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Mini-Review

Evolution of the phosphotransferase Spo0B: A structural perspective

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ABSTRACT

Bacteria use the phoshorylation-dependent two-component paradigm to detect signals and to produce appropriate responses. They rely on these signaling pathways for adapting to fluctuating environmental conditions for optimal growth and survival. Sporulation in *Bacillus subtillis* is carried out under the control of a more sophisticated version of the two-component system called the phosphorelay. A unique component in this system is the phosphotransferase protein Spo0B. The site of phosphorylation in Spo0B is a histidine residue as in the histidine kinases. Additionally, Spo0B has structural resemblances to the autokinase domain of histidine kinases. These similarities suggest that Spo0B evolved from a histidine kinase with enhanced phosphotransferase activity. This manuscript examines the structural basis of evolution of Spo0B. The monomeric Spo0B comprises of two domains, an N-terminal helical hairpin followed by a C-terminal domain which folds like the ATPase domain of a histidine kinase. In the autokinase domain of a histidine kinase, the two sub-domains are connected by a long flexible linker. Spo0B appears to have evolved from an autokinase domain by deleting this long linker region and also by truncating a loop region from the ATPase domain necessary for ATP binding.

KEYWORDS: two-component system, phosphorelay, phosphotransferase, histidine kinases, molecular evolution of Spo0B.

INTRODUCTION

Bacteria make use of two-component systems to adapt to changes in living conditions. A typical two-component system comprises a histidine kinase (HK) and a response regulator. The histidine kinases are made up of a sensor domain and an autokinase domain that consists of a phosphotransferase subdomain and an ATP-binding subdomain. The initiation of developmental gene expression in the sporulation of *Bacillus subtilis* is directly controlled by a more sophisticated version called the phosphorelay which is an expanded version of the two-component system (Fig. 1) [1-3]. In this phosphorelay, there are five histidine kinases (KinA, KinB, KinC, KinD and KinE) responding to signals and they are dephosphorylated by a common response regulator, Spo0F. Phosphorylated Spo0F is the substrate for the Spo0B phosphotransferase [2, 4, 5], which serves to mediate phosphoryl transfer from Spo0F to Spo0A, the transcription factor. The sequence of phosphorylated amino acids in the phosphoryl transfer reactions that characterize the phosphorelay is His-Asp-His-Asp (Fig. 1).

Sporulation is a tightly regulated process. The presence of the extra components of the phosphorelay provides additional control stages for regulation [1] through inhibition of the catalytic mechanism [6] or through the action of phosphatases or other export mechanisms [7, 8]. Interestingly, the additional components have significant similarities with the prototypical two-component systems. The second component of the

