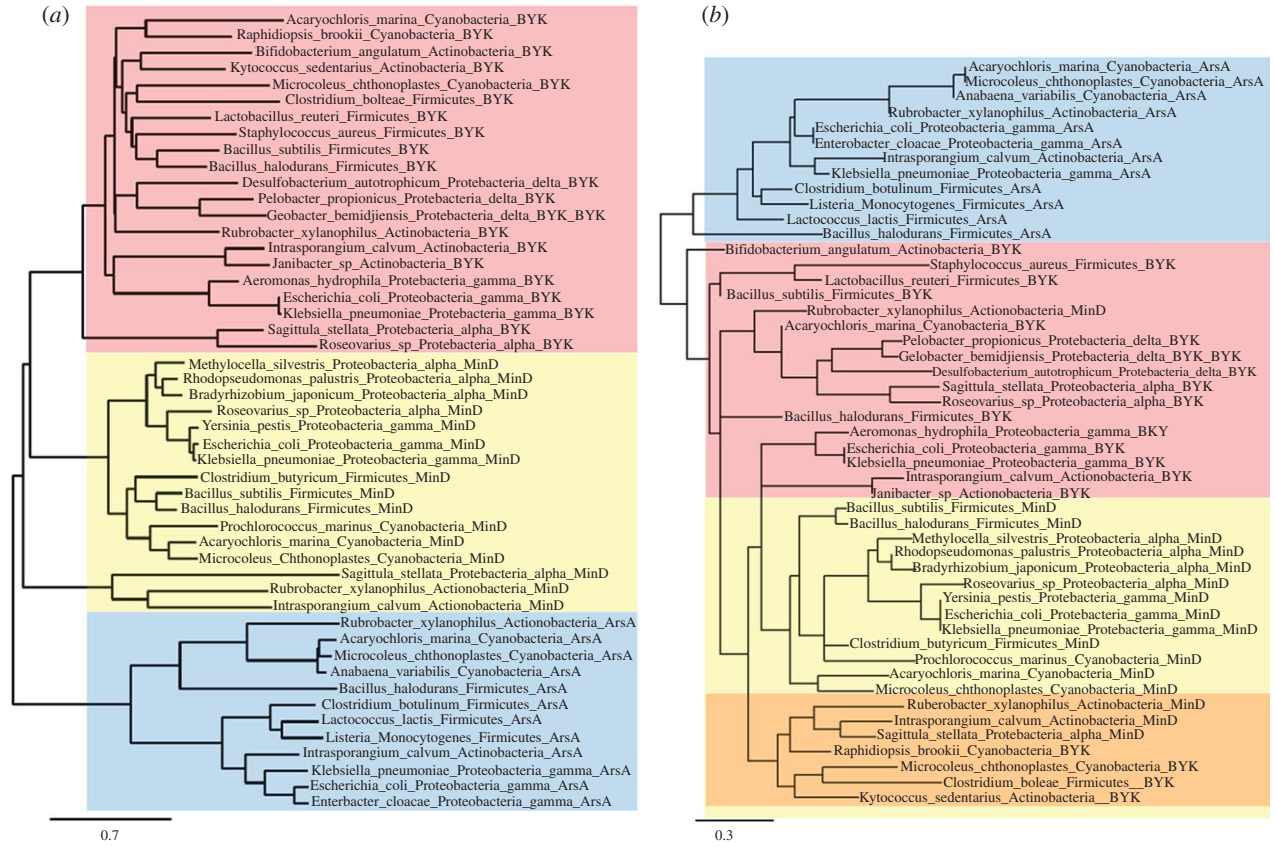

Figure 1. Evolutionary relationship among BY-kinase, ArsA and MinD protein families. Protein sequences were obtained from the following species. BY-kinases (catalytic domain): *Bacillus subtilis, Bacillus halodurans, Escherichia coli, Klebsiella pneumoniae, Pelobacter propionicus, Geobacter bemidjiensis, Sagittula stellata, Roseovarius* sp., *Acaryochloris marina, Microcoleus chthonoplastes, Aeromonas hydrophila, Raphidiopsis brookii, Clostridium bolteae, Lactobacillus reuteri, Staphylococcus aureus, Desulfobacterium autotrophicum, Bifidobacterium angulatum, Kytococcus sedentarius* and *Janibacter* sp. ArsA ATPases: *Lactococcus lactis, Clostridium botulinum, Listeria monocytogenes, Enterobacter cloacae, Anabaena virabilis, M. chthonoplastes, Acaryochloris marina, Intrasporangium calvum, Rubrobacter xylanophilus, K. pneumonia, E. coli* and *B. halodurans*. MinD proteins: *B. subtilis, B. halodurans, E. coli, K. pneumonia, Sagittula stellata, Roseovarius* sp., *R. xylanophilus, I. calvum, Acaryochloris marina, Microcoleus chtonoplastes, Prochlorococcus marinus, C. butyricum, Yersinia pestis, Rhodopseudomonas palustris, Methylocella silvestris* and *Bradyrhizobium japonicum*. (*a*) Neighbour joining tree obtained with BioNJ. BY-kinases are highlighted in pink, ArsA proteins in blue and MinD proteins in yellow. (*b*) Maximum-likelihood tree obtained with PhyML. Same colour-coding was used; a clade with mixed BY-kinases and MinD proteins is in orange. Alignments were prepared with ClustalW and trees were visualized using TreeDyn. All cited software is available at http://www.phylogeny.fr/ [45].

from *A. johnsonii*. An additional motif located at the very C-terminal end of the kinase domain further distinguishes BY-kinases from other P-loop proteins [52]. This motif, named the tyrosine cluster (YC), varies in length (from 10 to 20 amino acids) and contains several tyrosine residues (from three to seven) that constitute the autophosphorylation sites of BY-kinases. Albeit designated as autophosphorylation, the event actually necessitates at least two BY-kinase molecules, one phosphorylating the tyrosine cluster of the other, and *vice versa*. It is therefore also referred to as trans-phosphorylation. The sequence of the tyrosine cluster motifs is not conserved among BY-kinases. It has been shown that each tyrosine could be phosphorylated independently of the others without any preferential order [53]. The presence of these four motifs (Walker A, Walker A′, Walker B and YC) was used to constitute a typical signature of BY-kinases [16]. Bioinformatics resources allowed the annotation and collection of more than 2000 genes in sequenced bacterial genomes that are proposed to belong to the family of BY-kinases. A database (BYKdb) dedicate to BY-kinases is available at http://bykdb.ibcp.fr/BYKdb/ [54].

In Proteobacteria, BY-kinases are essentially found in the form of a membrane protein displaying a large extracellular loop and a cytosolic catalytic domain [17]. By contrast, BY-kinases of Firmicutes possess the cytoplasmic catalytic domain in a separate polypeptide that interacts with a separate membrane protein, homologous to the extracellular domain of proteobacterial BY-kinases, but shorter in size [55]. These two polypeptides are encoded by adjacent genes in Firmicutes. Firmicute-type cytosolic BY-kinases, however, require the membrane protein to undergo autophosphorylation [55]. More precisely, the cytosolic and C-terminal end of the membrane protein (about 30–50 residues) mimics the juxtamembrane region of BY-kinases from Proteobacteria and is sufficient to promote BY-kinase activation [56]. In agreement with this observation, the isolated cytoplasmic domain of a BY-kinase from Proteobacteria is also active *in vitro*. The cause and functional consequences of this separation remain unclear, although some recent leads suggest that it could allow

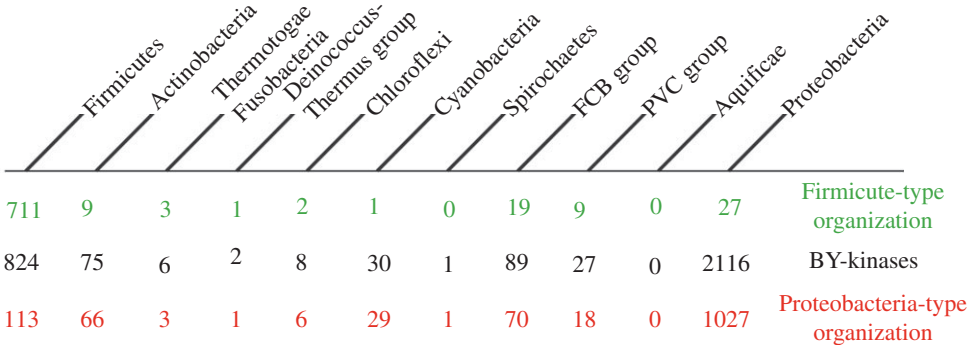

Figure 2. BY-kinase architectures among bacterial phyla. The number of BY-kinases predicted in the BYKdb [54] for each bacterial phyla is indicated in black. Those displaying a Firmicute-type organization are numbered in green, whereas those with a Proteobacteria-type organization are numbered in red.

interaction with other proteins to participate in additional regulatory functions. The two structural prototypes, Firmicute- and Proteobacteria-type architectures, are not strictly confined and some representatives of both can be found in other bacterial phyla, as predicted in BYKdb (figure 2).

5. STRUCTURAL ASPECTS OF BY-KINASES

The first structural insights concerning BY-kinases came from the low-resolution electron microscopy structure (14 Å) of the *E. coli* BY-kinase Wzc [57]. Wzc was described as 'molar tooth and its roots' forming a tetrameric ring that oligomerizes *via* the periplasmic domains and with non-interacting cytosolic domains freely protruding in the cytosol. The same organization was observed in the electron microscopy structure of Wzc in complex with the outer-membrane translocon Wza [58]. The Wzc–Wza complex formed a ring-shaped structure spanning the periplasm and connecting the inner and outer membranes of the bacteria. However, this structural organization did not explain the trans-autophosphorylation mechanism demonstrated previously [52].

