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Overview of protein phosphorylation in bacteria
with a main focus on unusual protein kinases in *Bacillus subtilis*

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Running title:

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1 Abstract

2 Protein phosphorylation is a post-translational modification that affects protein activity through the
3 addition of a phosphate moiety by protein kinases or phosphotransferases. It occurs in all life forms.
4 In addition to Hanks kinases found also in eukaryotes, bacteria encode membrane histidine kinases
5 that, with their cognate response regulator, constitute two-component systems and
6 phosphotransferases that phosphorylate proteins involved in sugar utilization on histidine and
7 cysteine residues. In addition, they encode BY-kinases and arginine kinases that phosphorylate protein
8 specifically on tyrosine and arginine residues respectively. They also possess unusual bacterial protein
9 kinases illustrated here by examples from *Bacillus subtilis*.

10

1 Introduction

2 Protein phosphorylation is a reversible post-translational modification (PTM) that is widespread in all
3 domains of life, eukaryotes, bacteria and archaea [1]. It is well characterized and its biological functions
4 are well documented; it plays important physiological roles [2-5]. This PTM is catalyzed by enzymes
5 called phosphotransferases or protein kinases [6-11]. Depending on their characteristics, these
6 enzymes specifically phosphorylate amino acid residues in proteins like serine (Ser), threonine (Thr),
7 tyrosine (Tyr), histidine (His), cysteine (Cys), aspartate (Asp) and arginine (Arg). The addition of
8 phosphoryl moieties regulates the activity of target proteins by, for example, inducing conformational
9 changes or modifying protein-protein interactions [12]. The added phosphoryl moieties on proteins
10 are removed either by protein phosphatases or phosphorylases.

11 Some of these enzymes are autophosphorylated or phosphorylate their substrates on His, Cys, Asp or
12 Arg residues and others are autophosphorylated or phosphorylate their substrates on Ser, Thr and Tyr
13 residues. It is noticeable that phosphorylations on His, Asp and Arg residues are relatively unstable at
14 acidic pH and sensitive to heat. By contrast phosphorylations on Ser, Thr, Tyr residues are more stable
15 [13]. In addition, the phosphorylation or autophosphorylation of a His or an Arg residue creates
16 phosphoramidate bond that has a standard free energy (ΔG) high enough to generate ATP out of ADP
17 [14, 15]. Consequently, a kinase that is autophosphorylated on these residues can be considered as a
18 high-energy phosphoenzyme intermediate mediating the transfer of a phosphoryl moiety from a
19 donor (the phosphotransferase) to an acceptor (its substrate) by contrast with phosphorylation of
20 proteins on Ser, Thr or Tyr that generates phosphoesters (Fig. 1) [13].

21 In this review, we will briefly describe the different types of phosphorylating enzymes that have been
22 identified so far in bacteria and we will illustrate this article by examples of unusual or atypical protein
23 kinases found in the Gram-positive model bacterium *Bacillus subtilis*.

24

25 Phosphorylation on His, Asp and Arg residues

1 In bacteria, two types of enzymes that phosphorylate their substrate(s) on His residues exist. The first
2 one belongs to the emblematic two components system (TCS). This system of phosphorylation was
3 considered as “The” system of phosphorylation in bacteria, TCS being the most abundant in
4 comparison to other systems [9-11]. TCS are generally composed of a sensor kinase (also called “His
5 kinase” or “phosphotransferase”) that autophosphorylates at a conserved His residue from ATP and a
6 response regulator that accepts this phosphoryl group on a conserved Asp residue. This Asp
7 phosphorylation, less stable than a phosphorylation on Ser, His or Thr, induces a conformational
8 change of the response regulator. The sensor kinases are frequently membrane proteins that respond
9 to environmental signals and phosphorylate response regulators that are transcriptional regulators.
10 Despite that the sensor kinases are highly specific of their cognate response regulators, several cross-
11 talks between TCSs have been reported; they may be due to the co-evolution of the participating
12 proteins [16, 17]. Although this phosphorelay system is qualified to be bacterial since TCSs are present
13 in a wide range of bacterial species and regulate a variety of cellular processes [18], they seem also
14 present in lower eukaryotes [19, 20].

