

Tyrosine-kinases in bacteria: from a matter of controversy to the status of key regulatory enzymes

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Abstract When considering protein phosphorylation in bacteria, phosphorylation of aspartic acid and histidine residues mediated by the two-component systems is the first to spring to mind. And yet other phosphorylation systems have been described in bacteria in the past 20 years including eukaryotic-like serine/threonine kinases and more recently tyrosine-kinases. Among the latter, a peculiar type is widespread among bacteria, but not in higher organisms. These enzymes possess unique structural features defining thus a new family of enzymes termed Bacterial tyrosine kinases (BY-kinases). BY-kinases have been shown to be mainly involved in polysaccharide production, but their ability to phosphorylate endogenous substrates indicates that they participate in the regulation of other functions of the bacterial cell. Recent advances in mass spectrometry based phosphoproteomics provided lists of many new phosphotyrosine-proteins, indicating that BY-kinases may be involved in regulating a large array of other cellular functions. One may expect that in a near future, tyrosine phosphorylation will turn out to be one of the key regulatory processes in the bacterial cell and will yield new insights into the understanding of its physiology.

Keywords Tyrosine-kinase · Phosphorylation · BY-kinase · Bacterial physiology · Regulatory process · Phosphoproteomics · Signaling

Historical Background

Protein phosphorylation has been originally detected years ago and has rapidly gained recognition as a key device in the regulation of multiple cellular functions of eukaryotic organisms (Hunter 2000; Pawson and Scott 2005). In contrast, interest in prokaryotic protein phosphorylation took much longer to gather momentum, the first experiments being performed in the 1960s (Rafter 1964). It was proposed that phosphorylation/dephosphorylation was a regulatory mechanism that emerged late in evolution to meet the specific needs of multicellular organisms (Kennelly and Potts 1996). Hence, existence of protein phosphorylation in bacteria was a matter of controversy for a decade and it is only in the early 1980s that it was established that bacteria also could harbor specific protein kinases and protein phosphatases (Garnak and Reeves 1979; Manai and Cozzzone 1979; Wang and Koshland 1978). Since that time, it has been demonstrated that bacteria possess three different protein-phosphorylating systems.

The first two of them, the “two-component systems (2CS)” and the “phosphotransferase system (PTS)” have been rapidly recognized as the hallmark of bacterial signaling (Deutscher et al. 2006; Klumpp and Krieglstein 2002). The 2CS are widespread among bacteria, but have been also found in certain eukaryotes such as plants, fungi, and yeasts. In 2CS, a protein called sensor-kinase autophosphorylates at a histidine residue at the expense of ATP, in response to a stimulus (input signal). Then the

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phosphoryl moiety is transferred to an aspartate residue of a second protein, called the response regulator. The phosphorylation of the response regulator controls its function as a gene regulator, whose action results in a cellular response. These systems are required for innumerable adaptive responses in bacteria and they represent a simple and efficient pathway of signal transduction. The second system, the PTS, is sometimes not considered as a protein-phosphorylating system *sensu stricto*, since its final purpose is not to modify a protein. Indeed, the phosphoryl moiety that is originally provided by phosphoenol-pyruvate (PEP) is passed through a chain of proteins, which are transitory phosphorylated on histidine, and finally transferred to a sugar. The phosphorylated form of certain components of the PTS is nevertheless involved in the regulation of a large array of functions including virulence of certain pathogens and nitrogen metabolism. Also, PTS-regulation-domain (PRD) in transcriptional antiterminators and activators are PTS regulatory targets that are (de)phosphorylated in response to the availability of carbon sources (van Tilbeurgh and Declerck 2001).