More recently, the crystal structures of the cytoplasmic domains of Etk, a paralogue of Wzc in *E. coli* [59], and of the orthologue CapB from the Firmicute *Staphylococcus aureus* [53] have been solved. Both structures displayed a Rossman fold typical of P-loop proteins [51]. Despite catalysing the same ATP-dependent phosphorylation of tyrosine residues, Etk and CapB did not show any structural similarity with eukaryotic tyrosine kinases [60]. CapB was fused to the C-terminal extremity (29 residues) of the transmembrane activator CapA that was shown to enhance the affinity of CapB for ATP and to stimulate its kinase activity [56]. When produced as a His-tagged recombinant protein in *E. coli*, Etk was phosphorylated, as well as the CapAB chimaera. Mass spectrometry analysis demonstrated that the four tyrosines of the CapB Y-cluster were heterogeneously phosphorylated [53]. In both structures, the crystallized kinase domain was monomeric and the phosphorylated Y-cluster was either disordered or involved in non-physiological crystal packing interactions. Again, these two structures did not give any insight into the trans-autophosphorylation mechanism. They however gave some interesting clues on the activation mechanism of BY-kinases from Firmicutes and Proteobacteria. As usually observed in P-loop proteins, conserved lysine and threonine residues from the Walker A motif and the aspartates from the Walker A′ and B motifs interacted with the phosphate moiety of the bound nucleotide and the associated magnesium ion (figure 3). Interestingly, the juxtamembrane N-terminal region of Etk_{cyto} superimposed with the C-terminal fragment of the trans-membrane activator CapA in the structure of the CapAB chimaera. The side chain of the penultimate residue of CapA, Phe221, was stacked on the base part of the bound ADP molecule, making a strong hydrophobic interaction stabilizing nucleotide binding and thus explaining the activation mode of CapB (figure 3). The C-terminal sequence of CapA orthologues is poorly conserved and it is not clear if this activation mechanism is conserved in all BY-kinases from Firmicutes. In Etk, the adenine base is stacked on the side chain of a phenylalanine residue located in another region of the protein, directly following the Walker A motif. This residue is conserved among BY-kinases from Proteobacteria, but not from Firmicutes, suggesting a distinct nucleotide-binding mode. Another difference observed between the two phyla concerns a short stretch of about 20 basic residues, called the RK-cluster, present in Etk but not in CapB [59]. The RK-cluster is 'inserted' in the region corresponding to the junction between CapA and CapB. The RK-cluster is also observed in other BY-kinases from Proteobacteria and it has been shown to be essential for the function of Wzc. Its N-terminal part is involved in the binding of the base part of the nucleotide whereas its disordered C-terminal part has been shown to be essential for the co-polymerase activity of Wzc in the exopolysaccharide synthesis machinery [61]. Its exact role, however, remains to be elucidated. Etk, like its paralogue Wzc, possesses outside its C-terminal Y-cluster an additional tyrosine that had been suggested to be involved in an intramolecular phosphorylation step, preceding trans-autophosphorylation of the Y-cluster [52]. The analysis of the Etk structure suggested that that this tyrosine points into the active site, and once phosphorylated it could flip out of the active site to allow ATP binding and subsequent phosphorylation of the Y-cluster [59,62]. However, its orientation seems quite incompatible with phosphate transfer, and this residue is not conserved

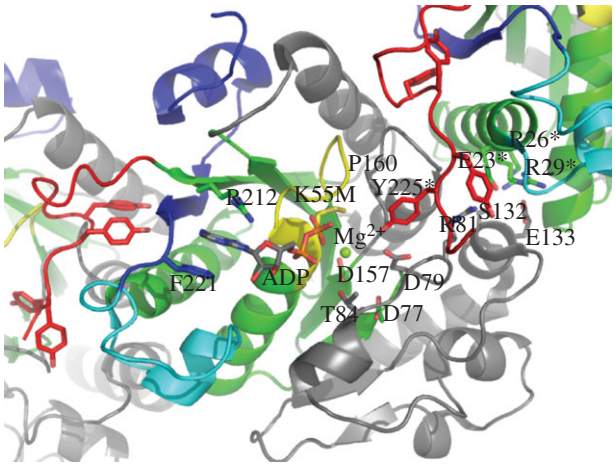

Figure 3. Close view of the CapAB active site. Bound ADP-Mg^{2+} is shown as sticks in the P-loop (in yellow) with the inactivating K55M mutation of the Walker A motif. The phosphorylatable residue Y225* from the neighbouring Y-cluster is highlighted as well as residues D157 and P160 from the extended Walker B motif and residues D77, D79 and R81 from the Walker A′ motif. Residue R81 together with the conserved residues S132 and E133 participate in octamer contact with residues E23*, R26* and R29* from the neighbouring subunit. The adenine base of the bound nucleotide is shown in stacking between residues R212 and the penultimate residue F221 from the CapA activator (in blue).

among all BY-kinases from Proteobacteria. Therefore, this putative additional phosphorylation step remains controversial and needs to be further investigated.

In order to decipher the trans-autophosphorylation mechanism of the Y-cluster, inactive mutants of the cytoplasmic domains of Wzc [61] and of the chimaera CapAB [53] were used. The P-loop lysine was replaced by a methionine, a mutation known to inhibit phosphate transfer without impairing ATP binding in P-loop proteins [63]. The crystal structures of these two non-phosphorylated kinase domains revealed the formation of a conserved ring-shaped octamer. In both cases, the last tyrosine of the Y-cluster of each subunit was bound into the active site of the neighbouring subunit, pointing towards the bound nucleotide, and thus explaining the trans-autophosphorylation mechanism of the BY-kinases (figure 3). The aromatic ring of the tyrosine is in a sandwich interaction between the proline residue of the extended Walker B motif and a lysine residue immediately following the Walker A′ motif of the neighbouring monomer. The second aspartate of this hhhhDXDXR motif directly interacts with the phosphorylatable hydroxyl of the tyrosine, most probably acting as an acid catalyst (figure 3). The flexibility of the C-terminal extremity is in agreement with the heterogeneous phosphorylation pattern observed by mass spectrometry but it is not clear why in both crystal structures the last tyrosine of the Y-cluster was preferentially bound into the neighbouring active site. The interaction of the Y-cluster of one subunit with the active site of the neighbouring subunit represents 50 per cent of the surface contact between two monomers. The second half of the interface encompasses the conserved arginine of the Walker A′ motifs and played a crucial role in stabilizing the octamer (figure 3) [53,61]. This region of contact involves others residues that are conserved among all bacteria, suggesting that the octamer is a conserved feature of BY-kinases.

Taken together, the crystal structures of the phosphorylated and non-phosphorylated forms of the BY-kinases suggest that the kinase domains dissociate upon phosphorylation, thus allowing endogeneous substrate proteins to interact with the active site of BY-kinases. However, in Proteobacteria, BY-kinases are trans-membrane proteins and the N-terminal periplasmic domains most probably remain associated in an oligomeric ring [64]. The co-polymerase function of the BY-kinases in the exopolysaccharide synthesis machinery has been shown to be dependent on a cyclic process oscillating between the non-phosphorylated and the phosphorylated form of the protein (see later text). This would thus correspond to cycling between a closed octameric ring-shaped conformation and an open conformation with independent cytoplasmic kinase domains linked to a trans-membrane crown, as observed in the low-resolution structure of phosphorylated Wzc [57]. In Firmicutes, the separation of the two domains into distinct polypeptide chains suggests that the phosphorylated kinase domains could dissociate from their cognate trans-membrane activator and interact freely with the endogenous substrates and/or cytosolic modulators.

6. COMPARISON WITH BACTERIAL ATP-BINDING PROTEINS

As indicated already, the closest structural homologue of BY-kinases is the bacterial cell division regulator MinD, which was successfully used as initial model for the resolution of the structure of *Staphylococcus aureus* CapAB. The Y-cluster of the BY-kinases has however no equivalent in the ATPase MinD, which does not autophosphorylate and has never been reported to phosphorylate any other protein. Interestingly, the fold is conserved between the two proteins with an r.m.s. distance of 1.66 Å over 158 aligned Cα atoms, but the C-terminal region of MinD superimposes with the N-terminus of the BY-kinase (figure 4). Such circular permutations can appear in evolution upon duplication of a precursor gene, when one or the other extremity of the resulting tandem protein is lost after introduction of a stop codon [65]. By contrast, BY-kinase structure is much more different from that of an atypical bacterial serine kinase, the bifunctional HPr kinase/phosphorylase (HPrK/P), which belongs also to the P-loop proteins. Indeed, the catalytic domain of HPrK/P displays a Rossman fold with an unusual topology similar to that already observed in the phosphoenolpyruvate carboxykinase [66] and forms a dimer of trimers surrounded by three dimers of N-terminal domains of unknown function [67]. The similarity between the HPrK/P and BY-kinases is therefore limited to the conserved P-loop type nucleotide-binding domain (figure 4) and they consequently belong to different classes of P-loop proteins. Disappointingly, the sequence and structure comparison of the BY-kinases with MinD ATPases and the HPrK/Ps does not provide any clear clues for distinguishing between P-loop proteins with and

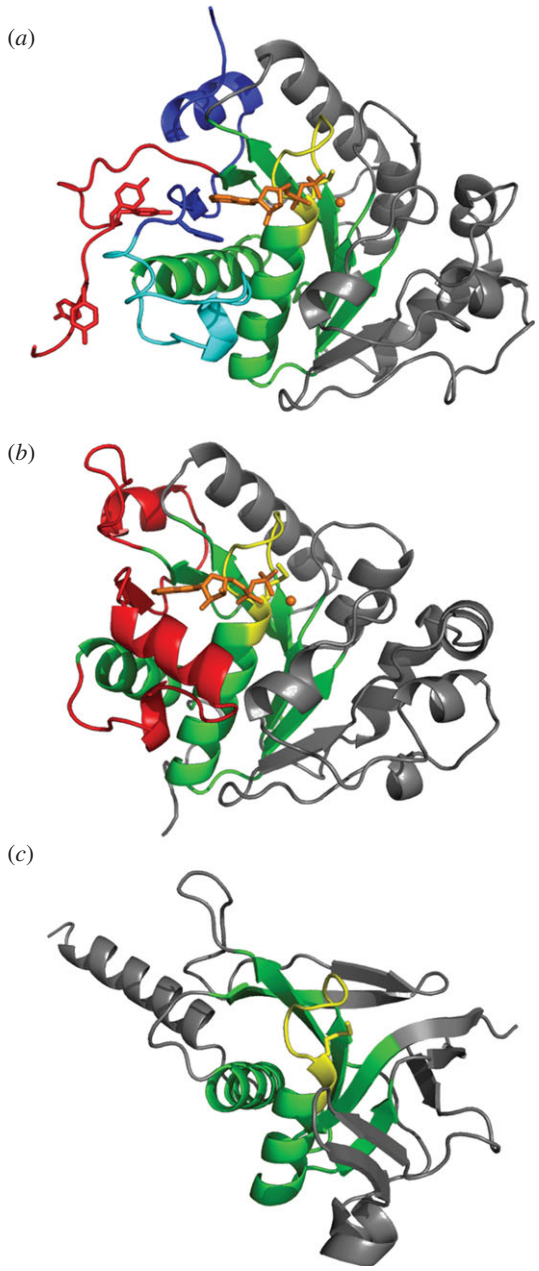

Figure 4. Structural comparison of CapAB with MinD and HPrK/P. The core of the BY-kinase CapAB conserved with MinD and HPrK/P is shown in green with the P-loop in yellow and the ADP-Mg in orange. (*a*) CapAB with the Y-cluster in red, the CapA fragment in blue and the N-terminus of CapB in cyan. (*b*) MinD with the C-terminal extremity superimposing on the CapA fragment and N-terminus of CapAB in red. (*c*) Catalytic domain of *Lactobacillus casei* HPrK/P with the conserved P-loop core in green and yellow (in the absence of nucleotide).

without protein kinase activity. It would be of utmost importance to find such clues in the future, because P-loop proteins are one of the largest protein families in all kingdoms of life, representing for instance 5 per cent of the human genome [68].