15 The second type of enzyme that phosphorylates protein(s) on His residues belongs to the
16 phosphoenolpyruvate : sugar phosphotransferase system (PTS) present in bacteria and in archaea [21,
17 22]. These enzymes are involved in the transport and concomitant phosphorylation of sugars but are
18 also able to phosphorylate catabolic enzymes, transporters and transcriptional regulators [6, 7, 23].
19 These phosphorylation reactions occur on conserved His residues (or Cys residues) and the initial
20 phosphoryl donor is the phosphoenolpyruvate (PEP). The phosphoryl group of PEP is usually
21 transferred via three proteins or protein complexes, Enzyme I, the phosphocarrier protein HPr and the
22 different units of Enzyme II, to the transported carbohydrate. It is notable that the first steps of this
23 phosphorylation cascade are reversible; indeed the phosphoryl derivatives of the cytoplasmic proteins
24 or domains of the PTS (EI, HPr, EIIA and EIIB) exhibit similar energy [24]. In addition, depending on their
25 phosphorylation state, they can phosphorylate conserved His residues of catabolic enzymes,

1 transporters and transcriptional regulators, all related to carbohydrate utilization, or interact with
2 them in a phosphorylation-dependent manner to regulate their activity [7, 8].
3 The bacterial Arg kinases have been recently identified in comparison to TCS and PTS phosphorylation
4 systems. They are poorly characterized except McsB from *B. subtilis* [25]. By a two-dimensional
5 chromatography with phosphoamino acid standards, this protein was initially proposed to be a Tyr
6 kinase [26] but in 2009, it was identified as an Arg kinase [15]. This protein, with YwLE, its cognate
7 phosphatase, is involved in stress response, protein quality control and in spore germination [15, 25,
8 27-29]. Indeed, phosphorylation of proteins on Arg residues by McsB from ATP serves as a tag for
9 ClpCP, a protease composed of the ClpC ATPase that recognizes and unfolds substrates then
10 translocates them to the ClpP active protease [25]. McsB seems to be the single Arg protein kinase in
11 *B. subtilis* because deletion of its gene abolishes all Arg phosphorylations detectable in this bacterium
12 (217 sites in 134 proteins) [29, 30]. The analysis of McsB sequence and structure showed that it has a
13 weak similarity to phosphagen kinases (PhKs), enzymes that catalyze the reversible phosphate transfer
14 between ATP and guanidino substrates. In addition, the PhK domain of Arg kinases can phosphorylate
15 the free amino acid Arg that can be used as substrate to regenerate ATP from ADP [31]. This reaction
16 plays a central role in cellular energy homeostasis. Concerning McsB, the phosphorylation reaction is
17 catalyzed by the PhK-like catalytic domain [32]. Since McsB possesses several substrates [30], we
18 wonder how this enzyme can recognize its targets; it was proposed that the specificity of McsB for its
19 substrates and against isolated arginine is determined by the accessibility of the active site,
20 interactions with the peptide backbone and electrostatic effects [32].

21

22 **Phosphorylation on Ser, Thr and Tyr residues**

23 Depending on their sequences, the nature of the residues they phosphorylate and their structures,
24 bacterial Ser, Thr or Tyr protein kinases can be classified into 3 types. Two seem specific of prokaryotes
25 whereas Hanks kinases are conserved in both eukaryotes and prokaryotes [33].

1 Until 1991, before the first bacterial Hanks kinase has been discovered in *Myxococcus xanthus* [34],
2 Hanks kinases were considered to be specific of eukaryotes. But now, this family of kinases is widely
3 represented in bacteria [33, 35] and for example, the *Mycobacterium tuberculosis* genome encodes 11
4 serine/threonine protein kinases [36]. The Hanks kinases are defined by the properties of their catalytic
5 domain. Indeed, the sequence of their catalytic domain possesses 12 specific signatures [37, 38]. These
6 enzymes oligomerize and autophosphorylate from ATP to be active. In eukaryotes, they phosphorylate
7 proteins on Ser, Thr and Tyr residues whereas in bacteria, they only phosphorylate Ser and Thr
8 residues. From a structural point of view, the catalytic domain of Hanks kinases possesses a
9 characteristic two-lobed catalytic core structure with the active site located in a deep cleft formed
10 between the two lobes (Fig. 2) [37]. The N-terminal lobe is involved in binding and orienting the ATP
11 molecule that is the phosphoryl donor; the C-terminal lobe is responsible for binding to the protein
12 substrate and for the transfer of the phosphoryl group. The structural characteristics of the catalytic
13 domain are conserved in eukaryotes and prokaryotes. In addition, Hanks kinases have several
14 substrates [35]; for example, the well-characterized Hanks kinase PrkC from *B. subtilis* has about ten
15 characterized substrates.