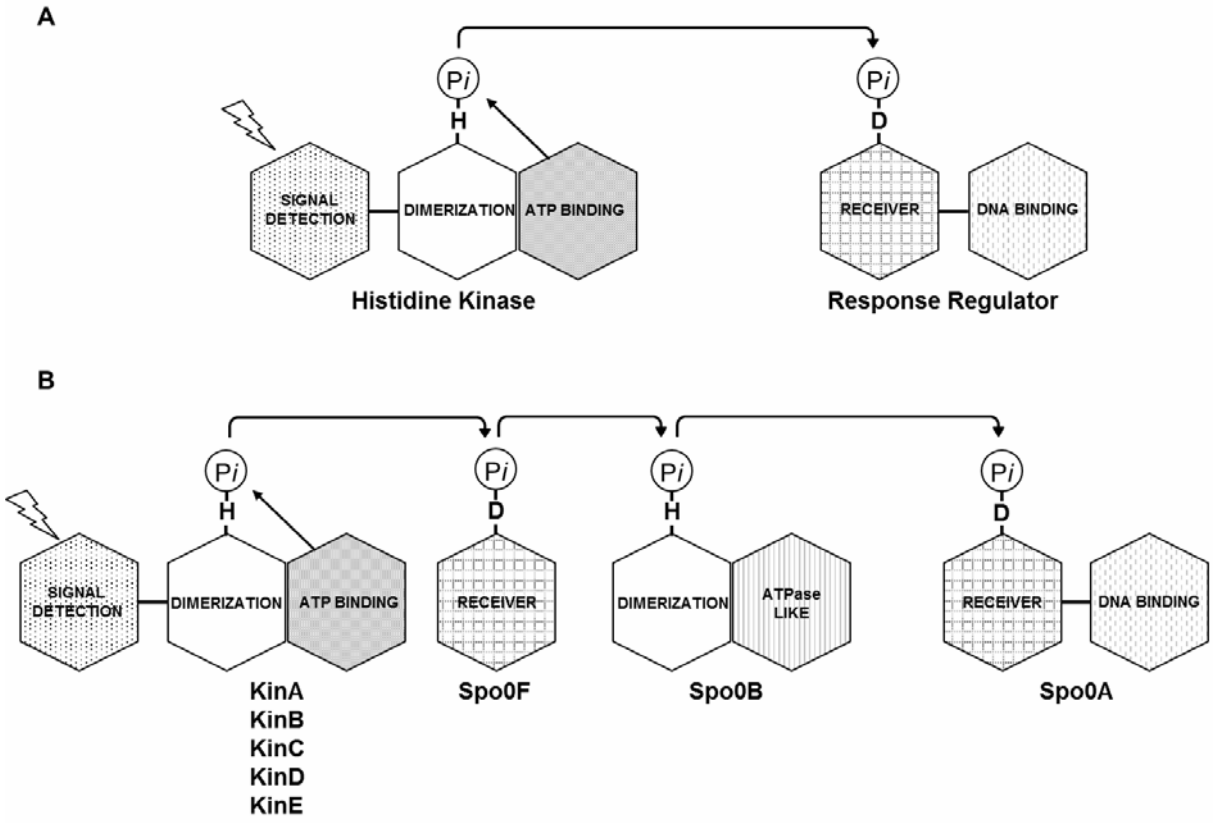

Fig. 1. Domain organization of two-component/phosphorelay signal transduction system. (A) In a typical two-component system, signal recognition by the sensor kinase induces the transfer of the γ-phosphate of ATP to a histidine residue of the phosphotransferase domain. The histidine kinase, in turn, phosphorylates an aspartate residue on response regulator. (B) In the sporulation phosphorelay system of *Bacillus subtilis*, the histidine kinase passes the phosphoryl group to an intermediate response regulator, Spo0F. Subsequently, the phosphotransferase, Spo0B, transfers it to the transcription factor, Spo0A.

phosphorelay, Spo0F, is very similar to N-terminal domain of the transcription factor Spo0A and the third component Spo0B is very similar to the autokinase domain of the histidine kinases (HKs). Additionally, HKs and Spo0B function as homodimers. These similarities indicate that Spo0F evolved from the transcription factor Spo0A and Spo0B from one of the histidine kinases. The uniqueness of Spo0B is that it can accept or donate the phosphoryl group with equal dexterity and do so 20 times more efficiently than KinA, the main histidine kinase in the phosphorelay [9].

The steps involved in the autophosphorylation and dephosphorylation of a histidine kinase are schematically depicted in Fig. 2 in order to compare and contrast them with the steps involved in phosphorylation-dephosphorization of Spo0B in the upcoming discussions. In response to an incoming signal, a histidine kinase autophosphorylates a histidine residue situated on the 4-helix bundle. This is accomplished through the transfer of the γ-phosphate from ATP bound to the ATPase domain of the HK. Thereafter, the ATPase domains swing away presumably accompanied by the dissociation of the ADP moiety. As the next step, the response regulator binds to the HK b4ringing the aspartate in proximity to the phosphorylated histidine allowing the response regulator to be phosphorylated and activated. It is obvious that the flexible liker between the 4-helix bundle and the ATPase domain is necessary for the normal function of a HK. Now we examine the structural architecture of Spo0B to understand how it evolved to attain this high efficiency for phosphoryl transfer.

