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Review Article

Giving a signal: how protein phosphorylation helps *Bacillus* navigate through different life stages

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Abstract

Protein phosphorylation is a universal mechanism regulating a wide range of cellular responses across all domains of life. The antagonistic activities of kinases and phosphatases can orchestrate the life cycle of an organism. The availability of bacterial genome sequences, particularly *Bacillus* species, followed by proteomics and functional studies have aided in the identification of putative protein kinases and protein phosphatases, and their downstream substrates. Several studies have established the role of phosphorylation in different physiological states of *Bacillus* species as they pass through various life stages such as sporulation, germination, and biofilm formation. The most common phosphorylation sites in *Bacillus* proteins are histidine, aspartate, tyrosine, serine, threonine, and arginine residues. Protein phosphorylation can alter protein activity, structural conformation, and protein–protein interactions, ultimately affecting the downstream pathways. In this review, we summarize the knowledge available in the field of *Bacillus* signaling, with a focus on the role of protein phosphorylation in its physiological processes.

Keywords: *Bacillus*, phosphorylation, protein kinases, protein phosphatases, sporulation, germination, biofilm, virulence

Introduction

The *Bacillus* genus comprises ~300 annotated species, which are divided into different phylogenetic clusters based on 16S rRNA and evolutionary relationships (Ash et al. 1991, Parte 2018). Members of the *Bacillus* genus are rod-shaped, mostly aerobic, or facultative anaerobes, Gram-positive spore-forming bacteria. Two of the most studied clades in these clusters are the *Bacillus subtilis* and *Bacillus cereus* clades. The subtilis clade comprises ten species including *B. subtilis*, *B. amyloliquefaciens*, *B. aerophilus*, *B. pumilus*, *B. atrophaeus*, *B. licheniformis*, *Bacillus sp.* 586, *Bacillus sp.* 916, *Bacillus sp.* JS, and *Bacillus sp.* BT1B_CT2, and the pathogenic species such as *B. anthracis*, *B. cereus*, and *B. thuringiensis* are classified as part of the cereus clade (Rooney et al. 2009, Bhandari et al. 2013). Among these, *B. subtilis*, commonly found in soil environments and plant roots, is the most characterized species. It is used as a model organism to decipher physiological and regulatory mechanisms in all the *Bacillus* species (Vlamakis et al. 2013, Harwood et al. 2018, Ravikumar et al. 2018, Richts et al. 2019, Errington and Aart 2020). Subtilis clade members have a wide range of industrial and medical applications owing to their phenotypic and genotypic variations (Cui et al. 2018, Harwood et al. 2018, Jezewska-Frackowiak et al. 2018, Su et al. 2020).

The *Bacillus* life cycle is composed of three distinct phases: spore formation (the dormant stage), spore germination, and a replicating vegetative phase (Soule 1932, Sella et al. 2014). *Bacillus* spores, formed under nutrition-deprived conditions, are metabolically inactive entities that can remain viable for extended periods (Graham-Smith 1930, Khanna et al. 2020). These endospores are highly resistant to stress conditions encountered in nature and, therefore, can be found in a wide range of environments including water, air, and soil. Under favorable conditions, these spores germinate into the metabolically active vegetative form (Watabe 2013, Bressuire-Isoard et al. 2018, Christie and Setlow 2020). Pathogenic *Bacillus* species proliferate inside their vertebrate hosts (Nicholson et al. 2000, Nicholson 2002, Arora et al. 2017a). When pathogenic Bacilli infect their hosts, the spores germinate and secrete toxins such as enterotoxin and emetic toxin, as well as phospholipases and proteases that help the bacteria to survive in the host environment (Moayeri et al. 2015, Sharma et al. 2017, Ehling-Schulz et al. 2019, Enosi Tuipulotu et al. 2021). Another survival strategy adopted by the members of this genus is biofilm formation. A biofilm is a matrix of exopolysaccharides, proteins, and nucleic acids that acts as a shield and protects the bacterium from various antibiotics and the host's immune response (Hobley et al. 2015, Hall and Mah 2017, Huang et al. 2020, Shemesh and Ostrov 2020). The transition of *Bacillus* from one phase to another depends on environmental and nutritional conditions, which are sensed by the bacterial regulatory proteins. The *Bacillus* regulatory network involves various sensory proteins present on the surface and their post-translationally modified forms, particularly phosphorylation. This review article focuses on the role of protein phosphorylation events during transitioning of *Bacillus* species between different stages as well as during infection in the human host. We tried to include the most relevant

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articles but may have missed some studies due to limitations of search engine criteria and article length.

Post-translational modifications (PTMs) play important roles in providing the required proteomic diversity in bacteria, despite smaller genome sizes. PTMs such as phosphorylation, glycosylation, acetylation, and methylation influence almost all aspects of cellular physiology (Arora et al. 2010, 2021, Higgins and Dworkin 2012, Sajid et al. 2015, Manuse et al. 2016, Mijakovic et al. 2016, Khan et al. 2018, Macek et al. 2019, Bonne Kohler et al. 2020, Singhal et al. 2020, Wang and Cole 2020). Among these PTMs, protein phosphorylation is one of the most widely documented and investigated modifications across the bacterial system (Fig. 1). There are dedicated protein kinases and phosphatases that are categorized based on the phosphate group transfer from ATP to specific amino acids, such as serine (Ser)/threonine (Thr) (Ser/Thr protein kinase-STK and Ser/Thr protein phosphatase-STP), tyrosine (Tyr) (bacterial protein Tyr kinase-BY kinase and protein Tyr phosphatase-PTP), histidine (His)/aspartate (Asp) (two-component system-TCS/His sensor kinase-phosphorelay), srginine (Arg) (Arg kinase and Arg phosphatase), cysteine (Cys) [phosphotransferase system (PTS) regulated protein (such as EIIA) phosphorylation of Cys residues using PEP as energy source], and atypical protein kinases that are unlike characterized kinases but still possess kinase activity. The major phosphorylation systems are shown in the Featured image (Hoch 2000, Stock et al. 2000, Pereira et al. 2011, Capra and Laub 2012, Grangeasse et al. 2012, Fuhrmann et al. 2013, Chao et al. 2014, Deutscher et al. 2014, Mijakovic et al. 2016, Elhawy et al. 2021, Huang et al. 2021, Zhang et al. 2021). Interestingly, many proteins are phosphorylated on multiple sites, as also identified in most phosphoproteome-based studies (Macek et al. 2007, Elsholz et al. 2012, Ravikumar et al. 2014, Birk et al. 2021). For example, 214 Ser/Thr/Tyr phosphosites were identified in 153 proteins (Birk et al. 2021) and 121 Arg phosphosites were identified in 87 proteins of *Bacillus subtilis* (Elsholz et al. 2012). It also indicates promiscuous activities of several kinases, with overlapping specificity, and the primary reason for the fallible connection between different studies.

TCSs are ubiquitous among bacterial systems, consisting of two proteins, a sensor His kinase (HK) and a response regulator (RR). When an external stimulus is sensed, the sensor HK is activated by autophosphorylation of a conserved His residue. This signal is then subsequently transferred to its cognate RR, which is usually a transcription factor, resulting in differential altered gene expression (Fabret et al. 1999, Bijlsma and Groisman 2003, Capra and Laub 2012, Groisman 2016). TCSs are known to be involved in various cellular pathways in *Bacillus sp*, such as stress response (Darmon et al. 2002, Dhiman et al. 2014, Diomande et al. 2014, Mike et al. 2014, Groisman 2016, Gupta et al. 2018), protease regulation (Perchat et al. 2011, Gupta et al. 2017), sporulation (Stephenson and Hoch 2002, Fujita and Losick 2003, White et al. 2006, Gopalani et al. 2016, Peng et al. 2017), metabolic pathways (Birkey et al. 1998, Tanaka et al. 2003, Repizo et al. 2006, Geng et al. 2007, Myers et al. 2016, Aggarwal et al. 2017, van den Esker et al. 2017), cell wall regulation (Howell et al. 2003, Dobihal et al. 2019, Wu et al. 2019), biofilm formation (Verhamme et al. 2007, Stubbendieck and Straight 2017, Zhou et al. 2018), antibiotic tolerance (Kesel et al. 2013, Dintner et al. 2014, Zhang et al. 2015, Koh et al. 2021), and pathogenesis (Duport et al. 2006, Vetter and Schlievert 2007, Brillard et al. 2008, Gohar et al. 2008, Stauff and Skaar 2009, van Schaik et al. 2009).

Our understanding of phosphorylation events at different residues such as Ser, Thr, Tyr, Arg, or Cys has evolved over the last three decades. For example, the kinases responsible for Tyr phosphorylation were named bacterial tyrosine (BY) kinases and were identified based on sequence homology with known Tyr kinases, such as the YwqD/YwqE Tyr kinase and phosphatase pair in *B. subtilis* (Mijakovic et al. 2003, 2005). Subsequently, more such pairs were characterized in *B. subtilis* with roles predicted in various cellular pathways based on substrate identification in phosphoproteome studies (Mijakovic and Deutscher 2015).

The importance of Ser/Thr phosphorylation was soon realized and with the advancement in genome sequencing and -omics technologies, various Ser/Thr kinases (STKs) and Ser/Thr phosphatases (STPs) were identified and characterized in *B. subtilis* and other microbes (Fischer et al. 1996, Moszer 1998, Obuchowski et al. 2000, Vijay et al. 2000, Iwanicki et al. 2002, Madec et al. 2002, Shakir et al. 2010, Lima et al. 2011, Pereira et al. 2011, Arora et al. 2012, Bidnenko et al. 2013, Borriss et al. 2018, Rajagopalan et al. 2018, Baros et al. 2020). Global phosphoproteome and *in silico* analysis further helped in the identification of substrates regulated by Ser/Thr phosphorylation (Levine et al. 2006, Macek et al. 2007, Miller et al. 2009, Ravikumar et al. 2014, Pan et al. 2015, Rosenberg et al. 2015, Arora et al. 2017b, Zhang et al. 2018, Shi et al. 2020, Birk et al. 2021). The *Bacillus* genus encodes two classes of STKs that are categorized based on sequence homology and conserved sequence motifs. These are eukaryotic-like Ser/Thr kinases (eSTKs) or Hanks-type kinases and atypical STKs (non-eSTKs), and two-component kinases such as Ser/Thr kinases with specific single substrates (Pereira et al. 2011, Arora et al. 2012, Shi et al. 2014a, Mijakovic et al. 2016, Nguyen et al. 2016, Stancik et al. 2018, Zhang et al. 2021).