16 The first bacterial Tyr kinase was discovered more recently. Indeed, it was identified in 1997 in
17 *Acinetobacter johnsonii* [39] and was the first member of a new family of protein kinases also called
18 BY-kinase [40, 41]. This family of protein kinases is found in several bacteria including Cyanobacteria,
19 Firmicutes and Actinobacteria [42]. In proteobacteria, the BY-kinases are composed of an outside
20 sensory loop linked to the catalytic intracellular domain whereas in firmicutes the outside sensory loop
21 is separated from the catalytic intracellular domain. This catalytic domain contains Walker A and B
22 motifs found in many nucleotide binding proteins [43]. In addition, BY-kinases autophosphorylate on
23 several tyrosine residues in a C-terminal motif termed tyrosine cluster. BY-kinases are involved in
24 various processes and in particular, in regulation of the production of capsular polysaccharide and
25 colanic acid [42, 44, 45].

1 In addition to the BY-kinases and Hanks kinases, others protein kinases that do not belong to one of
2 these families are able to phosphorylate their substrate(s) on Ser or Thr or Tyr residue(s). They could
3 be classified as unusual or atypical protein kinases (APK) like the cell translocating kinase A (CtkA) from
4 *Helicobacter pylori* [46] involved in the induction of host inflammatory responses, the high-persistence
5 factor A (HipA) from *Escherichia coli* [47, 48] and the Isocitrate dehydrogenase kinase/phosphatase
6 (IDH K/P) from *E. coli* that regulates the glyoxylate pathway [49, 50]. All these enzymes do not possess
7 any similarities of sequence and structure with Hanks kinases (or BY-kinases) and do not necessarily
8 autophosphorylate but catalyze the phosphorylation of their substrate(s) on Ser or Thr residue(s). The
9 rest of this review will describe these APKs by focusing on enzymes found in *B. subtilis*.

10

11 **HPr kinase/phosphorylase, an ancestral APK that regulates catabolite repression**

12 The HPr kinase/phosphorylase (HprK/P) is the enzyme that catalyzes the phosphorylation and the
13 dephosphorylation of the HPr protein of the PTS, and its homologous protein Crh, on the conserved
14 Ser 46 residue in *B. subtilis* [51, 52] but it is not autophosphorylated. This key atypical enzyme,
15 extensively studied, is present in several bacteria; it plays a central role in the control of carbohydrate
16 uptake and in the preferential utilization of favorite carbon sources, a phenomenon known as
17 catabolite repression [52-54]. This is a homo-oligomeric bifunctional enzyme whose kinase activity is
18 stimulated by glycolytic intermediates like fructose 1,6-bisphosphate (FBP); it was shown that it
19 exhibits a strong positive cooperativity for ATP and FBP binding [55]. This enzyme is unusual by several
20 aspects. Although a HPr kinase activity has been discovered in 1983 [51], the identification of the gene
21 encoding the enzyme catalyzing this activity was identified fifteen years later [52]. Sequence analysis
22 showed that HprK/P does not possess any similarity with Hanks kinases but possess a Walker A motif
23 found in many proteins that bind ATP or GTP. This Walker A motif (G/A)xxxxGK(S/T) is characteristic of
24 the phosphate binding loop (P-loop) at the nucleotide binding site [43]. In addition, the structure of
25 HprK/P was the first structure of a bacterial Ser protein kinase to be characterized [56]. It confirms that
26 HprK/P is not related to Hanks kinases but belongs to another large structural family, the P-loop