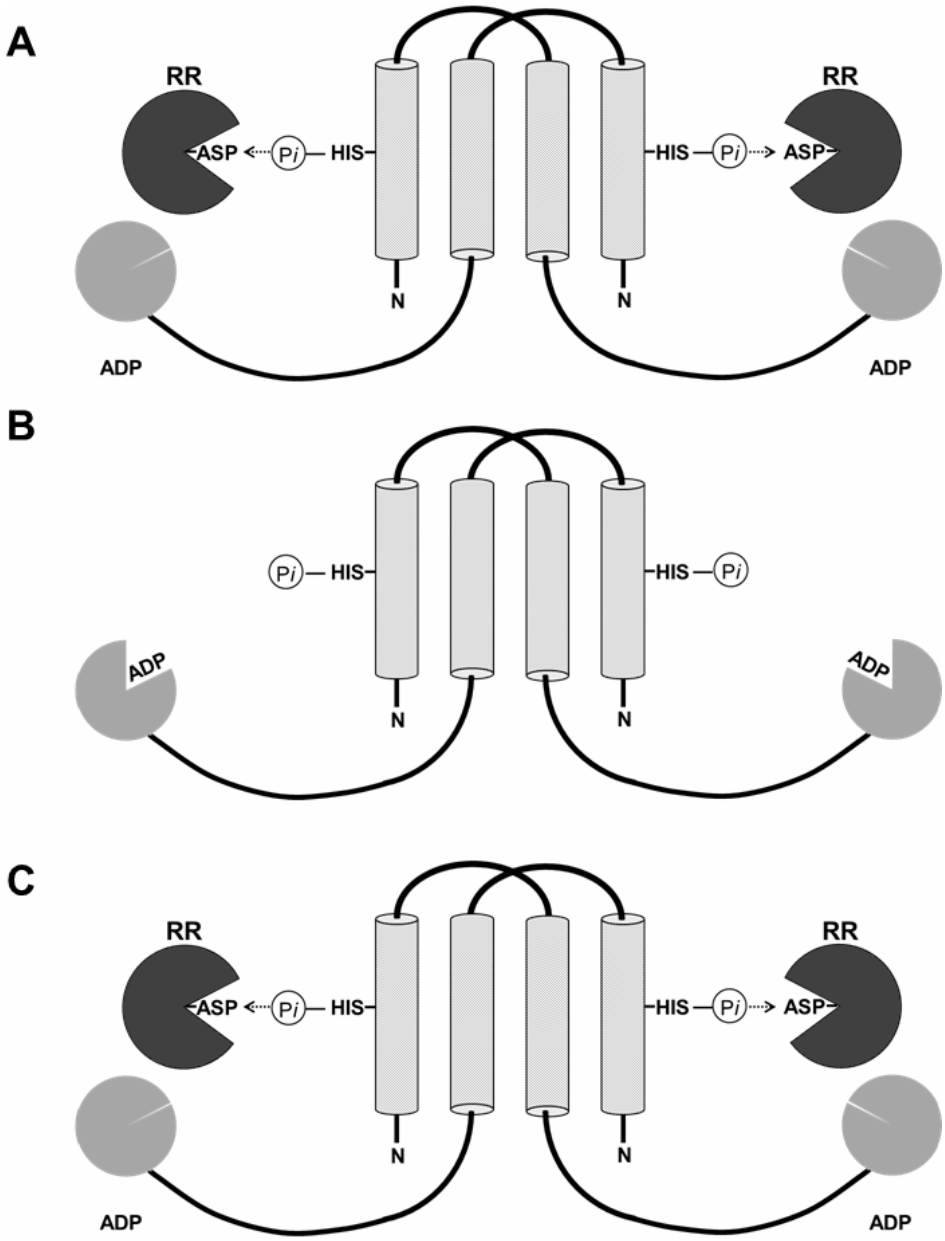

Fig. 2. Autophosphorylation of the histidine kinase and the transfer of the phosphoryl group to the response regulator. (A) The γ-phosphate from ATP is transferred onto a histidine residue in the 4-helix bundle. (B) The ATPase domain containing the ADP molecule reorients making the His-P accessible for the response regulator. (C) The response regulator (RR) associates with the 4-helix bundle and accepts the phosphoryl group from the histidine residue.