The global phosphoproteome of Arg phosphorylation in *B. subtilis* identified >100 proteins with phosphorylated Arg sites across diverse classes of proteins involved in metabolism, cellular architecture, sporulation, and various stress-induced responses (Elsholz et al. 2012, Schmidt et al. 2014, Trentini et al. 2014, Singh et al. 2015, Fuhrmann et al. 2016, Mijakovic et al. 2016, Zhou et al. 2019). In the phosphoproteome study by Elsholz et al. (2012), of the 87 proteins identified to be phosphorylated on Arg residues, 17 were phosphorylated on multiple sites. These proteins were ClpC, RpoB, AroA, MtnK, OdhA, BdhA, ComfA, ComGA, ComK, RpoC, RpsM, GltA, Gmk, GroEL, GudB, MenB, and MtnA (Elsholz et al. 2012). Interestingly, genetic deletion of Arg kinase McsB in *B. anthracis* caused alteration in vegetative cells and spore morphology, defective growth at elevated temperatures, and reduced sporulation and germination efficiencies (Singh et al. 2015). These studies signify the physiological relevance of Arg phosphorylation in the *Bacillus* species.

In the following sections, we summarize the role of protein phosphorylation during different stages and pathways in the *Bacillus* (Table 1).

Cellular architecture and growth

The Gram-positive bacterial cell wall is comprised mostly of multilayered peptidoglycan sheets and teichoic acids that serve as a protective barrier and help to maintain the bacterial shape and integrity. Bacterial growth and cellular integrity is a dynamic process that requires continuous hydrolysis of the cell wall to introduce breaks for the accumulation of newly formed peptidoglycan units into the cell wall (Vermassen et al. 2019). In *B. subtilis*, this controlled autolysis is carried out by endopeptidases such as CwlO and LytE, which are further regulated by the WalRK TCS (Yamaguchi et al. 2004, Salzberg et al. 2013, Dobihal et al. 2019, 2022). The WalRK regulon is mostly composed of genes encoding autolysins, autolysin inhibitors, ABC transporters, and genes in-

Table 1. Phosphorylation targets in *Bacillus* sp. in various cellular pathways.

Kinase/phosphatase	Substrate/target	Experimental validation	References
Cell morphogenesis			
Bsu PrkC	YvcK, YmfM (RodZ), WalR, CpgA, LtaS, YfnI, YvgJ, YqgS, FtsZ	I, II, III[a], III[b]	Foulquier et al. (2014), Ravikumar et al. (2014), Libby et al. (2015), Absalon et al. (2008), Pompeo et al. (2018a)
Bsu PrpC	YvcK, YmfM (RodZ), WalR, CpgA, YfnI, FtsZ	I, III[a], III[b]	Foulquier et al. (2014), Ravikumar et al. (2014), Libby et al. (2015), Absalon et al. (2008), Pompeo et al. (2018a)
Bsu WalRK	*cwlO*, *lytE*, *iseA*	I, III[b]	Salzberg et al. (2013), Dobihal et al. (2019)
Bas WalRK	*ftsE*, *eag*, *kinB3*	I	Dhiman et al. (2014)
DNA metabolism/regulation			
Bsu PtkA	SSBs, YorK, SalA, MinD, RecA, DivIVA	I, II, III[a], III[b]	Mijakovic et al. (2006), Macek et al. (2007), Jers et al. (2010), Shi et al. (2014b)
Bsu PtkB	MutL, RpoB, MinD, PolA	II	Shi et al. (2014b)
Bsu PtpZ	SSBs, MinD, PolA, RpoB, MutL	I, II, III[b]	Mijakovic et al. (2006), Shi et al. (2014b)
Bsu YabT	MinD, MutL, AbrB	I, II	Shi et al. (2014b), Kobir et al. (2014)
Bsu PrkD	SalA, AbrB	I, II	Shi et al. (2014b), Kobir et al. (2014)
Bsu PrkC	AbrB	I	Kobir et al. (2014)
Bsu STK	CodY, AbrB, RecA	III[a]	Macek et al. (2007), Soufi et al. (2010)
Bsu McsB	RecA, RpoB, Ssb, DivIVA	III[a]	Elsholz et al. (2012), Schmidt et al. (2014)
Bsu YwlE	RecA, RpoB, Ssb, DivIVA	III[a]	Elsholz et al. (2012), Schmidt et al. (2014)
Bsu DegSU	ComK	I	Dahl et al. (1992), Hamoen et al. (2003)
Metabolsim			
Bsu HprK/P	Crh	I, III[b]	Landmann et al. (2012)
Bsu PrkC	YwjH, GlnA, Icd, AlsD	I	Pietack et al. (2010)
Bsu PtkA	Ugd, Asd, Ldh, Eno, FatR, SalA	I, II	Jers et al. (2010), Mijakovic et al. (2003), Petranovic et al. (2009), Derouiche et al. (2013), Shi et al. (2014b), Derouiche et al. (2015)
Bsu PtpZ	Ugd	I	Mijakovic et al. (2003)
Bsu STK	Pyk, Asd, Eno, GlnA, Icd, YwjH	III[a]	Eymann et al. (2007), Macek et al. (2007)
Bsu McsB	Eno, GlnA	III[a]	Schmidt et al. (2014)
Bsu YwlE	Eno, GlnA	III[a]	Schmidt et al. (2014)
Bsu CitST	*citM*	I	Repizo et al. (2006)
Bsu GlnKL	*glsA*, *glnT*	I	Satomura et al. (2005)
Bsu YufLM	*maeN*, *yflS*	I	Tanaka et al. (2003)
Bsu PhoPR	ResDE	I	Birkey et al. (1998)
Bsu LytST	*ysbA*	I	van den Esker et al. (2017)
Bas PrkD	Pyk	I	Arora et al. (2012)
Bas PrkC	Eno	I	Virmani et al. (2019)
Bas PhoPR	*phoA*, *pst*	I	Aggarwal et al. (2017)
Protein synthesis			
Bsu PrkC	Ef-Tu, Ef-G, CpgA	I, III[a], III[b]	Absalon et al. (2009), Ravikumar et al. (2014), Gaidenko et al. (2002), Shah et al. (2008), Dworkin and Shah (2010), Ravikumar et al. (2014), Absalon et al. (2009), Pompeo (2012)
Bsu PrpC	Ef-Tu, Ef-G, CpgA	I, III[a], III[b]	Gaidenko et al. (2002), Ravikumar et al. (2014), Absalon et al. (2009), Ravikumar et al. (2014)
Bsu STK	Ef-Tu, Ef-G	III[a]	Levine et al. (2006), Eymann et al. (2007)
Bsu McsB	Ef-Tu, Ef-G	III[a]	Schmidt et al. (2014)
Bsu YwlE	Ef-Tu, Ef-G	III[a]	Schmidt et al. (2014)
Bsu WalRK	*sasA*	I	Libby et al. (2019)
Bas PrkC	Ef-Tu, Ef-G	I	Arora et al. (2017b), Arora (2013)
Bas PrpC	Ef-Tu	I	Arora et al. (2013)

Table 1. Continued

Kinase/phosphatase	Substrate/target	Experimental validation	References
Sporulation			
Bsu YabT	RecA, RacA, SsbA, AbrB, YabA, Ef-Tu	I, II, III[b]	Bidnenko et al. (2013), Shi et al. (2014b), Derouiche et al. (2016), Kobir et al. (2014), Garcia Garcia et al. (2018), Pereira et al. (2015)
Bsu SpoIIE	RecA, RacA	I, II	Shi et al. (2014b)
Bsu PrkA	σK	I	Yan et al. (2015)
Bsu CotH	CotB, CotG	I	Nguyen et al. (2016)
Bsu SpoIIAB	SpoIIAA, σF	I	Clarkson et al. (2004), Duncan and Losick (1993)
Bsu SpoIIE	SpoIIAA	I	Duncan et al. (1995)
Bsu KinA-E	Spo0F, Spo0B, Spo0A	I	Burbulys et al. (1991), Jiang et al. (2000)
Bsu RapA	Spo0F	I	Perego et al. (1994)
Bsu Spo0E	Spo0A	I	Ohlsen et al. (1994)
Bsu CotH	CotB, CotG	I	Nguyen et al. (2016)
Bas BAS1213–1214	KinD	I	Gupta et al. (2018)
Bce LytSR	*lrgAB*, *clhAB*$_2$	I	Chandramohan et al. (2009)
Bth LytSR	SpoIIP	I	Peng et al. (2017)
Germination			
Bsu PrkC	Ef-G	I, III[a], III[b]	Gaidenko et al. (2002), Shah et al. (2008), Dworkin and Shah (2010), Ravikumar et al. (2014)
Bsu PrpC	Ef-G	I, III[a], III[b]	Gaidenko et al. (2002), Ravikumar et al. (2014)
Bsu McsB	Tig, SigA	I, III[a]	Elsholz et al. (2012), Zhou et al. (2019)
Bsu YwlE	Tig, SigA	I, III[a]	Elsholz et al. (2012), Zhou et al. (2019)
Bsu CotH	CotB, CotG	I	Nguyen et al. (2016)
Bas PrkC	Eno	I	Virmani et al. (2019)
Biofilm			
Bsu KinC	Spo0A	I	Lopez et al. (2009), Shemesh et al. (2010)
Bsu KinD	Spo0A	I	Aguilar et al. (2010)
Bsu CssRS	TasA	I	Steinberg et al. (2020)
Bsu DegSU	YiT	–	Kobayashi and Ikemoto (2019)
Bsu PrkC	AhpA via AbrB, GroEL	III[a]	Zwick et al. (2017), Ravikumar et al. (2014)
Bsu PrpC	GroEL	III	Ravikumar et al. (2014)
Bsu YabT	YabA	I	Garcia Garcia et al. (2018)
Bas PrkC	GroEL	I, III[b]	Arora et al. (2017b)
Stress response			
Bsu McsB	CtsR, GroEL, ClpC, ClpP	I, III[a]	Fuhrmann et al. (2009), Elsholz et al. (2012), Schmidt et al. (2014)
Bsu YwlE	CtsR, GroEL, ClpC, ClpP	III[a]	Elsholz et al. (2012), Schmidt et al. (2014)
Bsu PtkA	DnaK	I, III[a]	Shi et al. (2016)
Bsu PtkB	DnaK	I, III[a]	Shi et al. (2016)
Bsu DesKR	*desA*	I	Cybulski et al. (2004)
Bsu LiaSR	*liaI*	I	Jordan et al. (2007), Kesel et al. (2013)
Bsu BceRS	*bceA*	I, III[b]	Dintner et al. (2014)
Bsu WalRK	*cwlO*, *lytE*	I	Takada et al. (2018)