1 proteins, containing kinases that phosphorylate small molecules, G-proteins and ATPases. The
2 structure of the substrate-enzyme complex revealed that HprK/P is composed of two trimers
3 associated in a hexamer that binds six HPr (or Crh) molecules [57]. Each monomer is composed of a N-
4 terminal domain and a C-terminal catalytic domain that possesses the two enzymatic activities. The
5 hexamer is maintained by contacts between C-terminal catalytic domains. The function of this N-
6 terminal domain is unknown but its fold is similar to the N-terminal domain of MurE/MurF, two
7 enzymes involved in peptidoglycan biosynthesis [56]. The catalytic C-terminal domain forms a globular
8 structure in which the Walker A motif forms the P-loop. In addition, when the protein is crystallized in
9 the presence of inorganic phosphate, this latter is found in the P-loop (Fig. 3) which is likely the binding
10 site for the phosphoryl moiety of the nucleotide substrate. The HprK/P catalytic domain shows
11 structural similarities with P-loop containing proteins and its closest structural homologue is the
12 phosphoenolpyruvate (PEP) carboxykinase from *E. coli* [58, 59]. In addition to its kinase activity, HprK/P
13 is able to dephosphorylate its two substrates, P-Ser-HPr and P-Ser-Crh in the presence of inorganic
14 phosphate [60]. By combining structural and biochemical analysis, this reaction, which was first
15 assumed to be hydrolytic, has been demonstrated to be phosphorolytic [57, 61]. HprK/P, which binds
16 phosphate at the same site as the β -phosphate of ATP, probably uses the inorganic phosphate instead
17 of a water molecule to carry out a nucleophilic attack on the phosphoryl bond in P-Ser-HPr or P-Ser-
18 Crh to produce pyrophosphate. This is the first case where an enzyme is able to catalyze the
19 dephosphorylation of a phosphorylated protein via a phospho-phosphorolysis mechanism. This
20 reaction is reversible; indeed, when pyrophosphate concentrations are high, HprK/P phosphorylates
21 its substrates from pyrophosphate as phosphoryl donor [61]. The authors proposed that, since HprK/P
22 possesses structural and functional similarities with several nucleotide kinases that phosphorylate
23 small molecules such as adenylate kinase or shikimate kinase and especially PEP carboxykinase, these
24 enzymes evolved from similar ancestors that probably used oligophosphates, the presumed ancestral
25 energy-rich phosphoryl donor. Interestingly, the FBP that stimulates HPr and Crh phosphorylation from
26 ATP does not affect the pyrophosphate-dependent HPr phosphorylation [61, 62]. This suggests that

1 stimulation of the enzyme by FBP appeared later in the evolutionary history of HprK/P, after it acquired
2 the ability to use ATP. Stimulation of ATP-dependent kinase activity of HprK/P by FBP renders ATP as
3 a more efficient phosphoryl donor than pyrophosphate [61].

4

5 **APKs involved in sporulation and germination of *B. subtilis***

6 One particularity of *B. subtilis* is its ability to sporulate. Spore formation is the result of an asymmetric
7 division that is induced by a nutritional stress. The spores are highly resistant to heat, desiccation or
8 radiation and can persist in the environment for a long time until conditions become favorable. This
9 process is highly controlled in particular by phosphorylation events via TCS and notably the
10 phosphorylation of the transcriptional regulator Spo0A. In addition to these phosphorylation events
11 and phosphotransfers via TCS, APKs are also involved in regulation of sporulation.

12 The first identified APK involved in sporulation is SpoIIAB. This small protein of about 16 kDa (less than
13 150 residues) acts as an anti-sigma factor by sequestering the sporulation specific sigma factor F in an
14 inactive complex [63]. It is also a protein kinase that does not possess similarities with any classical
15 kinases. It phosphorylates the anti-anti-sigma factor SpoIIAA on Ser residue [64]. In a few words, sigma
16 F should be free to be active and is inactivated when it is in complex with SpoIIAB (Fig. 4). The release
17 and activation of sigma F is due to the protein SpoIIAA that triggers the dissociation of the SpoIIAB-
18 sigma F complex [63, 64]. In addition, SpoIIAB interacts with SpoIIAA to phosphorylate it by using ATP.

19 This phosphorylation inactivates SpoIIAA that, in its phosphorylated form, has very low affinity for
20 SpoIIAB; in such condition, sigma F interacts with SpoIIAB and thus is inactive. Phosphorylation of
21 SpoIIAA by SpoIIAB thus prevents the activation of sigma F indirectly. SpoIIIE is another actor in this
22 feed-back regulation loop; it is a specific phosphatase that dephosphorylates phosphorylated SpoIIAA.

23 In its unphosphorylated form, SpoIIAA can again interact with SpoIIAB to liberate sigma F and can again
24 be phosphorylated. Thus, there is a competition between sigma F and SpoIIAA (the anti-anti-sigma
25 factor) for the binding to SpoIIAB that is regulated by the reversible phosphorylation of SpoIIAA (Fig.
26 4).

1 CotH, a component of the spore coat, is another APK whose role is connected to sporulation and found
2 in many sporulating and non-sporulating bacteria. This protein is not required for sporulation but is
3 involved in the revival of the spore (spore germination) in *B. subtilis*. Sequence analysis revealed that
4 this protein possesses significant sequence similarity with the APK HipA [47, 48] and also with the
5 eukaryotic Fam20C-related secretory pathway kinases [65]. The elucidation of the crystallographic
6 structure of CotH showed that it possesses N- and C-lobes. However, the two lobes are quite different
7 from those usually found in Hanks kinases; notably CotH possesses an atypical protein kinase-like fold
8 with a unique mode of ATP binding [65]. Moreover, using purified proteins, it was observed that CotH
9 autophosphorylates and is also able to phosphorylate CotB and CotG, two proteins of the spore coat
10 that were previously shown to interact with CotH [66]; these phosphorylations are necessary for spore
11 germination [65]. Up to now, the nature of the phosphorylated residue is unknown. However, Fam20C
12 phosphorylates its substrates with a clear preference for Ser residues and HipA is autophosphorylated
13 and phosphorylates its substrate, the Glu-tRNA-ligase, on a Ser residue; we can thus hypothesize that
14 CotH is probably a Ser kinase.