Spo0B can accept or donate the phosphoryl group without undergoing conformational changes

The Spo0B molecule functions as a dimer and it exists in the dimeric state both in solution and in the crystal lattice. The Spo0B monomer is made up of 192 amino acid residues with a molecular weight of 22,542 Da [10]. The structure of the monomer consists of two domains, N-terminal helical hairpin and a C-terminal domain [11]. The helical-hairpins from two protomers associate to form a tight 4-helix bundle to generate a Spo0B

dimer (Fig. 3A). The histidine residue at position 30 is the site of phosphorylation and is located approximately in the middle of the 4-helix bundle. The molecular structure of Spo0B is depicted in Fig. 3A. The secondary messenger Spo0F carrying the phosphoryl group associates with Spo0B and transfers the phosphoryl group to His30 on Spo0B. The geometry of association has been revealed by the crystal structures of Spo0F:Spo0B complexes [4, 5] (Fig. 3B). As the subsequent step in signal transduction, the transcription factor Spo0A binds to Spo0B to accept the phosphoryl group to get activated. The aspartate containing N-terminal domain of Spo0A is very similar to Spo0F and binding mode of Spo0A should be the same as that of Spo0F.

Similarities of Spo0B with histidine kinases (HKs)

HKs are multi-domain proteins and the structure of a HK can be broadly divided into two parts, (i) the N-terminal signal detection domain and (ii) the C-terminal autokinase domain. The autokinase domain consists of a histidine containing dimerization sub-domain and an ATP-binding sub-domain. Spo0B is a phosphotrasferase and although it does not have a signal detection-processing domain, it is remarkably similar to the autokinase domains of HKs. Histidine kinases, like Spo0B function as dimers and the mode of dimerization of Spo0B is similar to that of histidine kinases. The dimerization is mediated through the association of the helical hairpins from two monomers to form a four-helix bundle, even though the lengths of the helices vary in HKs from those in Spo0B. Additionally, the C-terminal domain of Spo0B is very similar to the ATP-binding domain of Kinases such as KinB [12], HK853 [13], YycG [14], EnvZ [15], PhoQ [16], CheA [17], HK853 [13], CheA [18], NtrB [19], and PrrB [20].

Spo0B appears to have evolved from a histidine kinase by eliminating the linker and the ATP lid

A structural comparison of a Spo0B monomer with a KinB monomer is shown in Fig. 4A. Here the C-terminal domain of Spo0B is overlaid on the ATP domain of KinB [12]. While the two C-terminal domains superpose well, the helical hairpins point to different directions, one to the left and the other to the top of the diagram. In Spo0B, the two sub-domains are tightly attached to one another, while in KinB, the helical hairpin is linked to the ATP domain through a long linker comprising 13 residues (Phe263-Asn275) giving it the flexibility to reorient as necessary. This flexibility is essential for the histidine kinases as it is involved in performing multiple tasks. In response to the incoming signal, the HKs have to autophosphorylate and this involves moving the ATP molecule close to the 4-helix bundle such that the γ-phosphate from the ATP can be transferred to the active histidine (Fig. 2). The next step of signal transduction is transferring the phosphoryl group to the subsequent component in the pathway such as Spo0F. This step involves the reorientation of the ATP domain so that the response regulator has access to the phosphorylated histidine on the 4-helix bundle (Fig. 2). Therefore a long linker is a pre-requisite for the function of HKs. On the contrary, Spo0B does not get autophosphorylated and the C-terminal domain does not have an ATP-binding site. In fact the two domains are stuck together forming a rigid structure.