Table 1. Continued

Kinase/phosphatase	Substrate/target	Experimental validation	References
Bsu CssRS	HtrA,HtrB	–	Westers et al. (2006), Noone et al. (2012)
Bsu RsbW	RsbV	I	Alper et al. (1996), Yang et al. (1996)
Bsu RsbP, RsbU	RsbV	I	Yang et al. (1996), Vijay et al. (2000)
Bsu RsbT	RsbS, RsbRA	I, III[b]	Yang et al. (1996), Chen et al. (2004), Kim et al. (2004b)
Bsu RsbX	RsbS, RsbRA	I	Yang et al. (1996), Chen et al. (2004)
Bas HssRS	–	–	Mike et al. (2014)
Bas HitRS	–	–	Mike et al. (2014), Pi et al. (2020)
Bas EdsRS	*clsT*	I	Laut et al. (2020)
Bas WalRK	–	–	Dhiman et al. (2015)
Bce CasKR	*desA*	I	Diomande et al. (2016)
Bce RsbK	RsbY	I	van Schaik et al. (2005), de Been et al. (2010)
Bth YvqEC	–	–	Zhang et al. (2015)
Bth YvcPQ	*yvcS1S2*, *yvcR*, *kapD*, *BMB171_C4835*	I	Zhang et al. (2016)
Virulence			
Bas PTS	AtxA	III[b]	Tsvetanova et al. (2007), Raynor et al. (2018), Bier et al. (2020)
Bas STK	AtxA via CodY and AbrB	I	Strauch et al. (2005), van Schaik et al. (2009), Chateau et al. (2013)
Bas PrpN	CodY	I, III[b]	Gangwal et al. (2022)
Bas PrkC	CodY	I	Gangwal et al. (2022)
Bas HprK/P	HpR	I	Poncet et al. (2004), Choi et al. (2006)
Bas BrrAB	*lef*, *pag*, *cya*, *atxA*	I	Vetter and Schlievert (2007)
Bas BAS2109–2108	Proteases	I, III[b]	Gupta et al. (2017)
Bce ResDE	Enterotoxins (without PlcR)	I	Duport et al. (2006)
Bce YvfTU	Via PlcR	I	Brillard et al. (2008)

Protein kinases and phosphatases are represented by different colors. Pink: STK (Ser/Thr kinases); light blue: STP (Ser/Thr phosphatases); dark blue: BY-K (*Bacillus* Tyr kinases); orange: PTP (*Bacillus* Tyr phosphatases); maroon: Arg kinases; gold: Arg phosphatases; green: histidine (His)/aspartate (Asp) (two-component system-TCS/His sensor kinase-phosphorelay); red: atypical STKs and two-component like Ser/Thr kinases and phosphatases. *Bacillus* species are abbreviated as *B. subtilis* (Bsu), *B. anthracis* (Bas), *B. cereus* (Bce), and *B. thuringiensis* (Bth).
The experimental validation of proteins/targets is shown as:
I: *in-vitro*; II: interactomics-based study; III[a]: phosphoproteome; and III[b]: *in-vivo*.

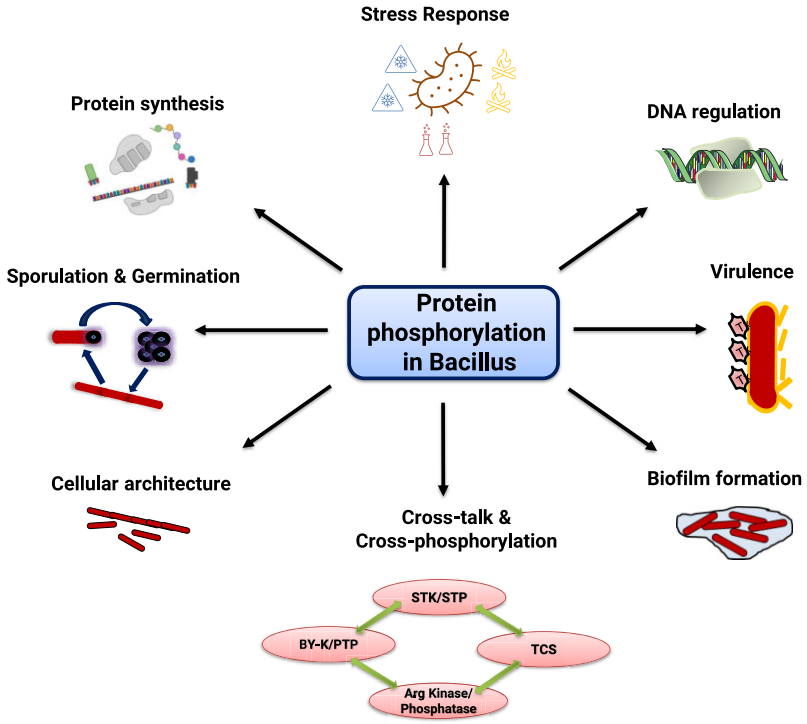

Figure 1. Network of protein phosphorylation in *Bacillus*. Protein phosphorylation in *Bacillus* regulates several aspects of their life cycle, including sporulation, germination, biofilm formation, protein synthesis, DNA regulation, cell architecture, stress responses, and virulence. The detailed regulatory processes are discussed in the text along with the unique aspects of cross-talk and cross-phosphorylation within the kinases of different classes.

volved in teichoic acid synthesis (Salzberg et al. 2013). Phosphorylation of the WalR RR at Asp53 by its cognate HK, WalK, blocks the promoter of autolysin inhibitor *iseA*, thereby causing an increase in the expression of these endopeptidases (Libby et al. 2015, Dobihal et al. 2019). Moreover, mutant *walR* bacilli have an L-shaped morphology and a complete loss of the bacterial cell wall, implicating the role of WalR in cell wall morphogenesis (Dominguez-Cuevas et al. 2012). In *B. anthracis*, though the functional relevance of the WalRK TCS has not been well-explored, its expression is induced in the presence of an inhibitor of cell wall synthesis, fosfomycin (Dhiman et al. 2015). Furthermore, WalR has been shown to bind with the promoter regions of *eag* (S-layer protein EA1), *ftsE* (ABC transporter), and *kinB3* (HK) (Dhiman et al. 2014). Among these, EA1 is an important component of the S-layer surrounding the cellular envelope that imparts structural stability to the cell wall (Chateau et al. 2020, Fioravanti et al. 2022). Phosphorylation of WalR at Thr101 by PrkC, a membrane-localized eSTK with surface-exposed PASTA (peptidoglycan and Ser/Thr kinase-associated) domain, is important for the regulation of cell wall homeostasis during the stationary phase in *B. subtilis* (Libby et al. 2015). The two-site phosphorylation of WalR at Asp53 and Thr101 by WalK and PrkC, respectively, enhances WalR activity and blocks the expression of SasA, a protein involved in antibiotic tolerance (Libby et al. 2019) (Fig. 2).

Lipoteichoic acid (LTA) is another important component of the cell wall, i.e. threaded across peptidoglycan layers and is required for optimal bacterial growth and physiology. Synthesis of LTA in *B. subtilis* involves four enzymes: LtaS, YfnI, YvgJ, and YqgS, which are phosphorylated by PrkC (Pompeo et al. 2018a). CpgA (YloQ), a multidomain GTPase, is another target of the PrkC/PrpC pair that plays an important role in the maintenance of bacterial shape and peptidoglycan deposition in *B. subtilis* (Cladiere et al. 2006, Absalon et al. 2008, 2009). Though the studies on CpgA phosphorylation lack functional and genetic evidence, they suggest a possible link between peptidoglycan biogenesis and Ser/Thr phosphorylation (Fig. 2).

In the vegetative stage, division at the septal region results in the generation of two daughter cells termed vegetative cells. These vegetative cells display a specific rod-shaped morphology, which is mainly determined by various proteins associated with the cell wall and cytoskeleton (Jones et al. 2001, Errington and Wu 2017, Angeles and Scheffers 2021). MreB, an actin-like cytoskeletal protein is a key factor that determines the characteristic rod-shape of *Bacilli*, and the absence of *mreB* in *B. subtilis* alters the localization of penicillin-binding protein (PBP1), resulting in bulging cell phenotype (Formstone and Errington 2005, Carballido-Lopez 2006, Kawai et al. 2009). Interestingly, overexpression of YvcK, a protein with a similar localization pattern as MreB, was able to rescue the *mreB* mutant phenotype with proper PBP1 localization (Gorke et al. 2005, Foulquier et al. 2011). Ser/Thr phosphorylation-mediated regulation of YvcK (Thr304) by the PrkC/PrpC pair is shown to be critical for the rod-shaped morphology in *mreB* mutant cells of *B. subtilis*. A null mutant strain of *mreB* overexpressing the phosphoablative mutant YvcK (Thr304Ala) exhibits a bulging-type phenotype with mis-localized PBP1. However, *mreB* mutant bacteria overexpressing the phosphomimetic mutant YvcK (Thr304Glu) were able to restore rod-shaped morphology (Foulquier et al. 2014). Another cell shape-determining protein, RodZ (YmfM), is a target of the PrkC/PrpC pair in *B. subtilis*, although its role and functional implication is still unknown (Alyahya et al. 2009, Ravikumar et al. 2014, van Beilen et al. 2016) (Fig. 2).

Ser/Thr phosphorylation of the cell division protein FtsZ is important for efficient septum formation in prokaryotes (Garcia et

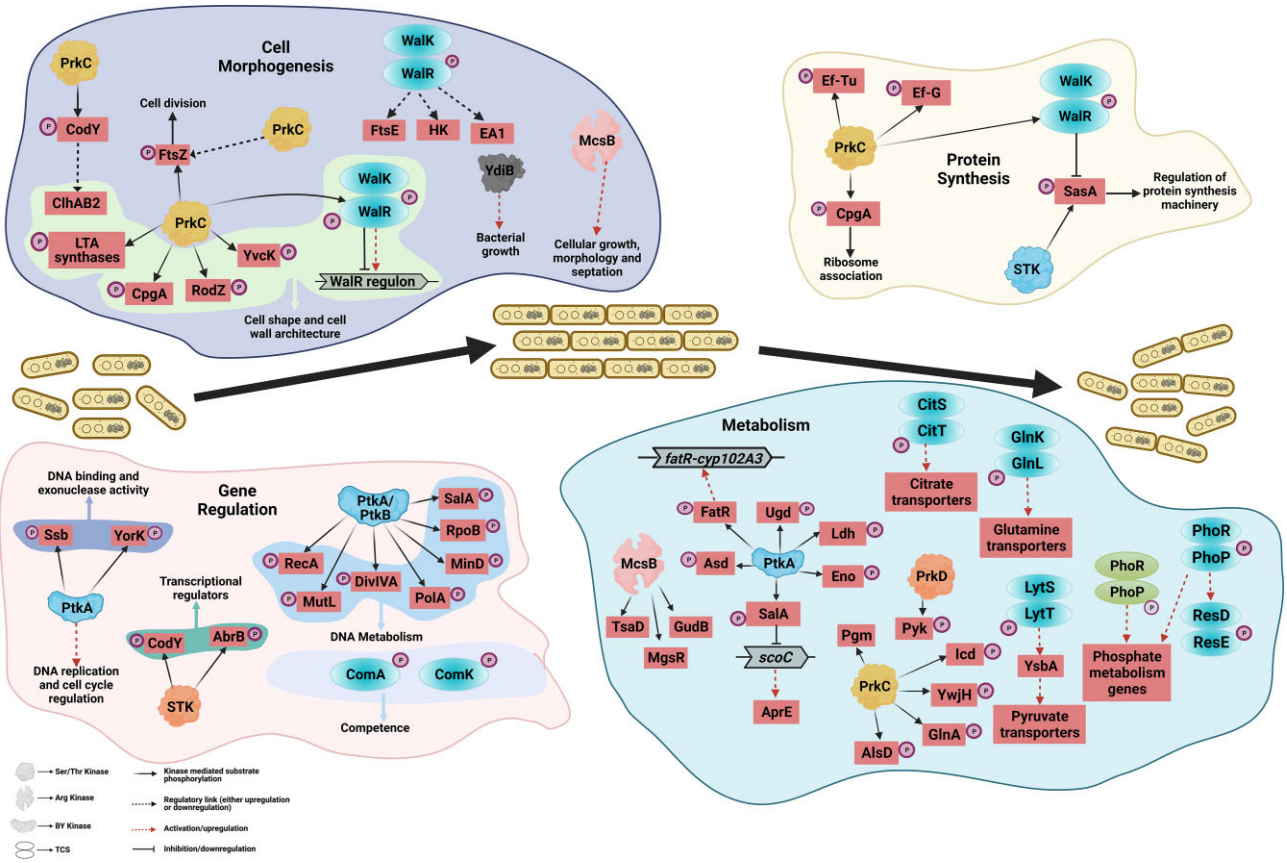

Figure 2. Role of protein kinases in *Bacillus* cell development and growth. The schematic diagram shows multiple proteins being regulated by phosphorylation during general cell development and growth with four specific processes illustrated: cell morphogenesis, protein synthesis, DNA regulation, and metabolism. Different classes of kinases are involved in each process that targets the substrates, which are involved in multiple pathways. Black solid arrows represent kinase-mediated substrate phosphorylation, red dotted arrows represent activation/upregulation, black lines with terminal bars represent inhibition or downregulation, and black dotted arrows represent regulatory links (either upregulation or downregulation).