15 The last APK involved in sporulation that will be described in this review is PrkA (that is not homologous
16 with PrkA from *Listeria monocytogenes*). This protein possesses homologs in many bacteria [67]; it was
17 suggested to be a protein kinase from sequence analysis that revealed a distant homology with Hanks
18 kinases [68]. In fact, PrkA is composed of two domains, a N-terminal domain related to ATPase
19 Associated with diverse cellular Activities (AAA+) containing a Walker A motif (this motif being also
20 found in several APKs) and a potential C-terminal kinase domain. In *B. subtilis*, no autophosphorylation
21 of PrkA was detected but addition of purified PrkA in a *B. subtilis* crude extract induced the
22 phosphorylation of an unidentified 60-kDa protein at a Ser residue [68]. PrkA was also shown to be
23 inhibited by an inhibitor of ATP-binding proteins [69]. Thus, even if it was clear that PrkA was involved
24 in a phosphorylation event, there were only few arguments to classify PrkA as a protein kinase. But
25 very recently, it was shown that, *in vitro*, the PrkA homologue from *E. coli*, YeaG was able to directly
26 phosphorylate the isocitrate lyase demonstrating that this protein is indeed a protein kinase [70]. The

1 cellular role of *B. subtilis prkA* gene was investigated; it is expressed during sporulation from the
2 sporulation specific sigma factor sigma E and its deletion results in a decrease of sporulation [71]. In
3 addition, analysis of PrkA cellular localisation revealed a characteristic localisation of a protein
4 associated with the spore coat. It was proposed that PrkA regulates, via the transcriptional repressor
5 ScoC, the expression of the sporulation specific sigma factor sigma K [69]. But, up to now, the
6 mechanism by which PrkA regulates ScoC and sigma K, the potential substrate(s) that is (are)
7 phosphorylated by PrkA, and the domain of PrkA responsible of the kinase activity are unknown.

8

9 **UbK constitutes a new family of conserved APK**

10 Recently, an APK, UbK (for Ubiquitous bacterial Kinase) that phosphorylates substrates on Ser/Thr/Tyr
11 residues has been identified [72]. This protein, found in several bacteria, was previously proposed to
12 be an ATPase [73]. But recent data obtained in *B. subtilis* and *Streptococcus pneumoniae* indicate that
13 this family of proteins in fact constitutes a new family of Ser/Thr/Tyr protein kinases [72, 74]. In *B.*
14 *subtilis*, Ubk protein (also named YdiB and TsaE) was shown to be able to autophosphorylate on Ser,
15 Thr and to a lesser extent on Tyr and to phosphorylate the YdiE protein, a protein that was proposed
16 to participate in a chemical modification of some specific tRNAs [72]. The 3D-structure of Ubk was
17 solved and showed that this kinase possesses a unique ATP-binding fold unrelated to classical protein
18 kinases, even if Ubk has a conserved HxD motif also found in Hanks kinases [72]. Concerning the cellular
19 role of Ubk in *B. subtilis*, it was firstly described as an essential protein [75]. Reinvestigation of its
20 cellular role showed that *ubk* gene is not essential [76] but its deletion or mutations induce a delay of
21 growth and a modification of resistance to oxidative stress [77]. However, here again, the mechanism
22 by which Ubk regulates resistance to oxidative stress as well as the consequence of YdiE
23 phosphorylation are unknown.

24

1 In conclusion, bacteria possess several systems to phosphorylate proteins and consequently regulate
2 a variety of cellular processes. Even if some of them are well characterized, the dynamics of the
3 phosphorylation events *in vivo* remains relatively unstudied. However, with the progress in live-cell
4 imaging, this challenge will be probably solved in the near future. In addition, some APK remain poorly
5 characterized. Their biochemical characterization, the identification of their substrates and the
6 determination of their role in bacterial physiology are important challenges for future studies. The
7 results will allow to reach a complete point of view concerning our knowledge on this key PTM that is
8 protein phosphorylation.