The third phosphorylating system found in bacteria closely resembles the “classical” ATP/GTP-dependent system predominant in eukaryotes (Cozzzone 1998; Shi et al. 1998). In this system, proteins are phosphorylated on serine and/or threonine or tyrosine. The first two identified enzymes catalyzing protein phosphorylation on serine residues were isocitrate dehydrogenase kinase/phosphatase (IDHK/P) and HPr kinase/phosphatase (HPrK/P) in *Escherichia coli* and *Bacillus subtilis*, respectively (Deutscher and Saier 1983; Garnak and Reeves 1979). From a structural standpoint, these two enzymes do not resemble eukaryotic protein-phosphorylating enzymes and it is only in the early 1990s that the first eukaryotic-like protein-kinase was identified in a bacteria, namely, *Myxococcus xanthus* (Munoz-Dorado et al. 1991). This protein kinase, the protein Pkn1, showed around 30% identity between its catalytic domain and those of representative eukaryotic protein kinases. Since, largely thanks to bacterial genomics, a repertoire of eukaryotic-like serine/threonine protein kinases (STPK) has been detected in various bacteria and many have been shown to be involved in the regulation of several cellular functions. STPK will not be discussed here and for review, readers are encouraged to see (Bakal and Davies 2000; Leonard et al. 1998).

Evidence of tyrosine phosphorylation

Once the existence of bacterial protein phosphorylation has been accepted, the existence of tyrosine phosphorylation in bacteria made the controversy reemerge. The first indication of a tyrosine-kinase activity in bacteria was reported in

E. coli, and then confirmed in several other bacteria, by showing the presence of phosphotyrosine in partial acid hydrolysates of proteins (Cortay et al. 1986). However, serious objections were raised as phosphotyrosine residues were thought to be due to protein nucleotydilation rather than phosphorylation (Foster et al. 1989). Then, differential labeling techniques proved the presence of phosphotyrosine in bacteria and in the next decade several tyrosine phosphorylation events were reported (Cozzzone 1993). For instance, in the antarctic psychrotrophic bacterium *Pseudomonas syringae*, phosphorylation of a 66-kDa protein is enhanced when the temperature increases, indicating a possible role of phosphorylation in bacterial adaptation to cold (Ray et al. 1994). A 88-kDa protein is tyrosine-phosphorylated in the cyanobacterium *Prochlorothrix hollandica* when the bacterial cell is adapted to high-light conditions, but is dephosphorylated upon shift from high to low-light (Warner and Bullerjahn 1994). Also, a 55-kDa protein modified at tyrosine is found in the pathogenic strain *Mycobacterium tuberculosis*, but not in a series of non-pathogenic *Mycobacterium* species, which may reflect the involvement of tyrosine phosphorylation in the process of host infection (Chow et al. 1994). However, in most cases, the nature of the phosphotyrosine-carrying proteins remained unknown and only a few of them were clearly identified. These include the two types of flagellin, a and b, which are required for the motility of *Pseudomonas solanacearum* (Kelly-Wintenberg et al. 1993), the multi-functional protein PutA from *Salmonella typhimurium*, which represses the proline-utilization operon and degrades proline to glutamate (Ostrovsky and Maloy 1995), the S-protein of the fish pathogenic bacterium *Aeromonas hydrophila* (Thomas and Trust 1995) and the virulence regulator TypA in the wall-less form of *E. coli* (Freestone et al. 1998). However, the characterization of the cognate tyrosine-kinase was missing in each case rendering the cellular role of tyrosine phosphorylation unclear. In the mid-1990s, the molecular cloning of a bacterial tyrosine-kinase gene definitively resolved the controversy about the existence of tyrosine-kinases in bacteria and this event could be considered as one of the most important milestones in the field of bacterial tyrosine phosphorylation. This was achieved in *Acinetobacter johnsonii* with the identification of the protein Ptk (Grangeasse et al. 1997). A considerable amount of progress has been made in the 11 years following the discovery of Ptk and tyrosine phosphorylation is now recognized as a key regulatory device in bacteria.

Tyrosine-kinases

A number of tyrosine-kinases have been identified and characterized in various bacteria since the identification of

the protein Ptk of *A. johnsonii*. Surprisingly, unlike eukaryotes, it seems that bacteria have developed several types of enzymes that catalyze protein phosphorylation on tyrosine (Grangeasse et al. 2007).