7. BIOLOGICAL ROLE OF BY-KINASE AUTOPHOSPHORYLATION

In the first phase of their characterization, BY-kinases were shown to function essentially as autophosphorylating enzymes and this feature was associated with their role in the biosynthesis and export of extracellular polysaccharide. Although the different groups working in the field agreed that the autokinase activity was required in this process, there were initially some apparent discrepancies. Thus, in *E. coli* K30, a positive regulation of the group I polysaccharide capsule biosynthesis by autophosphorylation of Wzc was observed [32], whereas in *E. coli* K12 production of the exopolysaccharide colanic acid was decreased when Wzc was phosphorylated [36]. When analysing the Gram-positive bacterium *S. pneumoniae*, a similar divergence was reported. In the RX1 strain, the BY-kinase CpsD was shown to be dephosphorylated to promote capsule synthesis, whereas an opposite situation was found in the *S. pneumoniae* D39 strain [34,69]. In *Acinetobacter lwoffii*, emulsan polysaccharide is produced to a relatively low extent when Wzc is phosphorylated, whereas in *E. amylovora* the overproduction of the low-molecular-weight-phosphatase AmsI induces a strong reduction in the exopolysaccharide amylovoran synthesis [70,71]. However, the identification of several bacterial phosphotyrosine phosphatases crucial for polysaccharide production shed new light on the regulatory function of BY-kinases in polysaccharide production (for example, the low-molecular-weight acidic phosphotyrosine phosphatase Wzb of *E. coli* and the manganese-dependent phosphotyrosine phosphatase CpsB of *S. pneumoniae* [72,73]). Hence, it was shown that both the phosphorylated and the non-phosphorylated forms of BY-kinases were alternately required for polysaccharide polymer production, explaining thus the divergent initial observations [74]. On this basis, the current model is that cycling between phosphorylated and non-phosphorylated forms of BY-kinases is required for proper synthesis and export of the polysaccharide polymer. According to their regulatory function in polysaccharide production, BY-kinases were classified as polysaccharide co-polymerases assisting the activity of the polymerase of the polysaccharide assembly membrane machinery [75]. However, the detailed mechanism by which BY-kinases interact with this machinery remains obscure. Several hypotheses have been proposed. Structural characterization of BY-kinases CapB and Wzc from *Staphylococcus aureus* and *E. coli*, respectively, showed that the non-phosphorylated form of BY-kinases forms a ring-shaped octamer which upon autophosphorylation dissociates to monomers. The monomers would remain in interaction with the trans-membrane domains. Therefore, an interesting possibility would be that BY-kinases might simply influence the amount of polysaccharide by serving as molecular scaffolds for the other proteins of the machinery (figure 5) [47]. Phosphorylation/dephosphorylation of BY-kinases would thus behave as a conformational switch that would most probably be transmitted to their trans-membrane domain. In turn, the latter would be affected in their interaction with other protein components of the polysaccharide assembly machinery, such as the polysaccharide unit polymerase, the lipid-linked repeat unit flippase or the lipid-sugar transferase [53]. Alternatively, one should also consider that BY-kinases could form a channel across the cytoplasmic membrane, large and hydrophilic enough to allow the polysaccharide polymer to cross the

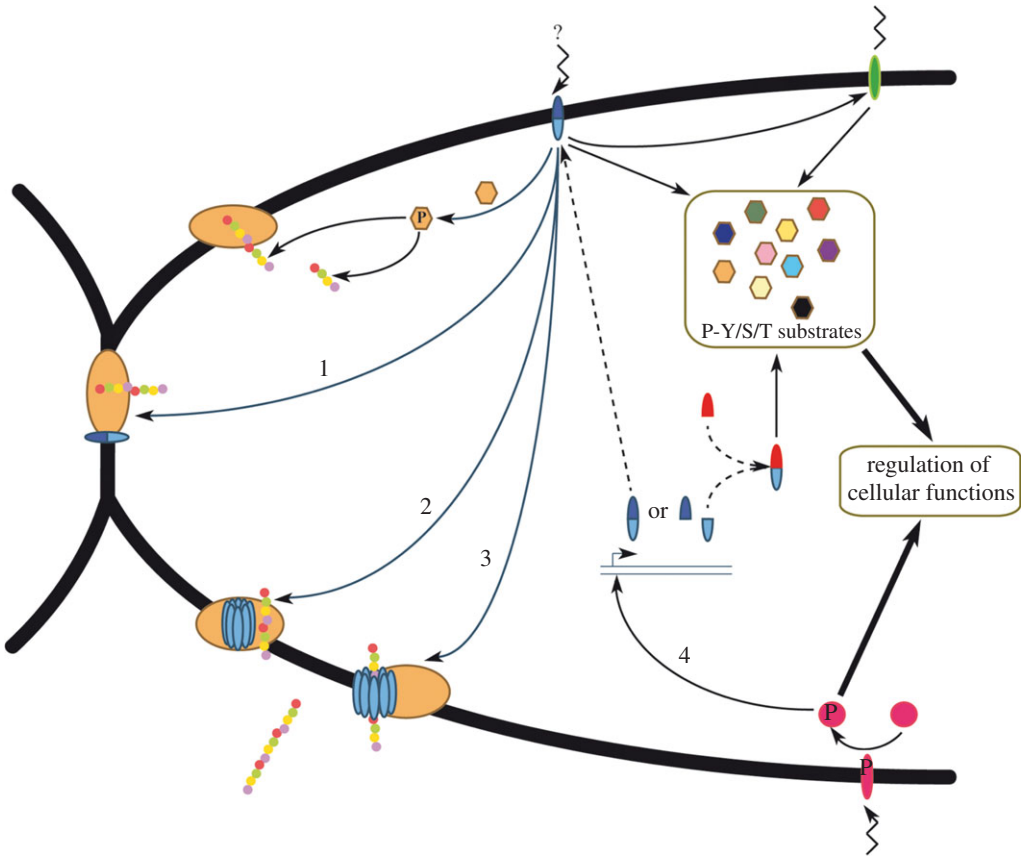

Figure 5. Role of BY-kinase-mediated phosphorylation in the bacterial cell. The polysaccharide membrane assembly machinery is shown as salmon-pink ovals. BY-kinases (blue oval) form a ring-shaped octamer, which upon autophosphorylation dissociates to monomers. This conformational switch is responsible for controlling polysaccharide biosynthesis and export, but the underlying mechanism remains unclear. Three possible scenarios, are shown with arrows numbered as followed: (1) targeting to the septum; (2) scaffolding of the assembly machinery; (3) forming a channel across the cytoplasmic membrane. Enzymes involved in polysaccharide unit synthesis are shown as a salmon-pink hexagon. Their phosphorylation by BY-kinases regulates the production of the polysaccharide polymer. Multicoloured hexagons represent proteins being phosphorylated by BY-kinases, and/or eukaryotic-like serine/threonine kinases (green oval) and/or histidine kinases of the two-component systems (fuchsia oval). Arrow 4 shows that two-component systems could regulate the expression of BY-kinases. Question mark shows the hypothetical stimulus triggering the activity of BY-kinase. The activation of cytoplasmic BY-kinases by proteins (red half-oval) distinct from their transmembrane membrane (dark blue half-oval) is also shown.

membrane [61]. Although cryo-electron-microscopic studies made with Wzc of *E. coli* K30 suggested that this channel would be filled by a lipid bilayer [57], one should note that in Gram-negative bacteria another protein is required for the polysaccharide to go across the outer membrane. This protein, named Wza in *E. coli*, also forms an octameric complex and was shown to interact directly with the Wzc oligomer, thus forming a complex that spans both the inner and the outer membranes [58,76]. This finding might suggest that the Wza–Wzc complex could form an octameric channel, directly constituting the polysaccharide secretion system (figure 5). This hypothesis is further supported by the structural similarities observed between the extracellular domain of BY-kinases and another class of polysaccharide co-polymerase involved in lipopolysaccharide biosynthesis [77]. These latter proteins were shown to regulate the length of O-antigens and they self-assembled into ring-shaped oligomers of variable sizes forming a large internal cavity [64]. BY-kinases would therefore play a more central role than assisting the Wzy polymerase in the translocation of capsular polysaccharides. Very recently, another study added a new facet to the role of BY-kinases in extracellular polysaccharide production. The BY-kinase CpsD that was previously found to be required for *S. pneumoniae* capsular polysaccharide production has been shown to localize at the bacterial division septum [78]. The authors further showed that in the absence of CpsD (named Wze in this study), capsular polysaccharides were still produced, but absent from the division septum. On this basis, it could be presumed that BY-kinases might also function as spatial regulators of capsular polysaccharide biosynthesis (figure 5). Considerable efforts are currently directed at understanding the exact role played by BY-kinases in polysaccharide export and the associated molecular mechanisms.