9

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12

13 **Competing interests**

14 The authors declare no competing interests.

15

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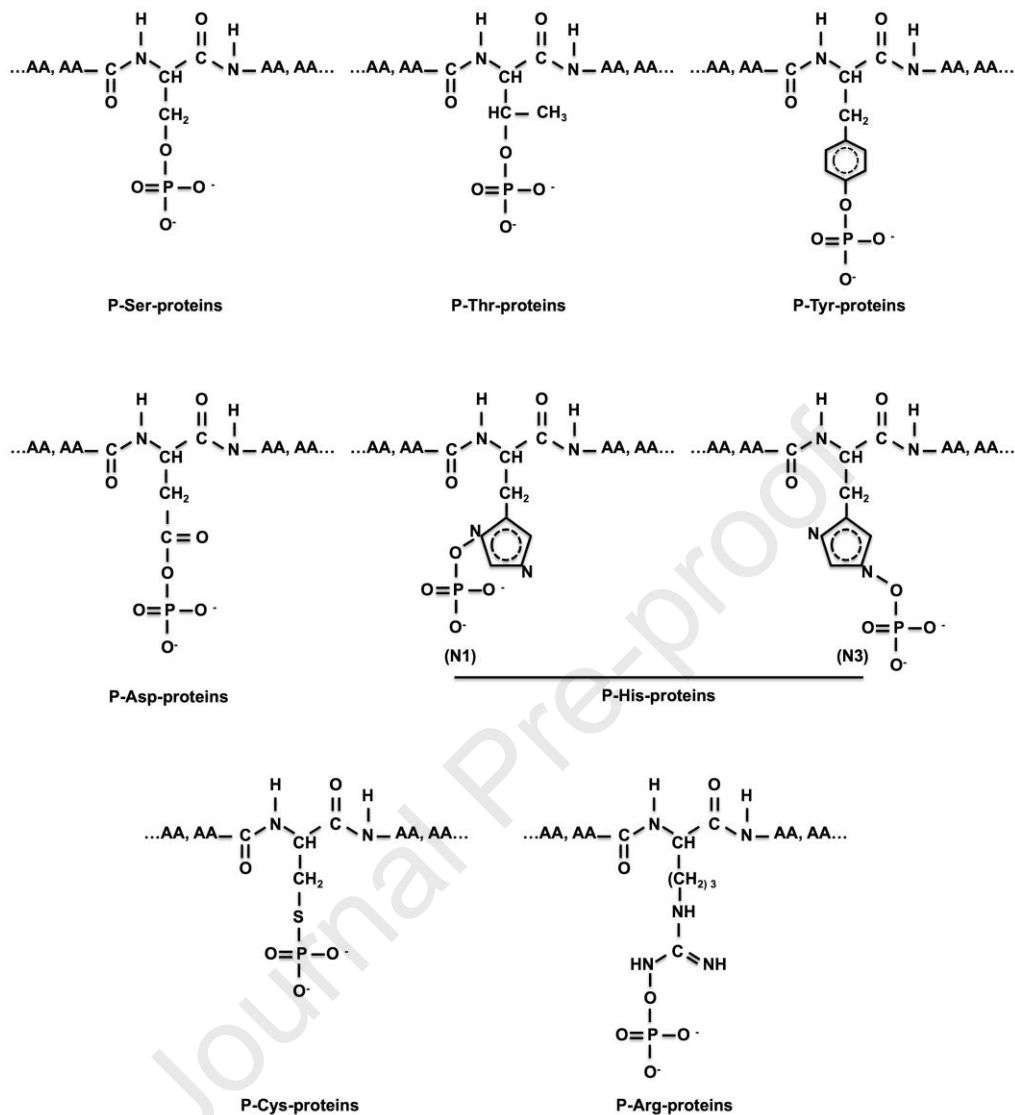
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Journal Pre-proof