A special tyrosine-kinase, homologous to the bacterial histidine-kinases of 2CS, has been characterized in *Caulobacter crescentus*. This protein, DivL, is autophosphorylated on a tyrosine residue substituting the conserved histidine found in 2CS sensor-kinases (Wu et al. 1999). In *Bacillus subtilis*, a protein utilizing a guanidine-phosphotransferase domain has been shown to catalyze tyrosine phosphorylation. Indeed, protein McsB has been shown to autophosphorylate and to phosphorylate proteins McsA and CtsR, thus allowing the control of the class III heat-shock regulon (Kirstein and Turgay 2005). Homologues of DivL and McsB in other bacteria were not found to catalyze tyrosine phosphorylation and this may account for the existence of tyrosine-kinases specific to certain bacterial species. Other bacterial proteins share amino acids sequence similarities with the catalytic site of eukaryotic tyrosine-kinases. Hence, protein MasK and Waap from *M. xanthus* and *P. aeruginosa*, respectively, have been shown to catalyze tyrosine phosphorylation, but their cellular function remains obscure (Thomasson et al. 2002; Zhao and Lam 2002). Their homologues were not found to possess a tyrosine-kinase activity in other bacteria. Considering the rather high number of bacterial genome sequenced, it is noteworthy that other eukaryotic-like tyrosine-kinases have not been detected. Nevertheless, it is possible that bacteria possess tyrosine-kinases that are indistinguishable by sequence from STPKs and that require further investigations to be detected.

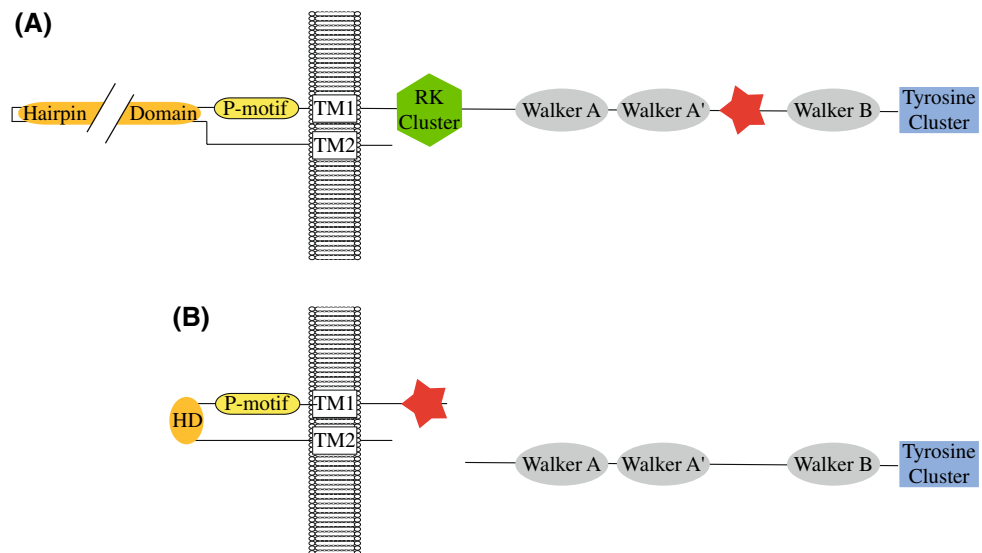
On the other hand, several proteins, homologous to the protein Ptk of *A. johnsonii*, have rapidly been shown to harbor a tyrosine-kinase activity (Bender et al. 2003; Grangeasse et al. 1998; Mijakovic et al. 2003; Morona et al. 2000a; Nakar and Gutnick 2003; Niemeyer and Becker 2001; Paiment et al. 2002; Soulat et al. 2006; Vincent et al. 1999). Numerous groups have since then performed a sizeable body of work and these enzymes have turned out to be the best described type of tyrosine-kinases in bacteria. They have been identified both in proteobacteria and firmicutes and in silico predictions also suggest their presence in actinobacteria (Grangeasse et al. 2007). These proteins do not share structural similarities with their eukaryotic counterparts and they curiously present some features in common with proteins of the MinD/Mrp ATPase family which have never been shown to possess a tyrosine-kinase activity (Leipe et al. 2002). They are involved in various cellular functions (see section “Cellular roles of BY-kinases”) and they have been classified recently in the same family, namely the BY-kinase family for Bacterial tyrosine kinase family (Grangeasse et al. 2007) (see section “BY-kinases”).