8. BY-KINASE-MEDIATED PHOSPHORYLATION OF BACTERIAL PROTEINS

Beyond their autophosphorylation, BY-kinases were shown to phosphorylate endogenous protein substrates on tyrosine residues and consequently to control some cellular processes. The first identified BY-kinase substrates were, quite serendipitously, found to participate in polysaccharide production (figure 5).

They are the uridine diphosphate (UDP)-sugar dehydrogenases, and their phosphorylation by BY-kinases has been documented in *E. coli*, *B. subtilis* and *Staphylococcus aureus* [37,55,79,80]. Upon phosphorylation, their dehydrogenase activity is enhanced. In *E. coli,* Wzc-mediated phosphorylation of Ugd has further been shown to regulate the amount of the exopolysaccharide colanic acid produced, whereas when phosphorylated on the same tyrosine residue by Etk, the synthesis of a modified sugar (UDP-4-amino-4-deoxy-L-arabinose) is increased. This sugar is a crucial element in bacterial resistance to antibiotics such as polymyxin and cationic peptides of the innate immune system, and Etk-mediated phosphorylation of Ugd was shown to efficiently induce bacterial resistance [37]. Ugd phosphorylation by the two BY-kinases Wzc and Etk is therefore at the crossroads of two distinct biosynthetic pathways, illustrating thus the regulatory potential of tyrosine phosphorylation in bacterial physiology. The molecular mechanism of activation of UDP-sugar dehydrogenases by BY-kinases remains not clearly understood and it could involve precise positioning of either the catalytic amino acid or the phosphorylated tyrosine with respect to the bound substrate in the active site [81]. To illustrate this, the recent characterization of the structure of CapO of *Staphylococcus aureus* suggests that the phosphorylated tyrosine could participate in the correct positioning of the catalytic cysteine residue [82]. Other proteins involved in polysaccharide production were shown to be phosphorylated by BY-kinases. One could cite the *Streptococcus thermophilus* phospho-glycosyltransferase EpsE and the *K. pneumoniae* undecaprenolphosphate glycosyltransferase WcaJ that are necessary for capsular polysaccharide production [83,84]. However, in the absence of structural information, the activation molecular mechanism remains unknown. Nevertheless, note that by contrast with UDP-sugar dehydrogenases, which retain basal activity upon Tyr to Phe mutations at the phosphorylation site, mutation of the phosphorylation site of the phospho-glycosyltransferase EpsE seems to induce its inactivation.

BY-kinases are also capable of phosphorylating other endogenous proteins substrates. The BY-kinase Etk was found to phosphorylate the heat-shock sigma factor σ32 and the antisigma σ E factor RseA and thus to participate in the heat shock response [85]. Similarly, the BY-kinase Wzc was found to phosphorylate the integrase (Int) of certain coliphages, thus suggesting that lysogenization could be regulated by Wzc-mediated phosphorylation of Int [86]. Several BY-kinases, not encoded by genes of polysaccharide loci, were also shown to phosphorylate diverse proteins or to be involved in other cellular processes. The BY-kinase PtkA of *B. subtilis* was shown to phosphorylate several proteins in addition to the UDP-sugar dehydrogenases TuaD and Ugd. Single-stranded DNA binding proteins SsbA and SsbB proteins are phosphorylated on tyrosine and this stimulates their ability to bind DNA [87]. Considering that cells deficient for PtkA expression exhibited defects in the initiation of DNA replication and chromosome distribution, PtkA could be involved in regulating DNA metabolism [48]. The single-stranded DNA exonuclease YorK and the aspartate semialdehyde dehydrogenase Asd are also activated by PtkA-dependent phosphorylation [49]. A variety of proteins phosphorylated by PtkA are still of unknown function, but, interestingly, a number of them displayed aberrant cellular localization in cells deficient for PtkA expression [49]. This suggested that besides controlling enzymes activity, BY-kinases would have another mode of action by ensuring proper localization of their targets. In the coming years, the use of cell biology approaches should help us to understand the role of BY-kinase mediated tyrosine phosphorylation and decipher the underlying regulatory processes.

9. FUTURE PROSPECTS AND CHALLENGES

In the last 15 years, evidence was provided that tyrosine phosphorylation of proteins is an important post-translational modification involved in the regulation of different biological processes in bacteria [88]. BY-kinases are widespread and account for most tyrosine phosphorylation events in bacteria. Nevertheless, numerous aspects remain obscure and it seems that we are only at the onset of understanding the biological significance of tyrosine phosphorylation.

One big question concerns the regulation of BY-kinase activity. The presence of a large extracellular loop suggests, by analogy to eukaryotic tyrosine kinases receptors, that the outside domain of BY-kinase could transmit signals, thus affecting the kinase function. Unfortunately, such signals remain to be identified. Generally speaking, extracellular signals are known for some two-component systems, but are unknown for a vast majority of other bacterial protein kinases [89]. The interaction of the extracellular BY-kinase domain with the polysaccharide translocation complex may prevent it 'sensing' other signals. On the other hand, one can reasonably argue that the very interaction with the translocation complex could indeed act as a signal triggering BY-kinase activity. A recent study showed that distinct mutations in the extracellular domain of the BY-kinase ExoP of *Sinorhizobium meliloti* either increased or abolished ExoP autophosphorylation [90]. It suggested that tyrosine autophosphorylation of ExoP could be modulated by conformational changes of the N-terminal domain possibly mediated by protein–protein interactions inside the polysaccharide assembly complex. Nevertheless, this possibility is unlikely to be valid for the phosphorylation of the other BY-kinase substrates not involved in polysaccharide production. Structure predictions showed that the extracellular domain of BY-kinases from Proteobacteria would possess more β-structures than the extracellular domain of BY-kinases from Firmicutes [77]. It is tempting to speculate that this feature would confer an additional function to the periplasmic domain of BY-kinases of Proteobacteria, namely a sensor activity, to allow phosphorylation of other targets not involved in polysaccharide production. In Firmicutes, the 'split in two' BY-kinase organization would allow the cytoplasmic domain to interact with other activating proteins. This latter possibility is supported by the finding that under certain growth conditions, the BY-kinase PtkA dissociates from the cognate membrane domain, becomes cytosolic and interacts with other cytosolic proteins that are not its substrates, but modulate its

Table 1. List of phosphotyrosine-peptides reported in bacterial phosphoproteomic analyses. p, indicates phosphorylated S, T or Y.

species	proteins	functions	peptide sequences
Escherichia coli	AsnS	asparaginyl-tRNA synthetase	KFENPVpYWGVDLSSEHER
	Dps	DNA protection during starvation protein	SpYPLDIHNVQDHLK
	GroS	chaperonin, small subunit of GroESL	VGDIVIFNDGpYGVK
	Ppa	inorganic pyrophosphatase	AQIAHFFEHpYK
	TnaA	tryptophanase/L-cysteine desulfhydrase	NIFGpYQYTIPTHQGR
	TufA	elongation factor Tu	GYRPQFpYFR
	YgfB	predicted protein	NIAQLGYDEDEDQEELEMSLEEIIEpYVR
	RpsD[a]	30S ribosomal subunit protein S4	VVNIAp(SY)QVSPNDVVSIR pSDLSADINEHLIVELp(YS)K
	RpsE	ribosomal subunit protein S5	IFpSFpTALTVVGDGNGRVGFGpYGK
	RpsG	ribosomal subunit protein S7	VGGpSpTpYQVPVEVRPVR
	RpsL	ribosomal subunit protein S12	VpYTpTTPKKPNpSALRK
	RpsR	ribosomal subunit protein S18	NpYIpTEpSGKIVPSR
	RplE[a]	ribosomal subunit protein L5	LMpTEFNp(YNS)VMQVPR GNp(YS)MGVREQIIFPEIDYDKVDR
	RplM	ribosomal subunit protein L13	DWpYVVDATGK HKAEpYp(TPHVDT)GDYIIVLNADK AEp(YT)PHVDpTGDpYIIVLNADK
	RplP	ribosomal subunit protein L13	VLpYEMDGVPEELAR
	RplU	ribosomal subunit protein L21	MpYAVFQSGGK
	RplV	ribosomal subunit protein L22	VSQALDILpTpYpTNK
Bacillus subtilis	AhpF[a]	alkyl hydroperoxide reductase (large subunit)	RLp(YS)LPNVTVVK
	Asd	aspartate-semialdehyde dehydrogenase	KApYGLNK
	Eno[a]	enolase	P(YPIIS)IEDGLDENDWEGHK
	InfA	initiation factor IF-I	VTVELSPpYDLTR
	LctE	L-lactate dehydrogenase	NAApYhIIEK
	OppA	oligopeptide ABC transporter (binding lipoprotein)	DGSLHVEPIAGVp(YWY)K
	RocA[a]	delta-1-pyrroline-5 carboxylate dehydrogenase	p(TVTY)AHEPFTDFTEAK
	YjoA	conserved hypothetical protein	EMGHTELPFpYQQR
	YnfE	conserved hypothetical protein	MDEILKQYMVLpYKK
	YorK	single-strand DNA-specific exonuclease	EFILQLDGpYK
	YvyG	putative flagellar protein	pYIQAITQTEDDRIK
	YwqF[a]	UDP-glucose dehydrogenase	LNFETp(SY)EK
Streptococcus pneumoniae	Tuf	translation elongation factor Tu	FKGEVpYILTK
	PgK	phosphopglycerate kinase	VLIGGGMTYTFpYKAQGIEIGNpSLVEEDK
	PfkA	6-phosphofructokinase	MEDIVApSIKAGpYECGKK
	PflB	formate acetyltransferase	ISGpYCVNpTKpYLTPEQK
	GlnA	glutamine synthetase, type I	HApYNYpTAIMNPpTVNSYKR
	PurA	adenylosuccinate synthetase	pYQGGDNAGHpTIVIDGKK
	StkP	serine/threonine protein kinase	VTSVAMPSpYIGpSSLEFTK

(*Continued.*)