1 Figures

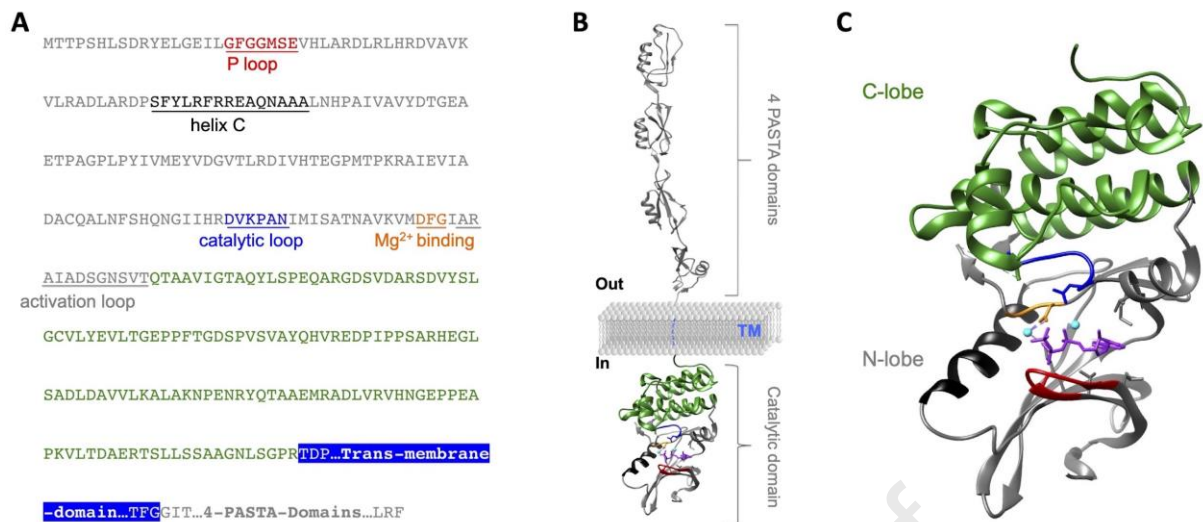


2

3 **Figure 1: Nature of phosphorylated residues in bacterial proteins.**

4 The phosphorylation of Ser, Thr and Tyr residues results in the formation of a phosphoester bound,
 5 the phosphorylation of Asp results in the formation of an acyl-phosphate, the phosphorylation of His
 6 and Arg results in the formation of a phosphoramidate and phosphorylation of Cys results in the
 7 formation of a phosphorothiolate. Phosphorylated His can exist as two distinct phosphoisomers, the
 8 1- or 3 isomer, resulting from covalent attachment of the phosphate to the N1 or N3 position of the
 9 imidazole core, depending on the specificity of the kinase; the phosphorylation of His on N1 is slightly
 10 less stable than the phosphorylation on N3.

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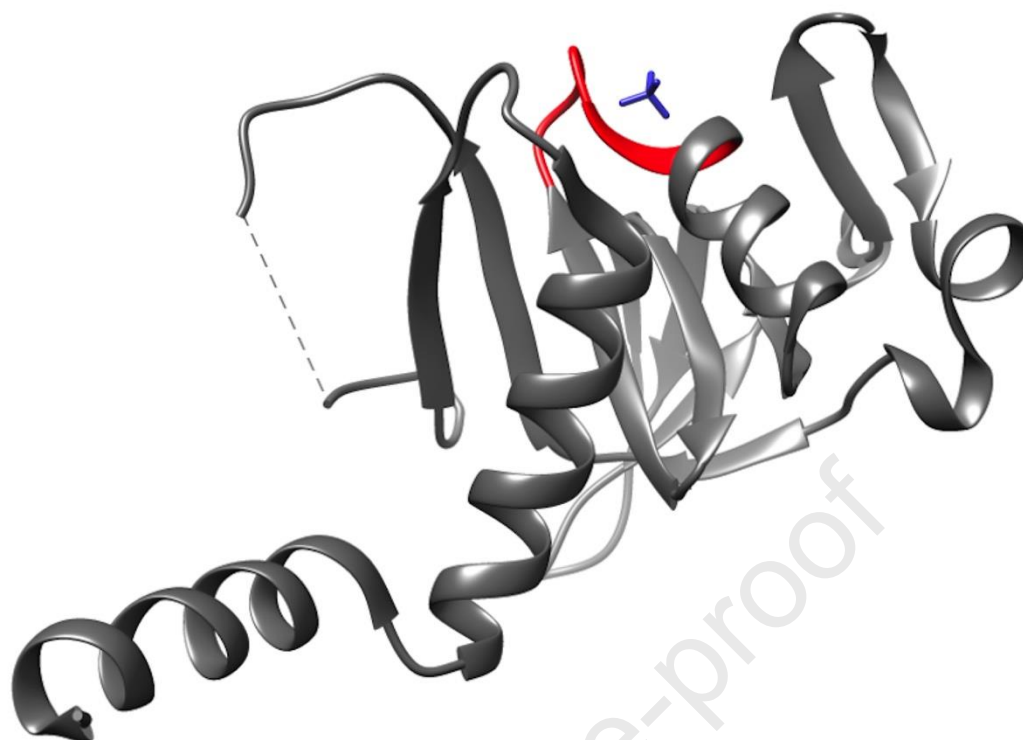
3 **Figure 2: Sequence and structure of the Hanks kinase, PknB, from *Mycobacterium tuberculosis***

4 **A-** Important residues of Hanks kinases are highlighted and the same color code is used in the
5 structure. In red: the P-loop, in black: the C helix, in blue: the catalytic loop, in orange: the magnesium
6 binding motif and in grey: the activation loop.