A view of the superposition of the C-terminal domain of Spo0B with the ATP domain of KinB is presented in Fig. 4B. These ATP-binding domains have the same fold as the C-terminal domain of Spo0B. These domains comprise two layers; one consisting mainly of 3 helices and the other made up of 5 β-strands. Fig. 4B clearly shows that there is excellent overall similarity between the two domains. The main difference is that KinB has a long loop called the ATP lid that forms part of the ATP-binding site. In Spo0B, this ATP lid is absent. Therefore the C-terminal domain of Spo0B must have evolved from the ATPase domain by eliminating the linker and the ATP lid.

Fig. 5 shows a sequence alignment of KinB with Spo0B based on the structural alignment of the C-terminal domain of Spo0B with the ATP domain of KinB [12] as depicted in Fig. 4B. As evident from Fig. 4, this sequence alignment also shows that the two most conspicuous differences are the absences of the long linker region and the ATP lid in Spo0B.

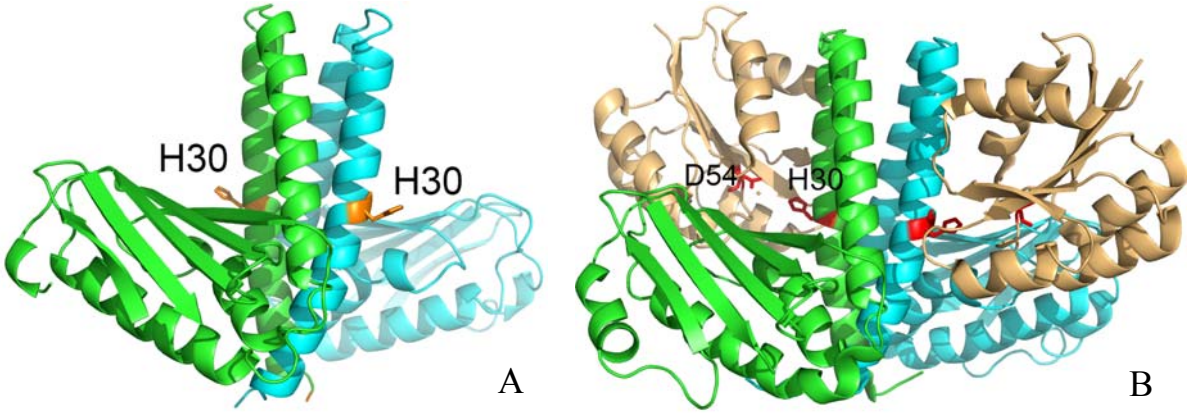

Fig. 3. The structure of Spo0B and its interaction with Spo0F. (A) The structure of the Spo0B dimer. The two protomers are colored in green and cyan. A protomer comprises two domains: the N-terminal α-helical hairpin made up of helices and C-terminal α/β domain consisting of 5 β-sheets and three helices. His 30, the site of phosphorylation, is labelled. (B) The figure shows the binding of Spo0F to Spo0B facilitating the phosphorylation of Spo0B [4]. Two Spo0F molecules (orange) bind to the spo0B dimer. The binding brings the Asp54 of Spo0B close to the His30 of Spo0B creating the ideal geometry for phosphotransfer.