Table 1. (*Continued.*)

species	proteins	functions	peptide sequences
	PrfA	petide chain release factor 1	pYAEAQGWRFEVMEApSMNGVGGFKEVVAMVSGQSVYpSK
	CbpC	choline binding protein C	TYYFGDIGEMVVGWQYLEIPGTGpYR
	Spr0084	conserved hypothetical protein	pYVpSKNELTQAEVIER
	InfA	initiation factor IF-I	NpYIRILAGDRVTVEMSPYDLTR
	FtsA	cell division protein	LEMRGLLpYpTGPR
	LytA	autolysin	pTGWVKpYKDpTWYYLDAK
Streptomyces coelicolor A3	SCO3843[a]	hypothetical protein	PGGp(YGY)PQPAGGQQPPAAPAPGGR
	SCO4973	putative uncharacterized protein	pYGGTEVK
	SCO5128[a]	putative membrane protein	GGAAp(YPSGT)PPYGTPTASDAGADAGRKTEp(LTT)R
	SCO5475	hypothetical protein	EAHAPAPAQAPAGGp(YGY)PPAGSR
	NarH	putative nitrate reductase	YLGVVLpYDADK
Lactococcus lactis	FusA[a]	elongation factor G	LYDGp(SY)HDVDSSETAFK
	GlnA	glutamine synthase	RLVPGYEAPVpYVAWAGR
	GroEL[a]	Chaperonin GroEL	Pp(SY)GSPLITNDGVTIAK
	MalE[a]	ABC-type sugar transport system	TPAp(SQY)DSQLK
	PknB	serine/threonine protein kinase	IMPANLINPpYDpTKPLIDKK
	Pta[a]	phosphotransacetylase	IAQRLGNFEAIGPILQGLNAPVp(SDLSRGSNEEDVY)K
Klebsiella pneumoniae	AhpC	alkyl hydroperoxide reductase	AAQpYVASHPGEVCPAK
	atpD[a]	ATP synthase subunit beta	AAPp(SY)EELSSSQELLETGIK
	CsrA	carbon storage regulator	IQAEKSQQSSpY
	DeoC	deoxyribose-phosphate aldolase	AAIApYGADEVDVVFPYR
	Efp	elongation factor P	ATpYYSNDFR
	FimA	type-1 fimbrial protein, A chain	YpYATGAATAGIANADATFK
	GalU	UTP-glucose-1-phosphate uridylyltransferase	GLGHAVLCAHPVVGDEPVAVILPDVILDEpYESDLSR
	ManB	phosphomannomutase	GELGEELNEDIApYR
	Mlc	putative NAGC-like transcriptional regulator	DALpYNGSLLIR
	PgK	phosphoglycerate kinase	pYAALCDVFVMDAFGTAHR
	PpsA	phosphoenolpyruvate synthase	LpYTSLGDAAVGR
	RplU	50S ribosomal protein L21	MpYAVFQSGGK
	RpoB[a]	DNA-directed RNA polymerase, beta subunit	P(STGSY)SLVTQQPLGGK
	RpoA	DNA-directed RNA polymerase alpha subunit	IApYNVEAAR
	RpsI	30S ribosomal protein S9	AENQpYYGTGR
	WcaJ	putative UDP-glucose lipid carrier transferase	ATLpYHR
	YhaH	putative cytochrome	FGPDPKPFSpY
	GpJ	putative prophage baseplate assembly protein	FNALpYEAIHVQGAK SSNNpYLGAAR
	YbfA	putative uncharacterized protein	AAHDFpY
	YeaG	putative uncharacterized protein	GpYLIPK
Helicobacter pylori	WecE	spore coat polysaccharide biosynthesis protein	FKDNPpYFpTPLHPLLKDK
	virB4	DNA transfer protein	NpYAPKLLp(SS)K

(*Continued.*)

Table 1. (*Continued.*)

species	proteins	functions	peptide sequences
	SpeA[a]	arginine decarboxylase	GLN-4p(YGLEAGSKSELIIAMSY)TNPK
	Dxs	deoxyxylulose-5-phosphate synthase	KSKSAILSPTEApYSNTLLELAK
	SpoT	penta-phosphateguanosine-3′-pyrophosphohydrolase	NECNIVGVpSpYLGYK
	UvrD	DNA helicase II	p(TLTS)RLApYLIGVCGVPp(SENTLTLTFTNKAS)K
	RpoB	DNA-directed RNA polymerase, beta subunit	pYpTVREAMER
	Eco57Ir	Type IIS restriction enzyme R and M protein	LpYQELKQNPNK
	PepQ	proline dipeptidase	pYSLEGKITEGFSDDGEPKGp(SS)GMPMLSVLR
	Tgt	tRNA-guanine transglycosylase	YpSKApYLHHLFRAK
	HP0468	conserved hypothetical protein	GVSpYNENIALK
	HP0112	conserved hypothetical protein	HNRLLPRDpYLGpYR
	HP0408	conserved hypothetical protein	pTLpYAFpSLIDSQYCSK
	HP0586	conserved hypothetical protein	DNApYVFRIKDLK
	HP1055[a]	conserved hypothetical protein	p(YFVSYFGTRFY)GDLLLGGGALK
Pseudomonas putida	HrpA	ATP-dependent helicase	DLQLSLNKEPADpYPKLHK
	TxrB	thioredoxin reductase	VIILGSGPAGYSAAVpYAAR
	Pfl01_2957	pyridine nucleotide-disulfide oxidoreductase	MLDANLKAQLKSpYLER
	CcmE	cytochrome **c**-type biogenesis protein	TVTITpYR
	PgaA[a]	predicted outer membrane protein	AGHp(YT)PALSVLR
	FlgL[a]	flagellar hook-associated protein	VVLTNGIVGp(T)Aap(TY)NAK
	Pfl01_2213	amino acid adenylation	FLDDPFNSGRMpYR
	Pfl01_4493	multidrug efflux protein	LPQDAEDPVLSKEAADASALMpYISFFSK
Pseudomonas aeruginosa	ThiC	thiamin biosynthesis protein	GIITPEMEpYIAIRENMK
	PA3965	probable transcriptional regulator	pYDLRILEELQR
	SucC	Succinyl-CoA synthetase beta chain	NLVTpYQTDANGQPVSK
	PvdL	probable non-ribosomal peptide synthetase	MWFLWQLEPDpSPApYNVGGLARLSGPLDVAR
	SbcB	exodeoxyribonuclease i	pYRARNFPEpTLNVAER
	PA0920	hypothetical protein	LIDRITApYR
	PA3238	hypothetical protein	pTFpYEVVLSK
	PA3347	hypothetical protein	NATpYLDSSALGMLLLLR
Campylobacter Jejuni	Cft	ferritin	TpYEHEQFIpTK
	Cj0691	hypothetical protein	pYNEVCEANEALR
	ThiJ	4-Me-5(β-hydroxyethyl)-thiazole phos. synthesis	KLCGDEIpYQK
Listeria monocytogenes	Atpa1	ATP synthase subunit alpha 1	IIDTTGKMLpYEESEEK
	FusA	elongation factor G	VMTDPpYVGR
	lmo0208	UPF0145 protein	SQGpYEDELINAR
	lmo0310	hypothetical protein	NKELISENpYNLDLEIKNK
	lmo1871	hypothetical protein	GpYRNVINEALR
	lmo2324	hypothetical protein	pYFIEVEKQARK
	lmo2373	hypothetical protein	IPVDVIDMAApYGTMNGEK

[a]Proteins with ambiguous phosphorylated tyrosine site.

kinase activity (Mijakovic *et al.* 2012, unpublished data). One can also not exclude that, when expressed, BY-kinase might be constitutively active, especially in Proteobacteria where the kinase domain is always linked to the extracellular domain.

Another intriguing question about BY-kinases concerns their ability to phosphorylate several substrates with distinct functions. Upon examination of BY-mediated protein phosphorylation sites, no clear-cut recognition motifs emerged in the immediate surroundings of the phosphorylated tyrosines. Moreover, if one considers the number of tyrosine-phosphorylation sites detected in recent phosphoproteomic studies (table 1), the conservation of phosphotyrosine sites is nearly non-existent [84,91–99]. The existence of a specific phosphorylation motif is therefore unlikely in proteins phosphorylated by BY-kinases, and it seems likely that they recognize structural motifs distant from the phosphorylated tyrosine. At present, no structure of BY-kinase/substrate complex has been published, and such structures are eagerly anticipated.

Recent bacterial phosphoproteomics studies (table 1) demonstrate that tyrosine phosphorylation has similar abundance in Bacteria (3–10% of phosphoproteome) and Eukarya (3–39% of phosphoproteome) [100]. One has to consider the fact that bacterial phosphoproteomes are incomplete as they represent only snapshots of tyrosine phosphorylation in the bacterial cell in a given environment and growth state. To illustrate this, a recent comprehensive analysis dedicated to *E. coli* ribosomal proteins surprisingly detected 14 tyrosine phosphorylation sites, whereas none were reported in the first phosphoproteomic analysis of *E. coli* published a year before (table 1) [95]. It will therefore come as no surprise if more phosphotyrosine-proteins are detected in the near future. Quantitative phosphoproteomic of strains with inactivated individual kinase genes, grown in specific conditions are now needed to link tyrosine kinases to their physiological substrates and unravel the cellular function of bacterial tyrosine phosphorylation [101,102].

10. CONCLUSION

Challenging as it may be, there is no doubt that the ongoing effort made in the field of bacterial tyrosine phosphorylation will lead to the characterization of new regulatory processes crucial for the bacterial cell. Moreover, evidence is provided at an increasing rate that connections and cross talk exist between BY-kinases and other phosphorylating systems (i.e. two-component systems and eukaryotic-like serine/threonine kinases) found in the bacterial cell. The field of bacterial post-translational modifications is rapidly expanding with the characterization of protein glycosylation, acetylation, methylation and pupylation [103]. It is evident that extensive cross talk exists among these various post-translational modifications in Eukarya [104], and we will undoubtedly have to tackle this kind of cross talk in bacterial protein phosphorylation networks, including but not limited to tyrosine phosphorylation.

This work was supported by grants of the CNRS (C.G. and S.N.), the University of Lyon (C.G.), the University of Paris-sud 11 (S.N.), the INRA (I.M.) and the ANR 08-BLAN-0143 (C.G.) and 10-BLAN-1303-01 (I.M., C.G. and S.N.).