Alltogether, these observations seem to indicate that bacteria have developed various domains for ATP-binding/hydrolysis and phosphorylation of tyrosine residues. One may even expect that proteins of still unknown function could harbor a new type of phosphorylation mechanism to act as tyrosine-kinase. This suggestion is supported by the finding that the protein of unknown function YihE indeed exhibits a eukaryotic-like kinase fold despite sharing no sequence homology with eukaryotic kinases (Zheng et al. 2007).

BY-kinases

BY-kinases are specific to bacteria in the sense that no other proteins sharing similarities with them have been found to catalyze tyrosine phosphorylation in the other kingdoms of life. Depending on the bacterial phyla, BY-kinases are found in the form of either membrane proteins or only associated to the membrane via protein-protein interactions (Grangeasse et al. 2007) (Fig. 1). Indeed, in proteobacteria, BY-kinases are inner-membrane proteins and consist of two main domains: a N-terminal domain bordered by two transmembrane helices which is located outside the cell and a C-terminal domain, following the second transmembrane helix, located in the cytoplasm (Doublet et al. 2002). Firmicutes share the same organization, except that the two domains are encoded by two distinct genes (Grangeasse et al. 2007). The two polypeptides thus encoded reconstitute a full protein like BY-kinases of proteobacteria. Also, it has been shown that the cytoplasmic protein alone is not active. It needs to interact with the C-terminal juxtamembrane region of the associated membrane protein to reconstitute the full cytoplasmic domain of proteobacterial BY-kinases and to show a tyrosine-kinase activity (Mijakovic et al. 2003; Soulat et al. 2006; Wugeditsch et al. 2001). One can observe that the trans-membrane domain external loop is shorter in size in firmicutes than in proteobacteria (Grangeasse et al. 2007). A mix of the two situations seems to exist in actinobacteria. Indeed, *in silico* predicted BY-kinases likely correspond to the proteobacterial type, but with an external loop similar in size to BY-kinases from firmicutes. When comparing to eukaryotic tyrosine-kinases receptors, one may expect that this outside domain could transmit (a) signal(s) thus affecting the kinase activity of BY-kinases. Unfortunately such (a) signal(s) remain(s) to be identified. In addition, very limited structural data are available for the external loop of BY-kinases, except that a proline-rich motif preceding the second trans-membrane helix seems to be important for their function in polysaccharide synthesis (Becker and Puhler 1998; Morona et al. 2000b; Obadia et al. 2007). Recently, the crystal structures of several

Fig. 1 Organization of BY-kinases in proteobacteria (a) and Firmicutes (b). The red star indicates either the internal phosphorylation site (a) or the hydrophobic residue which complements the active site of BY-kinases (b) found in proteobacteria or firmicutes, respectively. *HD* Hairpin domain, *P-motif* proline-rich domain, *TM1* and *TM2* transmembrane span 1 and 2



proteins homologous to the N-terminal domain of BY-kinases, namely proteins of the Polysaccharide Co-Polymerase 1 (PCP1) family, have been reported (Tocij et al. 2008). The BY-kinases' external loop likely adopts a similar fold, *i.e.*, an α/β -base domain and an α -hairpin domain (Fig. 1). Nevertheless, structural predictions suggest that some differences between BY-kinases N-terminal domains and PCP1 seem to exist. Indeed, a modified base domain and a shorter hairpin domain can be predicted in BY-kinases from proteobacteria and firmicutes, respectively. It might therefore be speculated that these structural differences may be involved in specific interactions with other membrane proteins in order to influence the tyrosine-kinase activity of BY-kinases. On the other hand, one cannot exclude that phosphorylation of BY-kinases would affect the organization of the N-terminal domain.