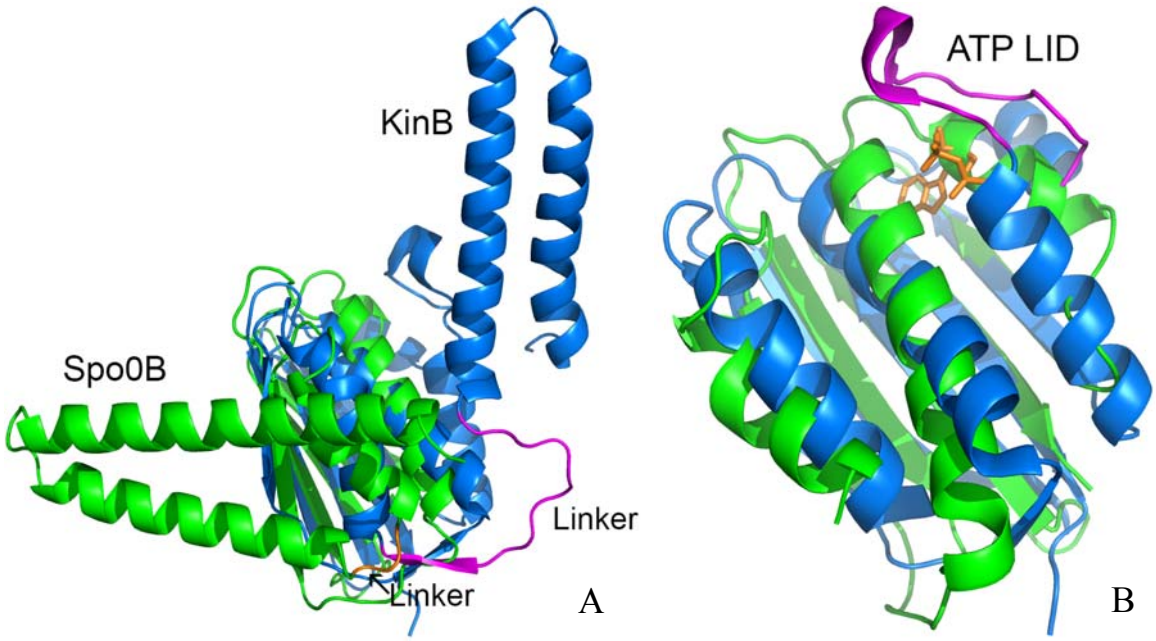

Fig. 4. Structural similarities and differences between monomers of Spo0B (green) and KinB (3D36) (marine blue). (A) Structural comparison of the two monomers by superposing the ATP-binding domain of KinB on the C-terminal domain of Spo0B. The C-terminal domain superposes well with the ATP domain, while the helical hairpins point to drastically different directions. In Spo0B, the helical hairpin and the C-terminal domain are connected by a very short linker (orange) comprising 3 amino acid residues. In KinB, the helical hairpin connects to the ATP domain through a longer linker (maroon) comprising 13 residues. (B) Thee structural superposition of the ATP domain (green) with the C-terminal domain of Spo0B (marine blue). KinB has an ATP-binding site and the crystal structure contains an ADP molecule in that site. A loop region (maroon) plays a key role in binding and the formation of the binding site for ATP. Interestingly this loop known as the ATP lid is absent in Spo0B.