REFERENCES

1 Krebs, E. G. & Fisher, E. H. 1956 The phosphorylase *b* to *a* converting enzyme of rabbit skeletal muscle. *Biochim. Biophys. Acta* **20**, 150–157. (doi:10.1016/0006-3002(56)90273-6)
2 Pawson, T. & Scott, J. D. 2005 Protein phosphorylation in signaling: 50 years and counting. *Trends Biochem. Sci.* **30**, 286–290. (doi:10.1016/j.tibs.2005.04.013)
3 Manai, M. & Cozzone, A. J. 1979 Analysis of the protein-kinase activity of *Escherichia coli* cells. *Biochem. Biophys. Res. Commun.* **91**, 819–826. (doi:10.1016/0006-291X(79)91953-3)
4 Wang, J. Y. & Koshland Jr, D. E. 1978 Evidence for protein kinase activities in the prokaryote *Salmonella typhimurium*. *J. Biol. Chem.* **253**, 7605–7608.
5 Garnak, M. & Reeves, H. C. 1979 Phosphorylation of isocitrate dehydrogenase of *Escherichia coli*. *Science* **203**, 1111–1112. (doi:10.1126/science.34215)
6 Hoch, J. A. 2000 Two-component and phosphorelay signal transduction. *Curr. Opin. Microbiol.* **3**, 165–170. (doi:10.1016/S1369-5274(00)00070-9)
7 Postma, P. W., Lengeler, J. W. & Jacobson, G. R. 1993 Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.* **57**, 543–594.
8 LaPorte, D. C. & Chung, T. 1985 A single gene codes for the kinase and phosphatase which regulate isocitrate dehydrogenase. *J. Biol. Chem.* **260**, 15 291–15 297.
9 Min, K. T., Hilditch, C. M., Diederich, B., Errington, J. & Yudkin, M. D. 1993 Sigma F, the first compartment-specific transcription factor of *B. subtilis*, is regulated by an anti-sigma factor that is also a protein kinase. *Cell* **74**, 735–742. (doi:10.1016/0092-8674(93)90520-Z)
10 Munoz-Dorado, J., Inouye, S. & Inouye, M. 1991 A gene encoding a protein serine/threonine kinase is required for normal development of *M. xanthus*, a Gram-negative bacterium. *Cell* **67**, 995–1006. (doi:10.1016/0092-8674(91)90372-6)
11 Pereira, S. F., Goss, L. & Dworkin, J. 2011 Eukaryote-like serine/threonine kinases and phosphatases in bacteria. *Microbiol. Mol. Biol. Rev.* **75**, 192–212. (doi:10.1128/MMBR.00042-10)
12 Zhao, X. & Lam, J. S. 2002 WaaP of *Pseudomonas aeruginosa* is a novel eukaryotic type protein-tyrosine kinase as well as a sugar kinase essential for the biosynthesis of core lipopolysaccharide. *J. Biol. Chem.* **277**, 4722–4730. (doi:10.1074/jbc.M107803200)
13 Thomasson, B., Link, J., Stassinopoulos, A. G., Burke, N., Plamann, L. & Hartzell, P. L. 2002 MglA, a small GTPase, interacts with a tyrosine kinase to control type IV pili-mediated motility and development of *Myxococcus xanthus*. *Mol. Microbiol.* **46**, 1399–1413. (doi:10.1046/j.1365-2958.2002.03258.x)
14 Duclos, B., Grangeasse, C., Vaganay, E., Riberty, M. & Cozzone, A. J. 1996 Autophosphorylation of a bacterial protein at tyrosine. *J. Mol. Biol.* **259**, 891–895. (doi:10.1006/jmbi.1996.0366)
15 Grangeasse, C., Doublet, P., Vaganay, E., Vincent, C., Deleage, G., Duclos, B. & Cozzone, A. J. 1997 Characterization of a bacterial gene encoding an autophosphorylating protein tyrosine kinase. *Gene* **204**, 259–265. (doi:10.1016/S0378-1119(97)00554-4)
16 Jadeau, F., Bechet, E., Cozzone, A. J., Deleage, G., Grangeasse, C. & Combet, C. 2008 Identification of the idiosyncratic bacterial protein-tyrosine kinase (BY-kinase) family signature. *Bioinformatics* **21**, 2427–2430. (doi:10.1093/bioinformatics/btn462)

17 Grangeasse, C., Cozzone, A. J., Deutscher, J. & Mijakovic, I. 2007 Tyrosine phosphorylation: an emerging regulatory device of bacterial physiology. *Trends Biochem. Sci.* **32**, 86–94. (doi:10.1016/j.tibs.2006.12.004)

18 Bach, H., Wong, D. & Av-Gay, Y. 2009 *Mycobacterium tuberculosis* PtkA is a novel protein tyrosine kinase whose substrate is PtpA. *Biochem. J.* **420**, 155–160. (doi:10.1042/BJ20090478)

19 Kirstein, J., Zuhlke, D., Gerth, U., Turgay, K. & Hecker, M. 2005 A tyrosine kinase and its activator control the activity of the CtsR heat shock repressor in *B. subtilis*. *EMBO J.* **24**, 3435–3445. (doi:10.1038/sj.emboj.7600780)

20 Wu, J., Ohta, N., Zhao, J. L. & Newton, A. 1999 A novel bacterial tyrosine kinase essential for cell division and differentiation. *Proc. Natl Acad. Sci. USA* **96**, 13 068–13 073. (doi:10.1073/pnas.96.23.13068)

21 Mattoo, A. R., Arora, A., Maiti, S. & Singh, Y. 2008 Identification, characterization and activation mechanism of a tyrosine kinase of *Bacillus anthracis*. *FEBS J.* **275**, 6237–6247. (doi:10.1111/j.1742-4658.2008.06748.x)

22 Fuhrmann, J., Schmidt, A., Spiess, S., Lehner, A., Turgay, K., Mechtler, K., Charpentier, E. & Clausen, T. 2009 McsB is a protein arginine kinase that phosphorylates and inhibits the heat-shock regulator CtsR. *Science* **324**, 1323–1327. (doi:10.1126/science.1170088)

23 Cortay, J. C., Duclos, B. & Cozzone, A. J. 1986 Phosphorylation of an *Escherichia coli* protein at tyrosine. *J. Mol. Biol.* **187**, 305–308. (doi:10.1016/0022-2836(86)90236-6)

24 Cozzone, A. J. 1997 Diversity and specificity of protein-phosphorylating systems in bacteria. *Folia Microbiol. (Praha)* **42**, 165–170. (doi:10.1007/BF02818973)

25 Warner, K. M. & Bullerjahn, G. S. 1994 Light-dependent tyrosine phosphorylation in the cyanobacterium *Prochlorothrix hollandica*. *Plant Physiol.* **105**, 629–633.

26 Ray, M. K., Kumar, G. S. & Shivaji, S. 1994 Tyrosine phosphorylation of a cytoplasmic protein from the antartic psychrotrophic bacterium *Pseudomonas syringae*. *FEMS Microbiol. Lett.* **122**, 49–54. (doi:10.1111/j.1574-6968.1994.tb07142.x)

27 Chow, K., Ng, D., Stokes, R. & Johnson, P. 1994 Protein tyrosine phosphorylation in *Mycobacterium tuberculosis*. *FEMS Microbiol. Lett.* **124**, 203–207. (doi:10.1111/j.1574-6968.1994.tb07285.x)

28 Kelly-Wintenberg, K., South, S. L. & Montie, T. C. 1993 Tyrosine phosphate in a- and b-type flagellins of *Pseudomonas aeruginosa*. *J. Bacteriol.* **175**, 2458–2461.

29 Ostrovsky, P. C. & Maloy, S. 1995 Protein phosphorylation on serine, threonine, and tyrosine residues modulates membrane–protein interactions and transcriptional regulation in *Salmonella typhimurium*. *Genes Dev.* **9**, 2034–2041. (doi:10.1101/gad.9.16.2034)

30 Dadssi, M. & Cozzone, A. J. 1990 Evidence of protein-tyrosine kinase activity in the bacterium *Acinetobacter calcoaceticus*. *J. Biol. Chem.* **265**, 20 996–20 999.

31 Hanks, S. K., Quinn, A. M. & Hunter, T. 1988 The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* **241**, 42–52. (doi:10.1126/science.3291115)

32 Wugeditsch, T., Paiment, A., Hocking, J., Drummelsmith, J., Forrester, C. & Whitfield, C. 2001 Phosphorylation of Wzc, a tyrosine autokinase, is essential for assembly of group 1 capsular polysaccharides in *Escherichia coli*. *J. Biol. Chem.* **276**, 2361–2371. (doi:10.1074/jbc.M009092200)

33 Ilan, O., Bloch, Y., Frankel, G., Ullrich, H., Geider, K. & Rosenshine, I. 1999 Protein tyrosine kinases in bacterial pathogens are associated with virulence and production of exopolysaccharide. *EMBO J.* **18**, 3241–3248. (doi:10.1093/emboj/18.12.3241)

34 Morona, J. K., Paton, J. C., Miller, D. C. & Morona, R. 2000 Tyrosine phosphorylation of CpsD negatively regulates capsular polysaccharide biosynthesis in *Streptococcus pneumoniae*. *Mol. Microbiol.* **35**, 1431–1442. (doi:10.1046/j.1365-2958.2000.01808.x)

35 Niemeyer, D. & Becker, A. 2001 The molecular weight distribution of succinoglycan produced by *Sinorhizobium meliloti* is influenced by specific tyrosine phosphorylation and ATPase activity of the cytoplasmic domain of the ExoP protein. *J. Bacteriol.* **183**, 5163–5170. (doi:10.1128/JB.183.17.5163-5170.2001)

36 Vincent, C., Duclos, B., Grangeasse, C., Vaganay, E., Riberty, M., Cozzone, A. J. & Doublet, P. 2000 Relationship between exopolysaccharide production and protein-tyrosine phosphorylation in Gram-negative bacteria. *J. Mol. Biol.* **304**, 311–321. (doi:10.1006/jmbi.2000.4217)

37 Lacour, S., Bechet, E., Cozzone, A. J., Mijakovic, I. & Grangeasse, C. 2008 Tyrosine phosphorylation of the UDP-glucose dehydrogenase of *Escherichia coli* is at the crossroads of colanic acid synthesis and polymyxin resistance. *PLoS ONE* **3**, e3053. (doi:10.1371/journal.pone.0003053)