The cytoplasmic C-terminal domain of BY-kinases has been well studied. This domain harbors the tyrosine-kinase activity and it has been shown that ATP molecules bind to this domain at a site different from that generally used by eukaryotic tyrosine-kinases (Doublet et al. 1999). This site comprises two amino acids sequences that resemble the Walker A (GXXGXGK[T/S]) and B (hhhhD) motifs normally found in nucleotide-binding proteins but not in eukaryotic protein-kinases (Leipe et al. 2003) (Fig. 1). Based on this homology, a general and common signature to BY-kinases has been proposed and more than 600 BY-kinases have been predicted in bacterial genomes (Jadeau et al. 2008). The Walker A motif of BY-kinases differs from the canonical Walker motif in that only the GK[S/T] amino acids are well conserved. Similarly, the BY-kinase Walker B motif is extended to ILVFM(3)DX(2)P. Also, the presence of an additional motif, termed Walker A' (ILVFM(3)DXDXR) has been observed (Fig. 1). This motif is important for Mg^{2+} binding. The BY-kinase

cytoplasmic domain is able to trans-phosphorylate on multiple tyrosine residues that are located in the very C-terminal end of the protein in a motif termed tyrosine cluster (Grangeasse et al. 2002; Morona et al. 2003; Paiment et al. 2002) (Fig. 1). Depending on the BY-kinases analyzed, the tyrosine cluster contains up to seven tyrosines that are potentially all phosphorylatable. An additional feature has been reported for BY-kinases from proteobacteria: a tyrosine located four amino acids away from the Walker A' motif, has been shown to be cis-phosphorylated (Grangeasse et al. 2002). Phosphorylation at this site has been shown to enhance phosphorylation of the tyrosine cluster. However, some BY-kinases possessing this "internal" tyrosine are not able to cis-phosphorylate at this position (Wugeditsch et al. 2001). Also, even if it is well conserved, a tyrosine is not systematically found at this position. Therefore, the occurrence and the role of cis-phosphorylation in BY-kinases of proteobacteria remain unclear and need further investigations.

Recently, the structures of two BY-kinases from firmicutes and proteobacteria have been reported for the first time (Lee et al. 2008; Olivares-Illana et al. 2008). This concerns the BY-kinases Etk and CapA/CapB from *E. coli* and *S. aureus*, respectively. These data explain at the molecular level some previous observations. First, concerning the cis-phosphorylation of the "internal" tyrosine of BY-kinases of proteobacteria, it has been suggested that it would induce a rotation of the phosphorylated tyrosine side chain rendering the catalytic site accessible to tyrosine cluster, or to any substrates, for trans-phosphorylation (Lee et al. 2008) (Fig. 1). This hypothesis is based on the two-step phosphorylation process originally described for BY-kinases of proteobacteria (Grangeasse et al. 2002), but it still needs to be confirmed. Another finding concerns the activation of BY-kinases found in firmicutes. Upon

interaction between the membrane domain and the cytoplasmic BY-kinase, the N-terminal membrane protein activates the cytoplasmic BY-kinase (Olivares-Illana et al. 2008). This activation is performed by a hydrophobic residue of the juxtamembrane domain, namely F221 in CapA of *S. aureus*, which complements the active site of CapB and interacts with the base moiety of ATP (Fig. 1). It is interesting to highlight the structural similarity between this juxtamembrane region of CapA and that of the N-terminal region of the cytoplasmic domain of Etk, in which the binding of the base moiety of the nucleotide, however, involves different residues. Previous studies had clearly established that BY-kinases are able to form oligomers (Collins et al. 2006; Doublet et al. 2002). These new structural data are in agreement with this oligomeric organization. In the case of Etk, a new motif, termed RK domain, rich in Arg and Lys, has been evidenced (Lee et al. 2008) (Fig. 1). This motif is well conserved in BY-kinases of proteobacteria, but it is not found in ones of firmicutes. It has been proposed that phosphorylation of the tyrosine cluster motif would modulate its ability to interact with the RK domain, thus resulting in structural changes in the oligomer. In firmicutes, and more especially CapB of *S. aureus*, it has been shown that the tyrosine cluster fits in the active site of the neighboring CapB molecule and that upon phosphorylation, the cytoplasmic domains of the oligomer dissociate (Olivares-Illana et al. 2008). It is clear that BY-kinases form oligomers; nevertheless, the levels of oligomerization seem to differ between BY-kinases from firmicutes and proteobacteria. Low resolution electron microscopy data indeed suggest that BY-kinases from proteobacteria form tetramers instead of the octamer observed in the crystal structure of CapAB, a BY-kinase from firmicutes. This observation could reflect differences in the functioning of BY-kinases, but one can not exclude that alternative oligomerization states could exist in vivo depending on the presence of other cellular partners.