al. 2016, Manuse et al. 2016, Hardt et al. 2017, Maurya et al. 2018, Rajpurohit et al. 2022). Interestingly, phosphoproteome analysis by Ravikumar et al. (2014) also identified FtsZ as a substrate of PrkC/PrpC in *B. subtilis*, and a study by our group reported septation defects and upregulated *ftsZ* levels in a *prkC* deletion mutant strain in *B. anthracis* (Dhasmana et al. 2021). These reports may provide a functional connection between PrkC and the regulation of cell division machinery in *Bacillus* genus via phosphorylation of FtsZ. In addition, a study on McsB Arg kinase (previously characterized as a Tyr kinase) in *B. anthracis* showed attenuated growth, elongated cell morphology (elongated cells), and defective septum formation in an *mcsB* null mutant strain, signifying the importance of Arg phosphorylation in bacterial growth and development (Mattoo et al. 2008, Singh et al. 2015). Furthermore, a new family of protein kinases belonging to the ubiquitous bacterial kinase family YdiB was shown to be important for normal bacterial growth (Karst et al. 2009, Nguyen et al. 2017).

Altogether, these studies highlight the widespread role of protein phosphorylation in the regulation of cell morphogenesis and growth in the *Bacillus* genus (Fig. 2).

Gene regulation

Protein phosphorylation is important in the regulation of various genetic processes including replication, transcription initiation, DNA condensation, and repair, via the phosphorylation of DNA/RNA binding proteins (Garcia-Garcia et al. 2016). Phosphoproteomics and interactomics studies have identified several proteins in *B. subtilis* that are phosphorylated at Ser/Thr/Tyr residues and are part of the gene regulatory network (Ravikumar et al. 2014, Shi et al. 2014b, Rosenberg et al. 2015). For instance, phosphorylation of a DNA single-stranded binding protein (Ssb) by the BY-kinase PtkA (formerly known as YwqD) and the YorK exonuclease specific for single-stranded DNA is important for DNA binding and exonuclease activities, respectively (Mijakovic et al. 2006, Jers et al. 2010) (Fig. 2). Inactivation of *ptkA* in *B. subtilis* causes the formation of multiple nucleoids the and accumulation of additional genetic material in the cells, indicating the role of Tyr phosphorylation in DNA replication and cell cycle regulatory pathways (Petranovic et al. 2007). Several other proteins involved in DNA and RNA metabolism that were identified as the target of BY-kinases (PtkA or PtkB) based on interactome analysis include RecA (recombinase), DivIVA (cell division protein), MinD (cell-division regulator), SalA (negative regulator of *scoC* expression, activator of PtkA kinase activity), PolA (DNA polymerase I), RpoB (DNA-directed RNA polymerase beta subunit), MutL (DNA mismatch repair protein), and TkmA/TkmB (protein tyrosine kinase activator) (Shi et al. 2014b). DivIVA is known to be regulated by protein phosphorylation in other bacterial species indicating that kinase-mediated regulation of cell division is conserved in diverse bacteria (Arora et al. 2014, Fleurie et al. 2014, Lee et al. 2014, Chaudhary et al. 2023).

The most studied Ser/Thr kinase and phosphatase pair involved in DNA regulation is the YabT kinase (Bidnenko et al. 2013) and the SpoIIE phosphatase (Duncan et al. 1995). These two en-

zymes are sporulation stage-specific and are critical to the development and maintenance of the spore genome (discussed in the sporulation section). Transcriptional regulators are another class of DNA-binding proteins that are targets of phosphorylation networks in the bacterial system (Dworkin 2015, Kalantari et al. 2015). The functional impact of Ser/Thr phosphorylation on the global transcriptional regulators CodY and AbrB has been studied using phosphorylation site mutants and DNA-binding experiments. Phosphorylation of AbrB at Ser86 hinders its DNA binding ability, leading to deregulation of target genes involved in the production of exoprotease, sporulation, and competence development in *B. subtilis* (Soufi et al. 2010, Kobir et al. 2014), while in both *B. subtilis* and *B. anthracis*, CodY phosphorylation was detected at a residue (Ser215) critical for its DNA binding activity (Joseph et al. 2005, Macek et al. 2007, Joon et al. 2017). Furthermore, our recent study in *B. anthracis* shows that CodY phosphorylation at Ser215 completely abolishes DNA binding with the promoter region of one of its target genes, *atxA*, leading to defects in toxin synthesis (Gangwal et al. 2022) (Fig. 2). Apart from these, protein phosphorylation also regulates various pathways involved in bacterial competence, the ability to uptake DNA from the environment, and its subsequent incorporation in the genome by specific protein channels and import machinery, leading to variations in the bacterial genotype and phenotype (O'Connell et al. 2022). In *B. subtilis*, the TCS RRs ComA and ComK are the most widely studied positive regulators of various early and late competence genes, respectively (Weinrauch et al. 1990, Dubnau et al. 1994, Maier 2020). ComK is shown to be phosphorylated by Arg kinase McsB and dephosphorylated by cognate phosphatase ywlE. Further, in the ywlE mutant of *B. subtilis*, ComK was found to be phosphorylated at six Arginine sites (R65, R157, R161, R165, R186, and R19). In the ywlE mutant, ComK-dependent gene expression was upregulated which suggests ComK activity is positively regulated by Arg phosphorylation (Elsholz et al. 2012). The phosphorylation status of another TCS RR, DegU, plays a critical role in the activation of competence genes and assists in the binding of ComK during competence (Dahl et al. 1992, Hamoen et al. 2003).

Cellular metabolism

Cellular metabolism plays a key role in bacterial growth and development via the modulation of important processes depending on the nutritional status and metabolic activity of the bacteria. In *Bacillus*, metabolic changes help to drive cellular machinery down to one of two paths, to produce either replicating vegetative cells or dormant spores. Glucose serves as a common energy source for heterotrophic bacteria and its absence triggers the uptake of other carbon sources, such as citrate, glutamine, pyruvate, or malate (Schilling et al. 2007). In *B. subtilis*, dedicated TCSs sense the presence of these additional carbon sources and thus increase the expression of membrane transporters facilitating their cellular intake. For example, CitST and GlnKL TCSs regulate the expression of Mg^{2+}–citrate transporters and glutamine transporters, respectively (Satomura et al. 2005, Repizo et al. 2006). The presence of citrate or glutamine causes autophosphorylation of the respective HKs, CitS, or GlnK, followed by the activation of their respective RRs, which in turn upregulate the expression of transporters involved in the uptake of citrate or glutamine (Satomura et al. 2005, Repizo et al. 2006).

In *B. subtilis*, in the absence of glucose as a major carbon source, LytST TCS is required for the uptake of pyruvate via inducing the transcription of YsbA, a protein involved in the upregulation of pyruvate transporters (van den Esker et al. 2017). Similarly, in a minimal medium, the presence of malate as the sole carbon source activates the YufLM TCS. Phosphorylated YufM binds to the promoter region of *maeN* and increases the surface expression of malate transporters to facilitate cellular growth on malate (Tanaka et al. 2003). In *B. anthracis*, PhoPR is another functional TCS, which is activated upon phosphate starvation. PhoP regulates the expression of phosphate metabolism-associated genes, e.g. *phoA* and *pst*, which help to overcome the limiting phosphate availability (Aggarwal et al. 2017). A similar PhoPR TCS exists in *B. subtilis*, which is activated under phosphate starvation and stimulates the degradation of teichoic acid in the cell wall, releasing phosphate ions, and hence fulfilling the metabolic need for phosphate (Pragai et al. 2004, Myers et al. 2016). Furthermore, phosphorylated PhoP directly increases the expression of another important TCS called ResDE, which is important for aerobic as well as anaerobic respiration during phosphate starvation conditions in *B. subtilis* (Birkey et al. 1998) (Fig. 2).

In *B. subtilis*, HPrK/P-mediated phosphorylation of the carbon-flux-regulating histidine protein Crh (a paralog of HPr) acts as a regulatory switch in carbon metabolism (Landmann et al. 2012). Additionally, PrkC phosphorylates four major metabolic enzymes, namely Transaldolase (YwjH), Glutamine Synthetase (GlnA), Isocitrate Dehydrogenase (Icd), and α-Acetolactate Decarboxylase (AlsD) (Pietack et al. 2010). *Bacillus anthracis* dual-specificity protein kinase (DSPK) PrkD phosphorylates pyruvate kinase (Pyk), an enzyme that catalyzes the concluding step of glycolysis, resulting in the inhibition of its specific activity (Arora et al. 2012). Pyk was also detected in the phosphoproteome study of *B. subtilis* (Macek et al. 2007) (Fig. 2). Since *pyk* mutant in *B. subtilis* produce more carbon dioxide and have a reduced growth rate (Fry et al. 2000), the role of Pyk phosphorylation in the growth, metabolism, and pathogenesis of different *Bacillus* species needs to be studied further.