38 Bechet, E., Guiral, S., Torres, S., Mijakovic, I., Cozzone, A. J. & Grangeasse, C. 2009 Tyrosine-kinases in bacteria: from a matter of controversy to the status of key regulatory enzymes. *Amino Acids* **37**, 499–507. (doi:10.1007/s00726-009-0237-8)

39 Leipe, D. D., Wolf, Y. I., Koonin, E. V. & Aravind, L. 2002 Classification and evolution of P-loop GTPases and related ATPases. *J. Mol. Biol.* **317**, 41–72. (doi:10.1006/jmbi.2001.5378)

40 Doublet, P., Vincent, C., Grangeasse, C., Cozzone, A. J. & Duclos, B. 1999 On the binding of ATP to the autophosphorylating protein, Ptk, of the bacterium *Acinetobacter johnsonii*. *FEBS Lett.* **445**, 137–143. (doi:10.1016/S0014-5793(99)00111-8)

41 Soulat, D., Jault, J. M., Geourjon, C., Gouet, P., Cozzone, A. J. & Grangeasse, C. 2007 Tyrosine-kinase Wzc from *Escherichia coli* possesses an ATPase activity regulated by autophosphorylation. *FEMS Microbiol. Lett.* **274**, 252–259. (doi:10.1111/j.1574-6968.2007.00841.x)

42 Leipe, D. D., Koonin, E. V. & Aravind, L. 2003 Evolution and classification of P-loop kinases and related proteins. *J. Mol. Biol.* **333**, 781–815. (doi:10.1016/j.jmb.2003.08.040)

43 Rosen, B. P., Bhattacharjee, H., Zhou, T. & Walmsley, A. R. 1999 Mechanism of the ArsA ATPase. *Biochim. Biophys. Acta* **1461**, 207–215. (doi:10.1016/S0005-2736(99)00159-5)

44 Ma, L., King, G. F. & Rothfield, L. 2004 Positioning of the MinE binding site on the MinD surface suggests a plausible mechanism for activation of the *Escherichia coli* MinD ATPase during division site selection. *Mol. Microbiol.* **54**, 99–108. (doi:10.1111/j.1365-2958.2004.04265.x)

45 Dereeper, A. *et al.* 2008 Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* **36**, W465–W469. (doi:10.1093/nar/gkn180)

46 Som, A. 2009 ML or NJ-MCL? A comparison between two robust phylogenetic methods. *Comput. Biol. Chem.* **33**, 373–378. (doi:10.1016/j.compbiolchem.2009.07.007)

47 Cuthbertson, L., Mainprize, I. L., Naismith, J. H. & Whitfield, C. 2009 Pivotal roles of the outer membrane polysaccharide export and polysaccharide copolymerase protein families in export of extracellular polysaccharides in Gram-negative bacteria. *Microbiol. Mol. Biol. Rev.* **73**, 155–177. (doi:10.1128/MMBR.00024-08)

48 Petranovic, D., Michelsen, O., Zahradka, K., Silva, C., Petranovic, M., Jensen, P. R. & Mijakovic, I. 2007 *Bacillus subtilis* strain deficient for the protein-tyrosine

kinase PtkA exhibits impaired DNA replication. *Mol. Microbiol.* **63**, 1797–1805. (doi:10.1111/j.1365-2958.2007.05625.x)
49 Jers, C., Pedersen, M. M., Paspaliari, D. K., Schutz, W., Johnsson, C., Soufi, B., Macek, B., Jensen, P. R. & Mijakovic, I. 2010 *Bacillus subtilis* BY-kinase PtkA controls enzyme activity and localization of its protein substrates. *Mol. Microbiol.* **77**, 287–299. (doi:10.1111/j.1365-2958.2010.07227.x)
50 Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. 1982 Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**, 945–951.
51 Saraste, M., Sibbald, P. R. & Wittinghofer, A. 1990 The P-loop: a common motif in ATP- and GTP-binding proteins. *Trends Biochem. Sci.* **15**, 430–434. (doi:10.1016/0968-0004(90)90281-F)
52 Grangeasse, C., Doublet, P. & Cozzone, A. J. 2002 Tyrosine phosphorylation of protein kinase Wzc from *Escherichia coli* K12 occurs through a two-step process. *J. Biol. Chem.* **277**, 7127–7135. (doi:10.1074/jbc.M110880200)
53 Olivares-Illana, V. *et al.* 2008 Structural basis for the regulation mechanism of the tyrosine kinase CapB from *Staphylococcus aureus*. *PLoS Biol.* **6**, e143. (doi:10.1371/journal.pbio.0060143)
54 Jadeau, F., Grangeasse, C., Shi, L., Mijakovic, I., Deleage, G. & Combet, C. 2011 BYKdb: the Bacterial protein tYrosine Kinase database. *Nucleic Acids Res.* **40**, D321–D324. (doi:10.1093/nar/gkr915)
55 Mijakovic, I. *et al.* 2003 Transmembrane modulator-dependent bacterial tyrosine kinase activates UDP-glucose dehydrogenases. *EMBO J.* **22**, 4709–4718. (doi:10.1093/emboj/cdg458)
56 Soulat, D., Jault, J. M., Duclos, B., Geourjon, C., Cozzone, A. J. & Grangeasse, C. 2006 *Staphylococcus aureus* operates protein-tyrosine phosphorylation through a specific mechanism. *J. Biol. Chem.* **281**, 14 048–14 056. (doi:10.1074/jbc.M513600200)
57 Collins, R. F., Beis, K., Clarke, B. R., Ford, R. C., Hulley, M., Naismith, J. H. & Whitfield, C. 2006 Periplasmic protein–protein contacts in the inner membrane protein Wzc form a tetrameric complex required for the assembly of *Escherichia coli* group 1 capsules. *J. Biol. Chem.* **281**, 2144–2150. (doi:10.1074/jbc.M508078200)
58 Collins, R. F., Beis, K., Dong, C., Botting, C. H., McDonnell, C., Ford, R. C., Clarke, B. R., Whitfield, C. & Naismith, J. H. 2007 The 3D structure of a periplasm-spanning platform required for assembly of group 1 capsular polysaccharides in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **104**, 2390–2395. (doi:10.1073/pnas.0607763104)
59 Lee, D. C., Zheng, J., She, Y. M. & Jia, Z. 2008 Structure of *Escherichia coli* tyrosine kinase Etk reveals a novel activation mechanism. *EMBO J.* **27**, 1758–1766. (doi:10.1038/emboj.2008.97)
60 Lee, D. C. & Jia, Z. 2009 Emerging structural insights into bacterial tyrosine kinases. *Trends Biochem. Sci.* **34**, 351–357. (doi:10.1016/j.tibs.2009.03.003)
61 Bechet, E., Gruszczyk, J., Terreux, R., Gueguen-Chaignon, V., Vigouroux, A., Obadia, B., Cozzone, A. J., Nessler, S. & Grangeasse, C. 2010 Identification of structural and molecular determinants of the tyrosine-kinase Wzc and implications in capsular polysaccharide export. *Mol. Microbiol.* **77**, 1315–1325. (doi:10.1111/j.1365-2958.2010.07291.x)
62 Lu, T., Tan, H., Lee, D., Chen, G. & Jia, Z. 2009 New insights into the activation of *Escherichia coli* tyrosine kinase revealed by molecular dynamics simulation and biochemical analysis. *Biochemistry* **48**, 7986–7995. (doi:10.1021/bi900811p)
63 Krell, T. *et al.* 2001 Biochemical and X-ray crystallographic studies on shikimate kinase: the important structural role of the P-loop lysine. *Protein Sci. Publ. Protein Soc.* **10**, 1137–1149.
64 Tocilj, A. *et al.* 2008 Bacterial polysaccharide co-polymerases share a common framework for control of polymer length. *Nat. Struct. Mol. Biol.* **15**, 130–138. (doi:10.1038/nsmb.1374)
65 Vogel, C. & Morea, V. 2006 Duplication, divergence and formation of novel protein topologies. *Bioessays* **28**, 973–978. (doi:10.1002/bies.20474)
66 Fieulaine, S., Morera, S., Poncet, S., Monedero, V., Gueguen-Chaignon, V., Galinier, A., Janin, J., Deutscher, J. & Nessler, S. 2001 X-ray structure of HPr kinase: a bacterial protein kinase with a P-loop nucleotide-binding domain. *EMBO J.* **20**, 3917–3927. (doi:10.1093/emboj/20.15.3917)
67 Marquez, J. A., Hasenbein, S., Koch, B., Fieulaine, S., Nessler, S., Russell, R. B., Hengstenberg, W. & Scheffzek, K. 2002 Structure of the full-length HPr kinase/phosphatase from *Staphylococcus xylosus* at 1.95 Å resolution: mimicking the product/substrate of the phospho transfer reactions. *Proc. Natl Acad. Sci. USA* **99**, 3458–3463. (doi:10.1073/pnas.052461499)
68 Lander, E. S. *et al.* 2001 Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921. (doi:10.1038/35057062)
69 Bender, M. H., Cartee, R. T. & Yother, J. 2003 Positive correlation between tyrosine phosphorylation of CpsD and capsular polysaccharide production in *Streptococcus pneumoniae*. *J. Bacteriol.* **185**, 6057–6066. (doi:10.1128/JB.185.20.6057-6066.2003)
70 Nakar, D. & Gutnick, D. L. 2003 Involvement of a protein tyrosine kinase in production of the polymeric bioemulsifier emulsan from the oil-degrading strain *Acinetobacter lwoffii* RAG-1. *J. Bacteriol.* **185**, 1001–1009. (doi:10.1128/JB.185.3.1001-1009.2003)
71 Bugert, P. & Geider, K. 1997 Characterization of the amsI gene product as a low molecular weight acid phosphatase controlling exopolysaccharide synthesis of *Erwinia amylovora*. *FEBS Lett.* **400**, 252–256. (doi:10.1016/S0014-5793(96)01398-1)
72 Vincent, C., Doublet, P., Grangeasse, C., Vaganay, E., Cozzone, A. J. & Duclos, B. 1999 Cells of *Escherichia coli* contain a protein-tyrosine kinase, Wzc, and a phosphotyrosine-protein phosphatase, Wzb. *J. Bacteriol.* **181**, 3472–3477.
73 Morona, J. K., Morona, R., Miller, D. C. & Paton, J. C. 2002 *Streptococcus pneumoniae* capsule biosynthesis protein CpsB is a novel manganese-dependent phosphotyrosine-protein phosphatase. *J. Bacteriol.* **184**, 577–583. (doi:10.1128/JB.184.2.577-583.2002)
74 Obadia, B., Lacour, S., Doublet, P., Baubichon-Cortay, H., Cozzone, A. J. & Grangeasse, C. 2007 Influence of tyrosine-kinase Wzc activity on colanic acid production in *Escherichia coli* K12 Cells. *J. Mol. Biol.* **367**, 42–53. (doi:10.1016/j.jmb.2006.12.048)
75 Whitfield, C. 2006 Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annu. Rev. Biochem.* **75**, 39–68. (doi:10.1146/annurev.biochem.75.103004.142545)
76 Dong, C., Beis, K., Nesper, J., Brunkan-Lamontagne, A. L., Clarke, B. R., Whitfield, C. & Naismith, J. H. 2006 Wza the translocon for *E. coli* capsular polysaccharides defines a new class of membrane protein. *Nature* **444**, 226–229. (doi:10.1038/nature05267)