Cellular roles of BY-kinases

Since they have been detected, BY-kinases have been shown to be involved in several cellular functions. Initially, they had been best studied for their ability to influence the biosynthesis of extracellular polysaccharides (Fig. 2). BY-kinases are encoded most often by genes that are part of large operons that direct the biosynthesis of these sugar polymers (Whitfield 2006). For instance, in *E. coli*, depending on the serotype analyzed, the BY-kinase Wzc encoding gene is located in a cluster controlling the production of either the exopolysaccharide colanic acid or the Group I capsular polysaccharide. Also, the second BY-kinase-encoding gene, *etk*, identified in *E. coli*, the

product of which is homologous to Wzc, is part of an operon determining the production of a Group 4 capsular polysaccharide (Peleg et al. 2005). It has been demonstrated that an active BY-kinase, as Wzc or Etk in *E. coli*, is required for polysaccharide synthesis. More precisely, the trans-phosphorylation of the tyrosine cluster is a key feature. Indeed, mutations affecting either the presence or the phosphorylation of the tyrosine cluster cause defects in the production of the cognate polysaccharide (Morona et al. 2003; Obadia et al. 2007; Paiment et al. 2002). More precisely, the overall level of phosphorylation of the tyrosine cluster, rather than the precise combination of residues accessible to phosphorylation, seems to be important for the extracellular polysaccharide synthesis. It has been also observed that BY-kinases influence not only the amount of the polymer produced, but also its size. It is thus speculated that BY-kinases could affect the functioning of the polysaccharide polymerase, namely the Wzy protein (Whitfield and Larue 2008). However, a direct interaction between Wzy and a BY-kinase has not been reported to date. A question arises as to which form of the BY-kinases, i.e. phosphorylated or non-phosphorylated, was active in polysaccharide production. Beside initial divergent observations, the main current model is that BY-kinases autophosphorylation does not act as an on/off mechanism, comparable to a switch. Rather, BY-kinase function requires its cycling between phosphorylated and non-phosphorylated forms to allow polysaccharide synthesis.

A major breakthrough in the characterization of the biological role of BY-kinases was made when they have been shown to phosphorylate endogenous proteins. UDP-sugar dehydrogenases were one of the first to be characterized (Fig. 2). Their activity is enhanced upon phosphorylation by the BY-kinases PtkA, Wzc and Etk, and CapB2 in *B. subtilis*, *E. coli*, and *S. aureus*, respectively (Grangeasse et al. 2003; Mijakovic et al. 2003; Soulat et al. 2007). More recently, a glycosyl-transferase was also found to be phosphorylated by the BY-kinase EpsD in *Streptococcus thermophilus* (Minic et al. 2007). These enzymes are involved in the synthesis of essential components in the biosynthesis of bacterial polysaccharides. In the case of *E. coli*, it has been demonstrated that beside the BY-kinase Wzc autophosphorylation, Wzc-mediated phosphorylation of Ugd also affect the production of polysaccharide (Obadia et al. 2007). Therefore, it is tempting to assume that BY-kinase could regulate two different steps of the polysaccharide synthesis pathway: an early step dedicated to polymer subunit production and a later step of transport and polymerization. However, one cannot exclude that phosphorylation of sugar-dehydrogenase or -transferase and phosphorylation of BY-kinases themselves are interdependent. Indeed, when looking at the crystal structure of the non-phosphorylated *S. aureus* CapB2