Several other reports on *B. subtilis* have identified the metabolic enzymes that are phosphorylated at Arg residues, indicating a possible role of the McsB/YwlE system in their regulation (Elsholz et al. 2012, Schmidt et al. 2014, Trentini et al. 2016, Zhou et al. 2019, Ogura 2020). A study by Ogura et al. (2004) showed glucose-mediated regulation of *mcsB* and *ywlE* expression in *B. subtilis*, suggesting that Arg phosphorylation plays a role in cellular growth in glucose-containing media. McsB and YwlE mediate reversible Arg phosphorylation of ClpCP protease and TsaD, a tRNA modification enzyme that regulates *ylxR* (a nucleoid-associated protein) expression through *PylxS* promoter. YlxR is known to regulate >400 genes, indicating the importance of glucose mediated induction of gene expression through McsB and YwlE (Ogura 2020). Interestingly, McsB has been shown to be involved in regulating protein turnover of its substrates, causing degradation of anomalous proteins through the ClpCP protease system, as discussed later in stress response section. *B. subtilis* McsB and its cognate Arg phosphatase YwlE were shown to reversibly phosphorylate and regulate Glutamate dehydrogenase GudB degradation in the cell (Stannek et al. 2014). The redox-sensitive modulator MgsR of SigB regulon, controls the expression of genes involved in oxidative or thiol-specific stress in *B. subtilis*. MgsR is phosphorylated by McsB, which mediates its degradation through Clp proteases (Lilge et al. 2020) (Fig. 2). Hajdusits et al. (2021) delineated that under stress conditions McsB forms a closed octamer-like compartment, which interconverts with monomers and other oligomers in a phosphorylation dependent manner. Interestingly, the active sites in the octamer are sequestered and only phosphorylate unfolded proteins that can enter the compartment, thus mediating their degradation. Contrarily, dimerized McsB can phospho-

rylate its substrates and regulate their activity (Hajdusits et al. 2021). Additional metabolic studies showed that PtkA-mediated phosphorylation of a Ugd family protein—UDP glucose dehydrogenase, is important for its catalytic activation in *B. subtilis* (Mijakovic et al. 2003, Petranovic et al. 2009) (Fig. 2). A similar PtkA-dependent phosphorylation mechanism activates aspartate semialdehyde dehydrogenase (Asd), converting aspartyl phosphate to aspartyl semialdehyde and inorganic phosphate (Jers et al. 2010). In different species of *Bacillus*, the role of UDP glucose dehydrogenase is implicated in the production of exopolysaccharides and cell wall organization, while Asd is involved in amino acid biosynthesis (Mijakovic et al. 2004, Jakobsen et al. 2009, Naerdal et al. 2011). In *B. subtilis*, PtkA has been shown to regulate the cellular localization of enzymes required for carbon metabolism, namely Ldh (Lactate dehydrogenase) and Eno (Enolase) (Jers et al. 2010). Phosphorylation of Eno by PrkC also affects its cellular localization and expression in *B. anthracis* (Virmani et al. 2019). Additionally, phosphorylation of Pgm by PrkC regulates its activity, indicating that PrkC regulates glycolysis at multiple steps (Virmani et al. 2023) (Fig. 2).

Protein phosphorylation can indirectly regulate metabolic enzymes through phosphorylation-mediated activation or inhibition of various transcriptional regulators. In *B. subtilis*, PtkA-mediated phosphorylation of a transcriptional regulator FatR, involved in the metabolism of polyunsaturated fatty acids, abrogates its DNA binding ability consequently leading to the derepression of the *fatR-cyp102A3* operon (Derouiche et al. 2013). PtkA-mediated phosphorylation of another transcriptional regulator, SalA, represses *scoC* (an aprE repressor) and activates the expression of a *B. subtilis* exoprotease, AprE (Derouiche et al. 2015). AprE is an important metabolic enzyme, i.e. required by growing bacteria for the supply of nutrients via extracellular protein degradation. In different *Bacillus* species, AprE synthesis is regulated by various transcriptional regulators including CodY, AbrB, DegU, ScoC, Hpr, SinR, and SalA that are in turn regulated by protein phosphorylation events (Ogura et al. 2004, Derouiche et al. 2015, Barbieri et al. 2016, Liu et al. 2020, Zhou et al. 2021, Zolfaghari Emameh et al. 2022). These studies, therefore, suggest a functional correlation between metabolism and protein phosphorylation.

Protein synthesis

The optimal functioning of protein synthesis machinery is vital for cell survival and growth. Protein synthesis comprises of three basic steps: initiation, elongation, and termination. After initiation, there is a multistep elongation cycle involving three major elongation factors, EF-Tu, EF-Ts, and EF-G (Xu et al. 2021). These are highly conserved proteins that are essential for the survival of bacteria. EF-Tu is a GTPase that has diverse functional roles ranging from translation to pathogenesis and can interact with a variety of macromolecules including RNA, nucleotides, and other proteins (Krab and Parmeggiani 2002, Maracci and Rodnina 2016, Harvey et al. 2019). It alternates between active (GTP-bound) and inactive (GDP-bound) states, which is responsible for accurate selection of aminoacyl-tRNA and its binding to the ribosome (Bourne et al. 1991, Schmeing et al. 2009, Sajid et al. 2011a, Talavera et al. 2018). These states of EF-Tu are controlled by EF-Ts, the guanine nucleotide exchange factor. EF-G is also an essential GTPase that translocates the ribosomes along the translating mRNA (Rodnina et al. 1997, Agirrezabala and Frank 2009).

PrkC/PrpC-dependent reversible phosphorylation of the elongation factors EF-G and EF-Tu has been widely reported in the *Bacillus* genus (Gaidenko et al. 2002, Levine et al. 2006, Shah et al. 2008, Absalon et al. 2009, Shah and Dworkin 2010, Arora et al. 2013, 2017). Ribosome-associated proteins have also been shown to be the targets of Ser/Thr phosphorylation in *B. subtilis* (Absalon et al. 2009, Pompeo et al. 2012). For example, the ribosome associated GTPase CpgA is phosphorylated by PrkC in *B. subtilis*. Mutation of the phosphorylated residue Thr166 decreases the GTPase activity of CpgA as well as its affinity to 30S ribosomal subunits. The *Bacillus* strains expressing the CpgA-Thr166Ala variant show growth defects and exhibit a curly morphology, indicating the importance of ribosome-associated protein phosphorylation in maintaining *B. subtilis* growth and morphology (Pompeo et al. 2012) (Fig. 2).

Bacillus sp. also utilize secondary messengers known as alarmones or hyperphosphorylated (p)ppGpp nucleosides for protein synthesis during nutrient deprivation (Bange and Bedrunka 2020). These alarmones act as a cue for starving bacteria to shut down various essential cellular pathways, such as transcription and DNA replication, thereby helping them to conserve their energy resources (Potrykus and Cashel 2008, Steinchen and Bange 2016, Gourse et al. 2018). In *B. subtilis*, the cellular level of these alarmones is determined by dedicated (p)ppGpp synthetases such as SasA (small alarmone synthetase A) (Nanamiya et al. 2008), which are known to be regulated by Ser/Thr phosphorylation (Libby et al. 2019). The expression of *sasA* is regulated by the WalR transcription factor, a component of the WalRK TCS. Interestingly, WalR itself is regulated by PrkC/PrpC-mediated reversible phosphorylation, resulting in further activation of WalR activity and repression of *sasA* expression (Libby et al. 2019). Thus, the protein synthesis machinery in *B. subtilis* is subjected to regulation by phosphorylation, either directly by phosphorylation of translational factors or by (p)ppGpp-mediated inhibition (Fig. 2).

Sporulation

The process of spore formation and revival provides an excellent model for understanding the developmental processes in the bacterial system. During sporulation, the entire *Bacillus* metabolic pathways are reset with the help of various regulatory modifications such as protein phosphorylation to form a dormant spore (Errington 2003, Hoch 2017, Khanna et al. 2020). In *B. subtilis*, initiation of sporulation is triggered by adverse environmental conditions, such as, nutritional stress, oxygen tension, or redox changes. These signals are perceived by KinA-E HKs, which phosphorylate Spo0A, the master regulator of sporulation (Fig. 3) (LeDeaux and Grossman 1995, LeDeaux et al. 1995, Jiang et al. 1999, Fujita and Losick 2005, Aguilar et al. 2010). The activation of Spo0A is managed by a phosphorelay cascade emanating from Kin HKs (Jiang et al. 2000). For example, KinA-mediated phosphorelay involves Spo0F, Spo0B, and Spo0A (Burbulys et al. 1991). Activated Spo0A, in turn, represses the expression of AbrB, a transcription repressor of sporulation (Strauch et al. 1990). On the other hand, phosphatases RapA and Spo0E reset Spo0F and Spo0A to an unphosphorylated state (Ohlsen et al. 1994, Perego et al. 1994, Perego 2001). In *B. anthracis*, out of nine sensor HKs, five have been characterized and shown to initiate sporulation via this classic phosphorelay (Brunsing et al. 2005). Apart from these Kin HKs, two functional TCSs, BAS1213–1214 and BAS0540–0541, are characterized in *B. anthracis* with a possible role in sporulation (Gopalani et al. 2016, Gupta et al. 2018). The BAS1214 HK senses oxidative stress and phosphorylates its cognate RR, BAS1213. The phosphorylated RR thus increases its expression and that of sporulation kinase D, which causes a reduction in sporulation efficiency (Gupta et al. 2018). In the BAS0540–0541 TCS, the overexpression of the BAS0540 RR

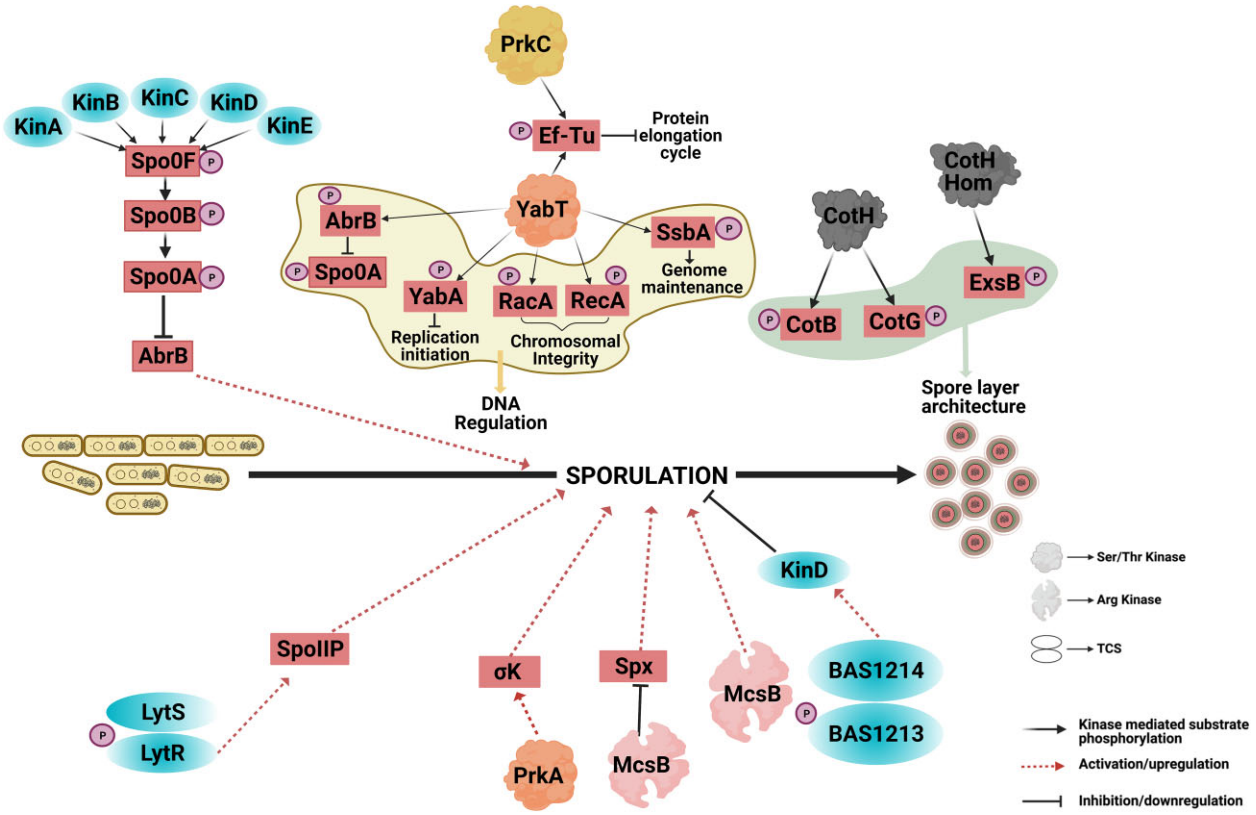

Figure 3. Role of protein kinases during sporulation in *Bacillus*. The schematic diagram shows several pathways being regulated by phosphorylation during sporulation. Every pathway involves multiple proteins' activity regulated through phosphorylation. Black solid arrows represent kinase-mediated substrate phosphorylation, red dotted arrows represent activation or upregulation, and black lines with terminal bars represent inhibition or downregulation.

causes a reduction in spore counts, although the molecular mechanism is still unknown (Gopalani et al. 2016).