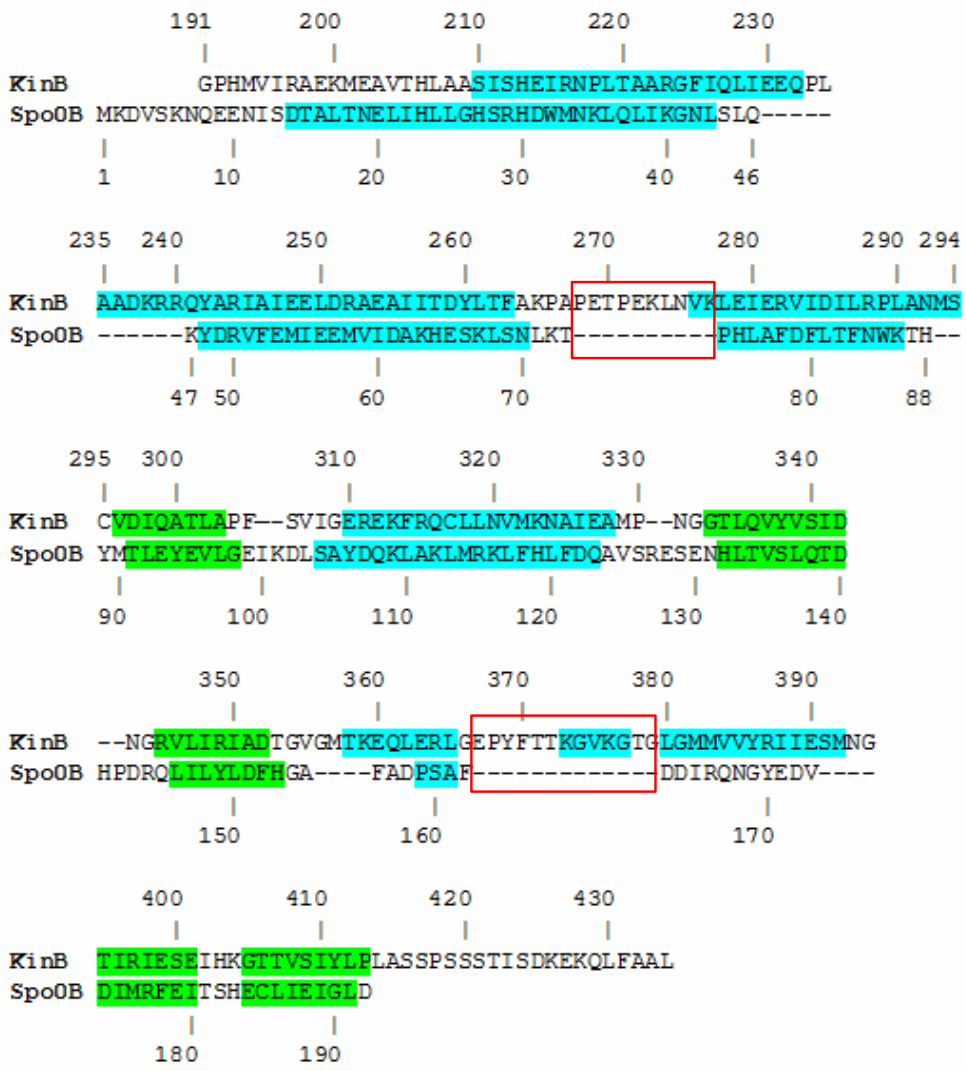

Fig. 5. Structure-based sequence alignment of Spo0B (*B. subtilis*) with KinB (*Geobacillus stearothermophilus*) (PDBID: 3D36). The α-helices are highlighted in turquoise and the β-sheets are highlighted in bright green. The most conspicuous difference between the proteins is the absence of the linker region and the ATP lid in Spo0B (shown in red boxes).

CONCLUSION

The main conclusions are the following: (i) The ATPase domains have a very large loop which embraces the ATP molecule and keeps it tightly bound to the protein. In Spo0B which does not bind to ATP, the corresponding loop is absent. (ii) In HKs, there is a linker region connecting the helical hairpin (4-helix bundle) region to the ATPase domain which allows it to move relative to one another to perform its different tasks. In Spo0B, however, this linker is practically absent making it a rigid unit with strong interactions between these domains. This architecture provides the ideal platform for Spo0F or Spo0A to interact and facilitate the phosphoryl transfer.

Structural elucidation of the proteins of the two-component system has played a vital role in providing insights into the mechanisms of bacterial signal transduction involved in bacterial adaptation and survival. A very large number of 3-dimensional structures of two-component proteins have collectively contributed to our understanding of the mechanism. Out of these proteins, two proteins in particular have played

very special roles in providing novel insights into the function of the two-component/phosphorelay systems. The first one is CheY [21, 22], the structure elucidation of which opened the doors for peeking into the structural basis of the two-component mechanism. The next major step was the elucidation of the crystal structure of Spo0B [11] which revealed many of the structural features of the histidine kinases. Undoubtedly the most important question in field was how does the signal get transmitted or how does the phosphoryl transfer take place. The answer came from the structure elucidation of Spo0B:Spo0F complex which showed how the two components in the two-component system associate and allow signal to move forward to switch on the regulatory pathways [4]. Thus Spo0B has shed much light in our understanding of the two-component/ phosphorelay signaling mechanisms.

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CONFLICT OF INTEREST STATEMENT

The author declares no competing financial interests.

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