7 **B-** This structure of PknB was drawn with UCSF Chimera software developed by the Resource for
8 Biocomputing, Visualization, and Informatics at the University of California, San Francisco
9 (<https://www.cgl.ucsf.edu/chimera/>) using the crystallographic structures of the extracellular domain
10 (PDB accession number: 2Kui) and the intracellular domain (PDB accession number: 1mru).

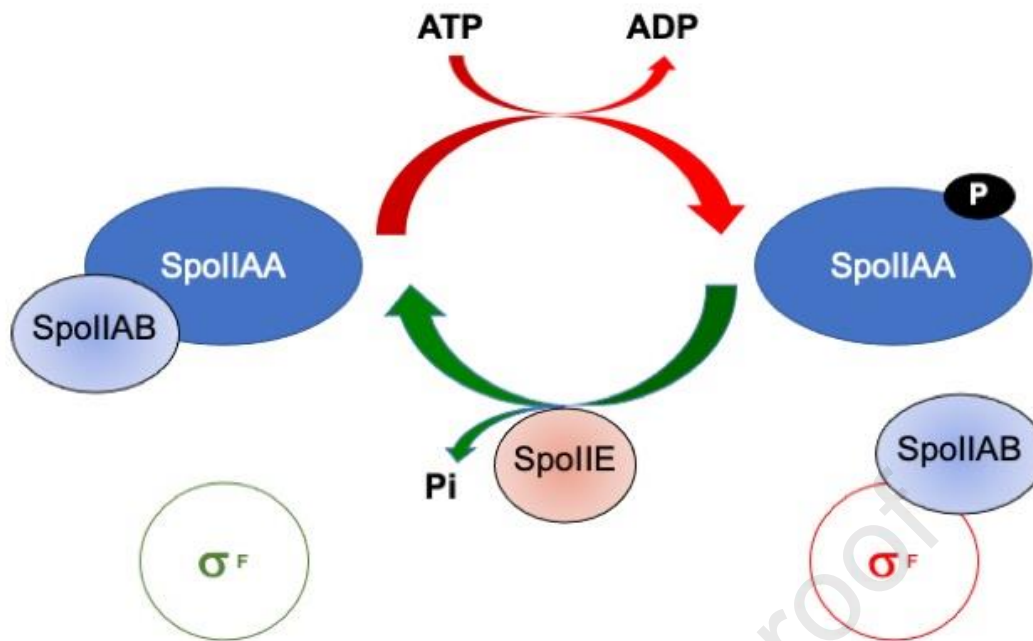
11 **C-** An enlargement of the intracellular domain with the N-terminal lobe shown in grey and the C-
12 terminal lobe shown in green. The activation loop is missing in this structure. In violet, the
13 phosphothio-phosphoric acid-adenylate ester (that mimics ATP binding) is represented as sticks with
14 two magnesium ions (in cyan) shown as spheres.

15



1
2 **Figure 3: Structure of the catalytic domain of HprK/P from *Lactobacillus casei* bound to phosphate.**
3 The catalytic domain of HprK/P from *L. casei* (PDB number 1JB1) possesses 59% of sequence identity
4 with that of *B. subtilis*. This structure was drawn with UCSF Chimera software
5 (<https://www.cgl.ucsf.edu/chimera/>). The P-loop is colored in red and the phosphate in blue.

6
7



1

2 **Figure 4: Regulation of sigma F by the APK SpoIIAB**

3 Sigma F is active in its free form (sigma F in green); it is inactivated when it is in complex with SpoIIAB
 4 (sigma F in red). The SpoIIAA protein triggers the dissociation of the SpoIIAB-sigma F complex thus
 5 activating sigma F. SpoIIAB is also able to interact with SpoIIAA and to phosphorylate this latter from
 6 ATP. Phosphorylated SpoIIAA has very low affinity for SpoIIAB. Thus, when SpoIIAA is phosphorylated,
 7 SpoIIAB can interact with sigma F that becomes inactive. SpoIIAB does not phosphorylate sigma F but
 8 sequesters it. Phosphorylation of SpoIIAA by SpoIIAB thus prevents the activation of sigma F
 9 indirectly. The specific phosphatase SpoIIIE dephosphorylates phosphorylated SpoIIAA and is another
 10 actor in this feed-back regulation loop.

11

12