The significance of TCSs is not limited to the initiation of sporulation, they are also involved in later stages of sporulation including forespore engulfment and mother cell lysis. In *B. anthracis*, LytSR TCS functions by dissipating proton motive force, acting as a signal for the induction of *lrgAB* and *clhAB*$_2$ transcription, which can affect sporulation efficiency and cell viability during the stationary growth phase (Chandramohan et al. 2009). In *B. thuringiensis*, the LytSR TCS is induced by the mother cell compartment sigma factor, SigE. Deletion of *lytS-lytR* causes defects in forespore engulfment by the mother cell. LytSR can induce the expression of a cell wall hydrolase, SpoIIP, however, the exact mechanism of this regulation is not known (Peng et al. 2017). Thus, multiple TCSs are important for controlling sporulation (Fig. 3).

The importance of Ser/Thr phosphorylation in context of sporulation in *B. subtilis* has been studied and reviewed extensively (Pompeo et al. 2016). Activation of prespore specific RNA polymerase (SigF) gene by Ser phosphorylation was one of the first discovered examples of regulatory Ser phosphorylation in *B. subtilis* (Min et al. 1993). This is achieved by the proteins present in the same operon: SpoIIAB (anti-SigF), SpoIIAA (anti-anti-SigF), and SpoIIE (serine phosphatase). SpoIIAB binds and inactivates SigF, while phosphorylation of SpoIIAA by SpoIIAB at a serine residue results in the release of SigF from the complex (Duncan and Losick 1993, Clarkson et al. 2004).

As cell-type-specific transcription factors are activated during different sporulating stages, the expression of specific kinases and phosphatases is also triggered during sporulation, indicating their requirement during this process (Kroos et al. 1999, Piggot and Hilbert 2004). For example, among the four characterized Hanks family Ser/Thr kinases in *B. subtilis*, the expression of YabT and PrkA peaks during sporulation initiation (Bidnenko et al. 2013, Yan et al. 2015). In *B. subtilis*, PrkA was shown to phosphorylate a 60-kDa protein in the crude extract at a Ser residue, but its functional relevance remained inconclusive due to lack of *in-vivo* data in the absence of this protein (Fischer et al. 1996). Later, the role of PrkA was linked to the sporulation process due to defective sporulation in *prkA* null mutant strain, possibly due to the downregulation of spore-specific transcription factor σK (Yan et al. 2015) (Fig. 3). Interestingly, another independent study in *B. subtilis* revealed sequence similarity of PrkA with ATP-dependent protease family proteins and PrkC-mediated regulation of PrkA protease activity was shown to be critical for the initiation of the sporulation process (Zhang et al. 2022). The expression of YabT kinase during sporulation initiation is required for the development and maturation of bacterial endospores in *B. subtilis* (Bidnenko et al. 2013). DNA binding proteins such as RecA, RacA, YabA, and single-stranded DNA binding proteins (Ssb) are the targets of YabT kinase (Bidnenko et al. 2013, Shi et al. 2014b, Derouiche et al. 2016, Garcia-Garcia et al. 2016, 2018) (Fig. 3). Among these, RecA (a DNA recombinase), and RacA (a chromosome-anchoring protein), are involved in maintaining the chromosomal integrity of the developing spore (Bidnenko et al. 2013, Shi et al. 2014b, Ramirez-Guadiana et al. 2016), while YabA is crucial for replication initiation during sporulation (Noirot-Gros et al. 2006, Garcia Garcia et al. 2018). *Bacillus subtilis* has two Ssb proteins, SsbA and SsbB, that are implicated in various pathways related to genome main-

tenance and natural competence (Lindner et al. 2004, Yadav et al. 2013, 2014, Paschalis et al. 2017). Mass spectrometry (MS) analysis revealed a novel phosphorylation site (Thr38) on the SsbA in *B. subtilis* (Derouiche et al. 2016), which was previously found to be phosphorylated on Tyr82 (Mijakovic et al. 2006) and Arg76 residues (Elsholz et al. 2012). This phosphorylation was primarily mediated by YabT, which enhanced the cooperative binding to single-stranded DNA (Derouiche et al. 2016). RecA is an SOS repair protein that plays an important role in bacterial DNA damage repair pathways (Nahrstedt et al. 2005, Million-Weaver et al. 2015, Torres et al. 2019). The deletion mutant of *yabT* and nonphosphorylatable mutant of RecA in *B. subtilis* exhibits same phenotype of increased sensitivity to DNA damage, indicating its role in DNA integrity maintenance (Bidnenko et al. 2013).

YabA negatively regulates replication initiation by decreasing cooperative binding of replication initiator DnaA to DNA (Noirot-Gros et al. 2006, Schenk et al. 2017). Phosphorylation of YabA by YabT kinase causes enhanced sporulation in *B. subtilis*, possibly correlating with decreased DNA replication and cell division (Garcia Garcia et al. 2018) (Fig. 3). YabT also phosphorylates the transition-phase transcriptional regulator AbrB, which is important for sporulation (Kobir et al. 2014). AbrB phosphorylation abrogates its DNA binding to the promoter of Spo0E, which is essential for maintaining the level of the active form of Spo0A-P (Molle et al. 2003, Shafikhani and Leighton 2004) (Fig. 3). YabT also phosphorylates EF-Tu, leading to decreased GTP hydrolysis and stabilization of interaction with the ribosome. This results in downregulation of protein synthesis by causing a halt in the protein elongation cycle in the cells undergoing a dormant state (Pereira et al. 2015). Furthermore, the functional implication of Ser/Thr phosphorylation in the context of sporulation has not been well-explored in the pathogenic *Bacillus* species. A recent report on *B. anthracis* demonstrated complete inhibition of the sporulation process in the absence of the STP PrpN (Gangwal et al. 2022). In a nutshell, these studies indicate the critical role of Ser/Thr phosphorylation in the sporulation pathway.

Bacillus spores have a multilayered structure consisting of exosporium (present in only a few species), a spore coat, cortex, and the core wall (Khanna et al. 2020). The spore coat is a thick sieve-like protein layer that protects the endospore. In *B. subtilis*, CotH, an atypical kinase present in the inner layer of the spore coat regulates the assembly of spore coat proteins (Naclerio et al. 1996, Nguyen et al. 2016, Scott and Newton 2016). CotH in *B. subtilis* and *B. cereus* acts as a kinase, phosphorylating two other spore coat proteins (CotB and CotG) on Ser residues, which is essential for efficient spore coat assembly (Nguyen et al. 2016, Freitas et al. 2020, Di Gregorio Barletta et al. 2022). Moreover, phosphorylation levels of CotB and CotG by CotH were shown to be sensitive to thermal variations, with higher efficiency at lower temperature (25°C) than at higher temperature (42°C) (Isticato et al. 2020, Di Gregorio Barletta et al. 2022). This makes CotH-mediated phosphorylation of CotB and CotG essential for proper spore coat morphogenesis (Fig. 3). Exosporium, an irregular-shaped layer consisting of hair-like projections is present in the outer layer of spore coat and is required for interaction with host cells and the surrounding environment (Bozue et al. 2007, Stewart 2015, Wang et al. 2016). In *B. anthracis*, ExsB, a CotG homolog present at the basal layer, is essential for stable attachment of exosporium to the spore coat. ExsB was found to be the highly phosphorylated exosporium protein, with at least 14 of its 19 Thr residues modified in its central region. This phosphorylation event is speculated to be controlled by a homolog of CotH, as occurs in the case of *B. subtilis* (McPherson et al. 2010, Freitas et al. 2020). Besides this, the deletion strain of McsB Arg kinase in *B. anthracis* showed defects in sporulation, in addition to cell growth and germination, confirming the role of Arg phosphorylation during the sporulation process (Singh et al. 2015) (Fig. 3).

Germination

The process of germination involves the progressive metabolic awakening of the dormant spore by reactivation of major biological processes including cell growth and protein synthesis machinery. This phenomenon is triggered by stimuli such as nutrient germinants that are sensed by surface receptors on the spore membrane and involve the temporal expression of about 30% of the bacterial genome as studied in the model organism *B. subtilis* (Keijser et al. 2007). Bacterial kinases are reported to be important in spore germination. The Ser/Thr kinase PrkC with surface-exposed PASTA domain, is a crucial germinant receptor, which mediates the muropeptide (small peptide fragments released from growing bacterial membranes)-dependent germination process in *B. subtilis* (Shah et al. 2008, Shah and Dworkin 2010, Squeglia et al. 2011) (Fig. 4). Furthermore, PrkC-mediated induction of the secretory peptidoglycan hydrolase YocH ensures the availability of muropeptides during the germination process by digestion of the surrounding bacterial peptidoglycan (Shah and Dworkin 2010). Mutant spores lacking *prkC* showed a muropeptide-dependent defective germination profile in *B. subtilis* and *B. anthracis* (Shah et al. 2008).

Following signal acquisition to exit dormancy, the protein machinery is activated to stimulate the growth of metabolically active vegetative cells (Sinai et al. 2015, Xing and Harper 2020). The translation factor EF-G is identified as a common substrate of PrkC/PrpC (Fig. 4), and its phosphorylation is important in the regulation of protein synthesis during germination in *B. subtilis* (Gaidenko et al. 2002, Shah et al. 2008, Shah and Dworkin 2010). As mentioned, the surface-exposed PASTA domain of PrkC can sense muropeptides, which activate PrkC and help in spore germination. Activated PrkC, in turn, transmits the signal inside the germinating spore and phosphorylates EF-G. This signaling module suggests the role of PrkC in exiting dormancy and facilitating spore germination through protein synthesis (Shah et al. 2008). Another translation factor, EF-Tu, was also identified as a common substrate of the PrkC/PrpC pair in *B. subtilis* and *B. anthracis*, though the functional relevance of this phosphorylation is still unknown (Absalon et al. 2009, Arora et al. 2013) (Fig. 4).