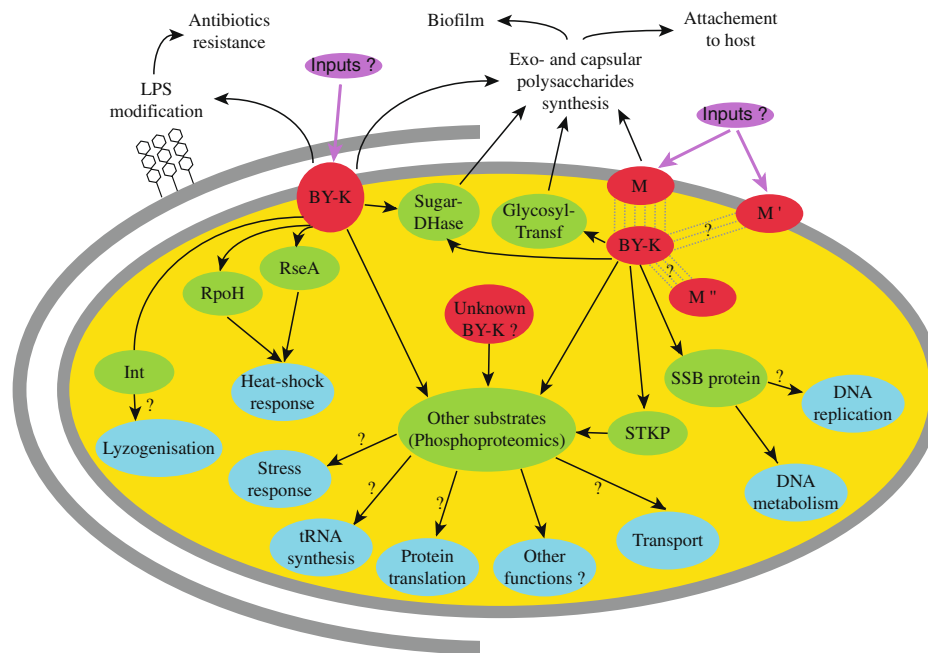


Fig. 2 Schematic model of the main BY-kinase mediated phosphorylation events and effects in a virtual chimerical proteobacteria-firmicute cell. BY-kinases (BY-K) and their modulators (M) are indicated in *red*. M' and M'' indicate potential alternative membrane and cytoplasmic modulators, respectively. Endogenous protein phosphorylated by BY-kinases and their cognate function are indicated in *green and blue*, respectively. Potential stimuli that could influence

BY-kinases activity are indicated in *purple*. Dashed-gray lines indicate interactions between BY-K and M. Question marks indicates expected relationship but which remain to be proved. Eukaryotic-like Ser/Thr kinases (STPK), lipopolysaccharide (LPS), integrase (Int), UDP-sugar-dehydrogenase (Sugar-DHase), glycosyl-transferase (Glycosyl-transf)

octamer, the tyrosine cluster fits in the active site of the neighboring CapB molecule and thus hinders access of the endogenous substrate to the catalytic site. Therefore, one can assume that any defect in tyrosine cluster phosphorylation and/or kinase activity should affect also the phosphorylation of endogenous substrates.

Other proteins are also phosphorylated by BY-kinases, among which heat-shock sigma factors RpoH and RpoE (Klein et al. 2003) (Fig. 2). RpoH and RpoE are the two sigma factors that control the expression of the two heat-shock regulons in *E. coli*. It was concluded that expression of a phosphorylation defective RpoH mutant confers a temperature-sensitive phenotype. Similarly, phosphorylation of RseA, an anti-RpoE factor is thought to alter its binding affinity toward RpoE. Altogether, these studies show that a BY-kinase can regulate the heat-shock response, and one can assume that tyrosine phosphorylation could be involved in other gene regulation processes. Another example comes from the work performed in *B. subtilis* and that concerns the phosphorylation by the BY-kinase PtkA of the single-stranded DNA-binding protein (SsbA) (Mijakovic et al. 2006) (Fig. 2). The role of SsbA phosphorylation is still unclear, but it has been observed that a PtkA-deficient *B. subtilis* strain accumulates extra chromosome equivalents and exhibits aberrant

initiation mass for DNA replication (Petranovic et al. 2007). These observations support the existence of a relationship between DNA metabolism and BY-kinases-mediated phosphorylation. Also, it has recently been observed that the integrase (Int) of certain phages is phosphorylated on tyrosine in *E. coli* (Kolot et al. 2008) (Fig. 2). BY-kinase Wzc has been shown to catalyze Int phosphorylation in vitro, but the in vivo Wzc-Int relationship remains unclear. Nevertheless, Int phosphorylation has been connected to reduced lysogenization and it is suggested that Wzc down regulates the activity of Int.