77 Morona, R., Purins, L., Tocilj, A., Matte, A. & Cygler, M. 2009 Sequence–structure relationships in polysaccharide co-polymerase (PCP) proteins. *Trends Biochem. Sci.* **34**, 78–84. (doi:10.1016/j.tibs.2008.11.001)

78 Henriques, M. X., Rodrigues, T., Carido, M., Ferreira, L. & Filipe, S. R. 2011 Synthesis of capsular polysaccharide at the division septum of *Streptococcus pneumoniae* is dependent on a bacterial tyrosine kinase. *Mol. Microbiol.* **82**, 515–534. (doi:10.1111/j.1365-2958.2011.07828.x)

79 Grangeasse, C., Obadia, B., Mijakovic, I., Deutscher, J., Cozzone, A. J. & Doublet, P. 2003 Autophosphorylation of the *Escherichia coli* protein kinase Wzc regulates tyrosine phosphorylation of Ugd, a UDP-glucose dehydrogenase. *J. Biol. Chem.* **278**, 39 323–39 329. (doi:10.1074/jbc.M305134200)

80 Soulat, D., Grangeasse, C., Vaganay, E., Cozzone, A. J. & Duclos, B. 2007 UDP-acetyl-mannosamine dehydrogenase is an endogenous protein substrate of *Staphylococcus aureus* protein-tyrosine kinase activity. *J. Mol. Microbiol. Biotechnol.* **13**, 45–54. (doi:10.1159/000103596)

81 Petranovic, D., Grangeasse, C., Macek, B., Abdillatef, M., Gueguen-Chaignon, V., Nessler, S., Deutscher, J. & Mijakovic, I. 2009 Activation of *Bacillus subtilis* Ugd by the BY-kinase PtkA proceeds via phosphorylation of its residue tyrosine 70. *J. Mol. Microbiol. Biotechnol.* **17**, 83–89. (doi:10.1159/000206635)

82 Gruszczyk, J. *et al.* 2011 Structure analysis of the *Staphylococcus aureus* UDP-N-acetyl-mannosamine dehydrogenase Cap5O involved in capsular polysaccharide biosynthesis. *J. Biol. Chem.* **286**, 17 112–17 121. (doi:10.1074/jbc.M110.216002)

83 Minic, Z., Marie, C., Delorme, C., Faurie, J. M., Mercier, G., Ehrlich, D. & Renault, P. 2007 Control of EpsE, the phosphoglycosyltransferase initiating exopolysaccharide synthesis in *Streptococcus thermophilus*, by EpsD tyrosine kinase. *J. Bacteriol.* **189**, 1351–1357. (doi:10.1128/JB.01122-06)

84 Lin, M. H., Hsu, T. L., Lin, S. Y., Pan, Y. J., Jan, J. T., Wang, J. T., Khoo, K. H. & Wu, S. H. 2009 Phosphoproteomics of *Klebsiella pneumoniae* NTUH-K2044 reveals a tight link between tyrosine phosphorylation and virulence. *Mol. Cell Proteomics* **8**, 2613–2623. (doi:10.1074/mcp.M900276-MCP200)

85 Klein, G., Dartigalongue, C. & Raina, S. 2003 Phosphorylation-mediated regulation of heat shock response in *Escherichia coli*. *Mol. Microbiol.* **48**, 269–285. (doi:10.1046/j.1365-2958.2003.03449.x)

86 Kolot, M., Gorovits, R., Silberstein, N., Fichtman, B. & Yagil, E. 2008 Phosphorylation of the integrase protein of coliphage HK022. *Virology* **375**, 383–390. (doi:10.1016/j.virol.2008.02.011)

87 Mijakovic, I., Petranovic, D., Macek, B., Cepo, T., Mann, M., Davies, J., Jensen, P. R. & Vujaklija, D. 2006 Bacterial single-stranded DNA-binding proteins are phosphorylated on tyrosine. *Nucleic Acids Res.* **34**, 1588–1596. (doi:10.1093/nar/gkj514)

88 Grangeasse, C., Terreux, R. & Nessler, S. 2010 Bacterial tyrosine-kinases: structure–function analysis and therapeutic potential. *Biochim. Biophys. Acta* **1804**, 628–634. (doi:10.1016/j.bbapap.2009.08.018)

89 Shah, I. M., Laaberki, M. H., Popham, D. L. & Dworkin, J. 2008 A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* **135**, 486–496. (doi:10.1016/j.cell.2008.08.039)

90 Jofre, E. & Becker, A. 2009 Production of succinoglycan polymer in *Sinorhizobium meliloti* is affected by SMb21506 and requires the N-terminal domain of ExoP. *Mol. Plant Microbe Interact.* **22**, 1656–1668. (doi:10.1094/MPMI-22-12-1656)

91 Soufi, B., Gnad, F., Jensen, P. R., Petranovic, D., Mann, M., Mijakovic, I. & Macek, B. 2008 The Ser/Thr/Tyr phosphoproteome of *Lactococcus lactis* IL1403 reveals multiply phosphorylated proteins. *Proteomics* **8**, 3486–3493. (doi:10.1002/pmic.200800069)

92 Macek, B., Mijakovic, I., Olsen, J. V., Gnad, F., Kumar, C., Jensen, P. R. & Mann, M. 2007 The serine/threonine/tyrosine phosphoproteome of the model bacterium *Bacillus subtilis*. *Mol. Cell Proteomics* **4**, 697–707. (doi:10.1074/mcp.M600464-MCP200)

93 Misra, S. K., Milohanic, E., Ake, F., Mijakovic, I., Deutscher, J., Monnet, V. & Henry, C. 2011 Analysis of the serine/threonine/tyrosine phosphoproteome of the pathogenic bacterium *Listeria monocytogenes* reveals phosphorylated proteins related to virulence. *Proteomics* **11**, 4155–4165. (doi:10.1002/pmic.201100259)

94 Ravichandran, A., Sugiyama, N., Tomita, M., Swarup, S. & Ishihama, Y. 2009 Ser/Thr/Tyr phosphoproteome analysis of pathogenic and non-pathogenic *Pseudomonas* species. *Proteomics* **9**, 2764–2775. (doi:10.1002/pmic.200800655)

95 Soung, G. Y., Miller, J. L., Koc, H. & Koc, E. C. 2009 Comprehensive analysis of phosphorylated proteins of *Escherichia coli* ribosomes. *J. Proteome Res.* **8**, 3390–3402. (doi:10.1021/pr900042e)

96 Macek, B., Gnad, F., Soufi, B., Kumar, C., Olsen, J. V., Mijakovic, I. & Mann, M. 2008 Phosphoproteome analysis of *E. coli* reveals evolutionary conservation of bacterial Ser/Thr/Tyr phosphorylation. *Mol. Cell Proteomics* **7**, 299–307. (doi:10.1074/mcp.M700311-MCP200)

97 Ge, R., Sun, X., Xiao, C., Yin, X., Shan, W., Chen, Z. & He, Q. Y. 2011 Phosphoproteome analysis of the pathogenic bacterium *Helicobacter pylori* reveals over-representation of tyrosine phosphorylation and multiply phosphorylated proteins. *Proteomics* **11**, 1449–1461. (doi:10.1002/pmic.201000649)

98 Parker, J. L., Jones, A. M., Serazetdinova, L., Saalbach, G., Bibb, M. J. & Naldrett, M. J. 2010 Analysis of the phosphoproteome of the multicellular bacterium *Streptomyces coelicolor* A3(2) by protein/peptide fractionation, phosphopeptide enrichment and high-accuracy mass spectrometry. *Proteomics* **10**, 2486–2497. (doi:10.1002/pmic.201000090)

99 Sun, X., Ge, F., Xiao, C. L., Yin, X. F., Ge, R., Zhang, L. H. & He, Q. Y. 2010 Phosphoproteomic analysis reveals the multiple roles of phosphorylation in pathogenic bacterium *Streptococcus pneumoniae*. *J. Proteome Res.* **9**, 275–282. (doi:10.1021/pr900612v)

100 Tan, C. S. *et al.* 2009 Comparative analysis reveals conserved protein phosphorylation networks implicated in multiple diseases. *Sci. Signal* **2**, ra39. (doi:10.1126/scisignal.2000316)

101 Mijakovic, I. & Macek, B. 2011 Impact of phosphoproteomics on studies of bacterial physiology. *FEMS Microbiol. Rev.* **36**, 877–892. (doi:10.1111/j.1574-6976.2011.00314.x)

102 Macek, B. & Mijakovic, I. 2011 Site-specific analysis of bacterial phosphoproteomes. *Proteomics* **11**, 3002–3011. (doi:10.1002/pmic.201100012)

103 Kobir, A., Shi, L., Boskovic, A., Grangeasse, C., Franjevic, D. & Mijakovic, I. 2011 Protein phosphorylation in bacterial signal transduction. *Biochim. Biophys. Acta* **1810**, 989–994. (doi:10.1016/j.bbagen.2011.01.006)

104 Derouiche, A., Cousin, C. & Mijakovic, I. 2011 Protein phosphorylation from the perspective of systems biology. *Curr. Opin. Biotechnol.* (doi:10.1016/j.copbio.2011.11.008)