Arg phosphorylation also plays an important role during spore germination in *B. subtilis* (Zhou et al. 2019). A transposon-based genetic screen in *B. subtilis* showed that genetic disruption of the Arg phosphatase gene *ywlE* results in severe germination defects (Zhou et al. 2019). In this study, the significance of Arg phosphorylation in spore germination was also corroborated by an accelerated germination process in the absence of Arg kinase-McsB. Furthermore, Arg phosphoproteome of spores identified 18 proteins, including the translation factor Tig and housekeeping sigma factor SigA. The impact of Arg phosphorylation on these two proteins in the context of germination was assessed by using phosphomimetic and phosphoablative mutants and germination defects were observed in Tig (Arg45Asp) and SigA (Arg365Asp) mutants. The study showed arginine dephosphorylation of Tig and SigA as an important regulatory step for the re-establishment of bacterial transcriptional and translational machinery by enabling Tig association with ribosomes and SigA activation during the germination process (Zhou et al. 2019). Interestingly, the deletion of *mcsB* in *B. anthracis* leads to reduced germination efficiency, thus high-

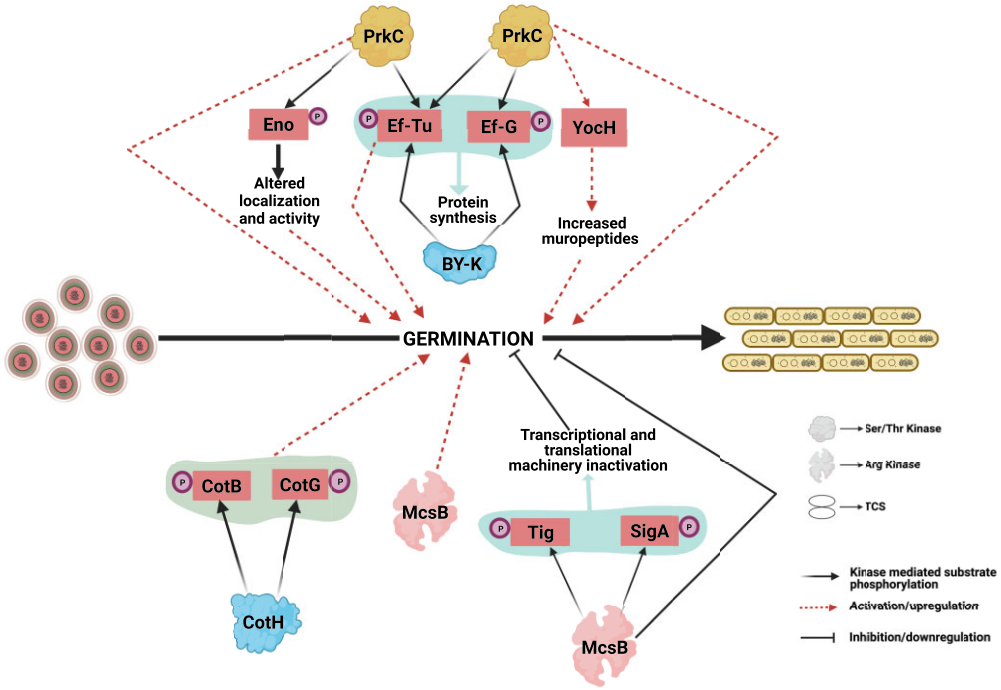

Figure 4. Role of protein kinases during spore germination in *Bacillus*. The schematic diagram shows multiple proteins regulated by phosphorylation during the spore germination process. The phosphorylation of these proteins is shown by black solid arrows. Red dotted arrows represent activation/upregulation, and black lines with terminal bars represent inhibition/downregulation.

lighting a strain-specific role of McsB in spore germination (Singh et al. 2015).

Apart from the protein synthesis pathway, the germination of bacterial spores involves the reprogramming of cellular metabolism. In fact, various studies have shown glycolytic enzymes as a target of Ser/Thr phosphorylation in *B. subtilis* and *B. anthracis* (Arora et al. 2012, Rosenberg et al. 2015, Virmani et al. 2019). In *B. anthracis*, phosphorylation of a glycolytic enzyme, enolase, by PrkC plays an important role in spore germination by modulation of enolase activity, expression, and cellular localization (Virmani et al. 2019) (Fig. 4).

In another class of atypical STKs, the *Bacillus* spore coat protein CotH was shown to be important during germination in *B. subtilis* (Naclerio et al. 1996). CotH-mediated Ser phosphorylation of two other spore coat proteins, CotB and CotG, is an essential requirement for spore germination (Nguyen et al. 2016). Although the mechanistic aspects of the observed germination defect have not been explored yet, it would be interesting to investigate the functional relevance of CotH in the *Bacillus* genus owing to the presence of its orthologs across different genera including spore-forming bacteria and even eukaryotic species (Nguyen et al. 2016) (Fig. 4).

Biofilm

Biofilm is a multicellular structure with complex cellular differentiation that helps bacteria to deal with external stresses such as nutrient deprivation, antibiotics, and low oxygen tension, allowing them to survive in adverse conditions (Hoiby et al. 2010, Kostakioti et al. 2013, Yin et al. 2019, Arnaouteli et al. 2021). As mentioned before, *B. subtilis* has five HKs (KinA–E) that autophosphorylate upon sensing environmental signals and regulate Spo0A phosphorylation (Burbulys et al. 1991). Apart from inducing sporulation, Spo0A is known to be vital in the formation of biofilm under low oxygen conditions, and deletion of *spo0A* restricts the cells to a monolayer pattern rather than a three-dimensional structure (Hamon and Lazazzera 2001, Mielich-Suss and Lopez 2015). Also, while KinA primarily controls sporulation, KinC-mediated activation of Spo0A is mainly linked to biofilm formation (Shemesh et al. 2010, Devi et al. 2015). With the aid of mathematical modeling, the activation of Spo0A by KinC was shown to be dependent on the bacterial growth phase and concentration of KinA (Chen et al. 2022). Another HK, KinD is reported to possess both kinase and phosphatase activities and is activated by lipoprotein Med (Aguilar et al. 2010, Banse et al. 2011). KinD-mediated phosphorelay fine-tunes Spo0A phosphorylation levels, thus becoming a switch to trigger either sporulation or biofilm formation (Aguilar et al. 2010). Small molecule inducers including L-malic acid released by tomato roots activate KinD, hence triggering the transcription of matrix-producing genes leading to bacterial biofilm formation on tomato roots (Chen et al. 2012). A combination of glycerol and manganese is also reported to initiate biofilm formation via specifically activating KinD-mediated signaling (Shemesh and Chai 2013). Also, in *B. subtilis* biofilm defects in *spo0A* mutants were rescued by mutations in *abrB* transcription factor, suggesting that Spo0A-mediated repression of *abrB* is essential for biofilm formation (Hamon and Lazazzera 2001).

In *B. subtilis*, the CssRS TCS stabilizes the biofilm by limiting the expression of the repressor *tasA*, thereby increasing the production of the extracellular matrix, which is crucial for successful biofilm formation (Steinberg et al. 2020). Also, the deletion of *cssRS* causes a sharp increase in the population of motile bacteria (Steinberg et al. 2020). Even after the biofilm is formed, another TCS, DegS-DegU induces the secretion of YIT toxin within the biofilm to destroy sensitive cells and attack any incoming competitor cells (Kobayashi and Ikemoto 2019). DegSU also controls

the expression of extracellular proteases and enzymes that are crucial for biofilms (Kobayashi 2007). In rhizobacterium, *B. amyloliquefaciens*, the ResDE TCS is reported to sense oxygen deprivation. It triggers biofilm formation by increasing the expression of terminal oxidases (Zhou et al. 2018). Apart from histidine kinases, Ser/Thr kinases also regulate biofilm formation and maintenance (Fig. 5).

The formation of biofilm, as already mentioned, depends on environmental cues to which the bacteria are exposed. These cues are sensed by surface proteins that mediate proper colonization. The Ser/Thr kinase PrkC, which possesses a surface-exposed sensor domain is crucial for biofilm formation. Interestingly, *prkC* deletion in *B. anthracis* also leads to the abrogation of biofilm formation. Mechanistic insights on the signaling pathway mediated by Ser/Thr phosphorylation driving biofilm formation in *B. anthracis* have emerged from the linkage of PrkC and one of its substrates, the GroEL chaperone (Arora et al. 2017b). GroEL has also been shown to be involved in biofilm formation in various other organisms like *Mycobacteria* and *Streptococci* (Ojha et al. 2005, Yin et al. 2019). MS studies revealed GroEL as one of the substrates of PrkC, and overexpression of native GroEL resulted in partial resumption of biofilm formation in a biofilm-defective *prkC* deletion strain (Fig. 5) (Arora et al. 2017b). Furthermore, phosphorylation of YabA by Ser/Thr kinase YabT negatively regulates biofilm formation by increasing the cellular level of Spo0A-P, the key regulator of genes involved in biofilm formation and sporulation (Garcia Garcia et al. 2018). Although the mechanism of tyrosine phosphorylation in biofilm formation is still unknown, null mutant strains of *ptkA* and *ptpZ* showed an altered biofilm phenotype in *B. subtilis* (Kiley and Stanley-Wall 2010). The biofilm formed in these conditions showed loss of "fruiting bodies" for sporulation and the absence of typical biofilm complex radial structures. The reason for this phenotype was attributed to the defective sporulation efficiency of the bacterial cells that were growing in the biofilm colony. Complete loss of biofilm formation was observed in the strains lacking both the BY kinases, *ptkA* and *ptkB* (Gerwig et al. 2014). Apart from this, flagellar motility of several *Bacillus* species that helps the bacteria to swim and slide across surfaces is often linked to biofilm initiation and development (Houry et al. 2010, Guttenplan and Kearns 2013, Liaqat et al. 2018, Li et al. 2022). Inhibition of motility promotes biofilm formation and in *B. subtilis*, flagellar motility and swarming properties of the bacteria are regulated by the phosphorylation status of the transcriptional regulator DegU by its cognate HK, DegS (Verhamme et al. 2007, Murray et al. 2009). Altogether, these studies indicate the central role of protein phosphorylation during biofilm formation in *Bacillus* (Fig. 5).

Stress response

Bacterial populations encounter various stressful conditions throughout their life cycle ranging from alterations in temperature, pH, osmolytes, nutrient deficiency, and exposure to antibiotics. To combat this, bacteria have evolved sophisticated stress responses that involve a wide range of cellular and morphological changes (Marles-Wright and Lewis 2007, Ultee et al. 2019, Cheng-Guang and Gualerzi 2020). This section presents current knowledge on the role of protein phosphorylation during stress conditions in *Bacillus* sp. including activation of SigB, the master regulator of general stress response (GSR) via a protein phosphorylation cascade involving Rsb (Regulator of sigma B) family proteins (Fig. 6).