Attributing one cellular function to one BY-kinase is rendered complicated as they are capable of phosphorylating several classes of protein substrates. For instance, BY-kinase Etk of *E. coli* is able to phosphorylate the UDP-glucose dehydrogenase (Ugd) in addition to sigma and anti-sigma factors RpoH and RseA (see above). Etk-mediated phosphorylation of Ugd has been shown to influence the ability of *E. coli* to resist to certain antibiotics, namely cationic antimicrobial peptides of the innate immune system (Lacour et al. 2006, 2008) (Fig. 2). Similarly, in *B. subtilis*, BY-kinase PtkA was found to phosphorylate both Ugd and Ssb (see above) (Mijakovic et al. 2003, 2006). If we look at the other side of the kinase/substrate relationship, one can imagine that a protein could

be phosphorylated by two distinct kinases. Such a situation has been recently described, and depending on the growth conditions of *E. coli*, protein Ugd can be phosphorylated by either Etk or Wzc (Lacour et al. 2008). In addition, recent site-specific phosphoproteomics studies performed with three bacterial models, namely *Lactobacillus lactis*, *E. coli*, and *B. subtilis*, have indicated that many more proteins could be tyrosine-phosphorylated (Macek et al. 2007, 2008; Soufi et al. 2008). In these studies, up to 10% of the phospho-sites detected are phosphotyrosines. It concerns proteins that carry out functions as diverse as tRNA synthesis, transport of amino acids, protein translation, amino acid synthesis, and stress response, but also on proteins of unknown functions (Fig. 2). Besides, these patterns of phosphotyrosine-proteins are expected to be incomplete, since they represent only “frozen snapshots” of phosphorylation in the bacterial cell in a given environment and growth state.

Concluding remarks

Despite a difficult start of this new field, it is now obvious that bacterial tyrosine phosphorylation represents an important regulatory device of bacterial physiology. In this review, we chose to focus essentially on tyrosine-kinases and their functions. Nevertheless, it must be kept in mind that for each kinase, there should be a complementary phosphotyrosine-phosphatase activity. We have not discussed about these enzymes, but it is noteworthy to mention that a number of them have been identified in bacteria. Like kinases, they share some structural similarities with their eukaryotic counterparts whereas others seem to be idiosyncratic to bacteria (for review see (Grangeasse et al. 2007)). In some cases, they have been shown to offset the BY-kinase functions, but their cellular role remains largely obscure. Henceforth, one may consider that the regulation of several physiological processes could depend only on the phosphorylation/dephosphorylation of a bacterial protein by its cognate tyrosine-kinase and phosphotyrosine-phosphatase. However, BY-kinases seem to be promiscuous enzymes, i.e., they are able to phosphorylate several endogenous proteins. In addition, the kinase activity of BY-kinases can likely be modulated by still unknown factors. Indeed, on the one hand, the indirect influence of AMPc on the activity of BY-kinase Ptk of *A. johnsonnii*, and on the other hand the possible existence of additional modulators of the BY-kinases of firmicutes, support this hypothesis. Therefore, very complicated BY-kinase dependent regulatory networks are expected to exist.

Tyrosine phosphorylation could also be considered as a self-sufficient regulatory device of particular bacterial functions. However, once again, one can expect that

crosstalk between BY-kinases and other phosphorylating/dephosphorylating bacterial enzymes will complicate this view. This hypothesis is supported by phosphoproteomics that clearly shows that certain tyrosine-phosphorylated proteins are also phosphorylated on serine and threonine. Also, in *L. lactis*, the probable STPK PknB is found to be tyrosine-phosphorylated (Soufi et al. 2008) (Fig. 2). Altogether, it is tempting to predict that beyond the existence of cellular functions peculiar to tyrosine phosphorylation, dynamic phosphorylation network involving S/T/Y phosphorylation, but also proteins of the 2CS and the PTS, will be involved in the global bacterial response and adaptation to its environment.

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