Protein Arg phosphorylation in the *Bacillus* is primarily linked to the maintenance of overall protein quality by regulated proteolysis of misfolded or aggregated proteins during stress conditions (Mijakovic et al. 2016). This is achieved by a regulatory system that involves McsB (Arg kinase)-mediated inhibition of CtsR (stress response transcriptional regulator) repressor activity, activation of the ClpCP proteolytic machinery, and phospho-Arg tagging of the misfolded proteins (Fuhrmann et al. 2009, Elsholz et al. 2011, Tao et al. 2012, Trentini et al. 2016). The kinase activity of McsB is further modulated by its repressor CtsR, binding of phospho-Arg polypeptides at its catalytic site, ClpC (stress response-related ATPase, AAA+ superfamily), McsA (Arg kinase activator protein), and finally by the activity of its cognate Arg phosphatase (YwlE) (Kirstein et al. 2005, 2007, Elsholz et al. 2011, 2012, Fuhrmann et al. 2013, Suskiewicz et al. 2019). Strains lacking *mcsB* and *clpC* in *B. anthracis* show growth defects at elevated temperature (43°C) (Singh et al. 2015). The importance of Arg phosphorylation in stress response pathways was demonstrated by the global phosphoproteome of the *B. subtilis* Arg phosphatase (ywlE) mutant strain. Phospho-Arg sites were detected in proteins involved in GSR (controlled by SigB), such as stress on the cell envelope by antibiotics and osmolarity changes, heat shock, cold shock, and oxidative stress (Elsholz et al. 2012). Given the widespread role of Arg phosphorylation in bacterial stress response, another Arg phosphoproteome study, focusing specifically on heat and oxidative stress, identified key bacterial stress RRs such as CtsR, GroEL, ClpC, and ClpP as targets of Arg phosphorylation in *B. subtilis* (Schmidt et al. 2014). Furthermore, the YwlE phosphatase is inactivated during oxidative stress conditions as a regulatory mechanism for the induction of stress-related genes and McsB-mediated Arg phosphorylation (Fuhrmann et al. 2016). These studies highlight the importance of protein Arg phosphorylation during stress conditions in *Bacillus*.

The documented role of Tyr phosphorylation in *Bacillus* stress pathways, on the other hand, is limited to only a few studies. The chaperone protein DnaK (Hsp70 family protein) is a widely studied heat shock protein, i.e. a member of chaperone machinery activated during stress conditions and is also reported to be a target of protein phosphorylation in other bacteria (Sherman and Goldberg 1993, Seeger et al. 1996, Peake et al. 1998, Mayer and Bukau 2005, Roncarati and Scarlato 2017, Rigo et al. 2020). DnaK was identified as a substrate in Ser/Thr/Tyr phosphoproteomes (Eymann et al. 2007). Later, it was identified that PtkA/PtpZ-mediated regulation of DnaK by phosphorylation at a Tyr residue was responsible for controlling its chaperone activity, which affects survival of *B. subtilis* under heat shock conditions. (Shi et al. 2016). Deletion mutants of two low molecular weight Tyr phosphatases, YwlE (also an Arg phosphatase) and YkfJ showed reduced bacterial resistance to ethanol stress in *B. subtilis*, suggesting their possible roles in generating resistance to stress (Musumeci et al. 2005).

Various environmental stresses encountered by bacteria activate specific TCSs. Two different TCS can activate the same set of downstream target genes. One such example is the Heme sensing TCS (HssRS) and the HssRS-interfacing TCS (HitRS) in *B. anthracis* (Mike et al. 2014, Pi et al. 2020). Alterations in cell envelope integrity activates HitRS, that in turn interacts with HssRS and coordinate heme and cell envelope stress response (Mike et al. 2014). Similarly, the HssRS and HitRS TCSs exist in *B. thuringiensis*, which indirectly control the growth in the presence of heme through the uncharacterized operon *hrmXY* (Schmidt et al. 2016).

In *B. subtilis*, adaptation to cold temperature is achieved by the DesKR TCS. DesK (HK) senses the ordered lipid pattern in the membrane and autophosphorylates, followed by DesR (RR)

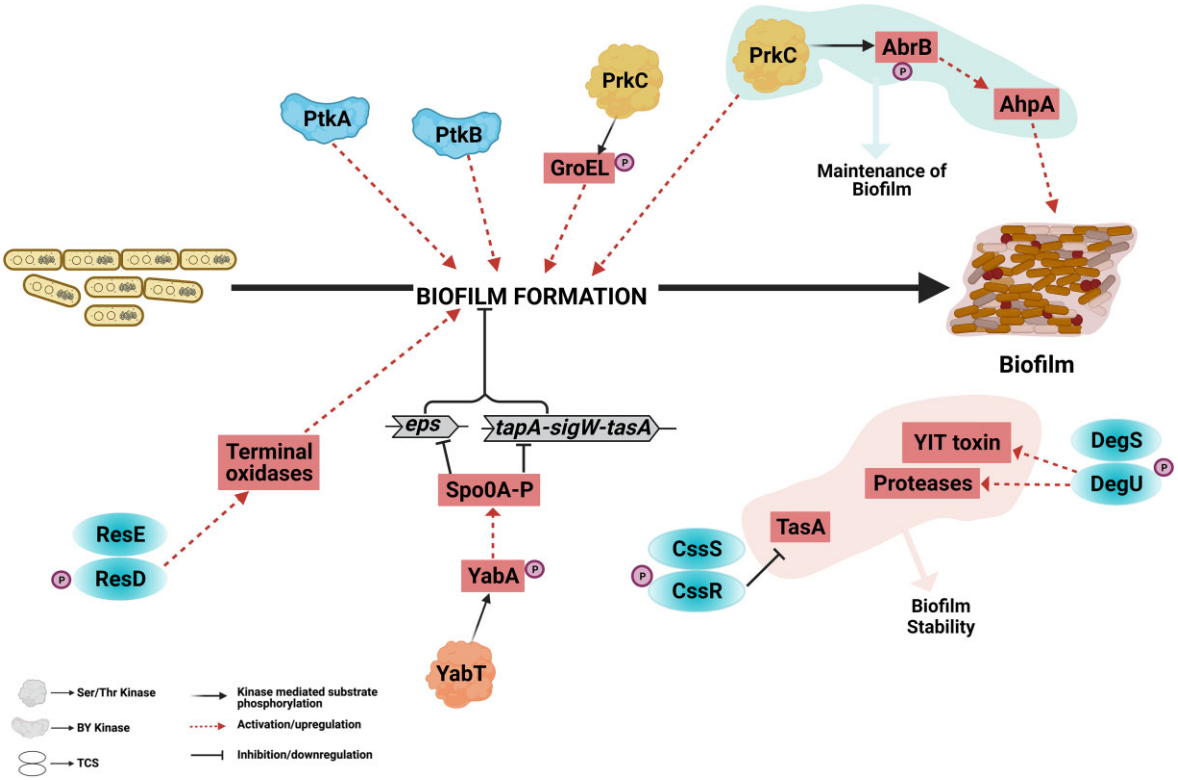

Figure 5. Role of protein kinases in *Bacillus* biofilm formation. Biofilm formation in bacteria is a complex process and requires regulation at several steps. The figure is a schematic representation of phosphorylation-mediated regulation of biofilm formation in bacteria. As shown, different classes of kinases and their substrates are required for biofilm formation. Black solid arrows represent kinase-mediated substrate phosphorylation, red dotted arrows represent activation/upregulation, and black lines with terminal bars represent inhibition/downregulation.

phosphorylation (Albanesi et al. 2004, Abriata et al. 2017). Active phospho-DesR then binds to the promoters of genes involved in the synthesis of the fatty acid desaturase DesA (Cybulski et al. 2004). DesK also responds to changes in pH; acidic pH breaks hydrogen bonds in the helix connecting the DesK transmembrane domain to the cytosolic domain, and thus abolishes the DesK-dependent synthesis of unsaturated acids, providing rigidity to the bacilli (Bortolotti et al. 2020). Apart from temperature and pH sensing, DesKR is highly expressed in low-pressure conditions in *B. subtilis* strain WN1106 (Fajardo-Cavazos et al. 2012). In *B. cereus*, adaptation to a cold environment occurs by higher expression of unsaturated fatty acid to maintain membrane fluidity at a lower temperature (Diomande et al. 2014). The CasKR TCS activates the expression of fatty acid desaturase DesA under cold conditions by ceasing repression of the *desA* promoter (Diomande et al. 2015, 2016).

In *B. anthracis*, a novel TCS named the Envelope Disruption System EdsRS is critical for managing the stress induced by targocil, an inhibitor that targets the cell envelope (Laut CL 2020). Compromised membrane integrity is detected by EdsS (HK), which results in its autophosphorylation and subsequent phosphorylation of EdsR (RR). Phosphorylated EdsR binds and activates the promoter of the BAS1661–BAS1663 operon encoding the cardiolipin synthase ClsT for the repair of the cell envelope in targocil-exposed vegetative cells. Exposure of *Bacillus* spores to targocil is reported to be highly toxic, as spore outgrowth requires rapid membrane generation (Laut CL 2020). In *B. thuringiensis*, the YvqEC and YvcPQ TCSs sense disturbances in the cell envelope and provide resistance against the cell wall targeting compounds vancomycin and bacitracin, respectively (Zhang et al. 2015, 2016). In *B. subtilis*, the LiaSR TCS manages the exogenous stress induced by the presence of peptide antibiotics targeting cell wall synthesis (bacitracin, nisin, vancomycin, and ramoplanin) (Mascher et al. 2004, Kesel et al. 2013). The LiaR-dependent promoter *liaI* is expressed during the transition from the exponential to stationary phase, suggesting an additional sensor signal for LiaS, other than antibiotics (Jordan et al. 2006, 2007). In *B. subtilis*, BceRS is a dedicated TCS that responds to the presence of bacitracin via its specific binding to BceB (permease) (Ohki et al. 2003, Dintner et al. 2014). BceS (HK) works in coordination with BceB (permease) and the level of BceS (HK) autophosphorylation is dependent on the BceA-bacitracin complex (Dintner et al. 2014).

WalRK plays an important role in cell wall homeostasis under physiological conditions in *B. subtilis*. Apart from this, WalRK is equally important for growth and cell proliferation under heat stress (Takada et al. 2018). WalK (HK) is activated upon heat stress and its activity is negatively modulated by two membrane proteins WalH and WalI (Takada et al. 2018). The presence of WalH and WalI is crucial to avoid overexpression of downstream endopeptidase genes, thus avoiding cell lysis. Even in *B. anthracis*, the expression of WalRK is induced either by temperature or in the presence of the bactericidal drug fosfomycin, an inhibitor of cell wall synthesis (Dhiman et al. 2015). In *B. subtilis*, the CssRS TCS is important in managing secretion stress, which happens when misfolded proteins accumulate outside the membrane and interfere with protein secretion. This inefficient protein secretion is detected by CssRS, which then increases the expression of the chaperonic proteases HtrA and HtrB (Westers et al. 2006, Noone et al. 2012).

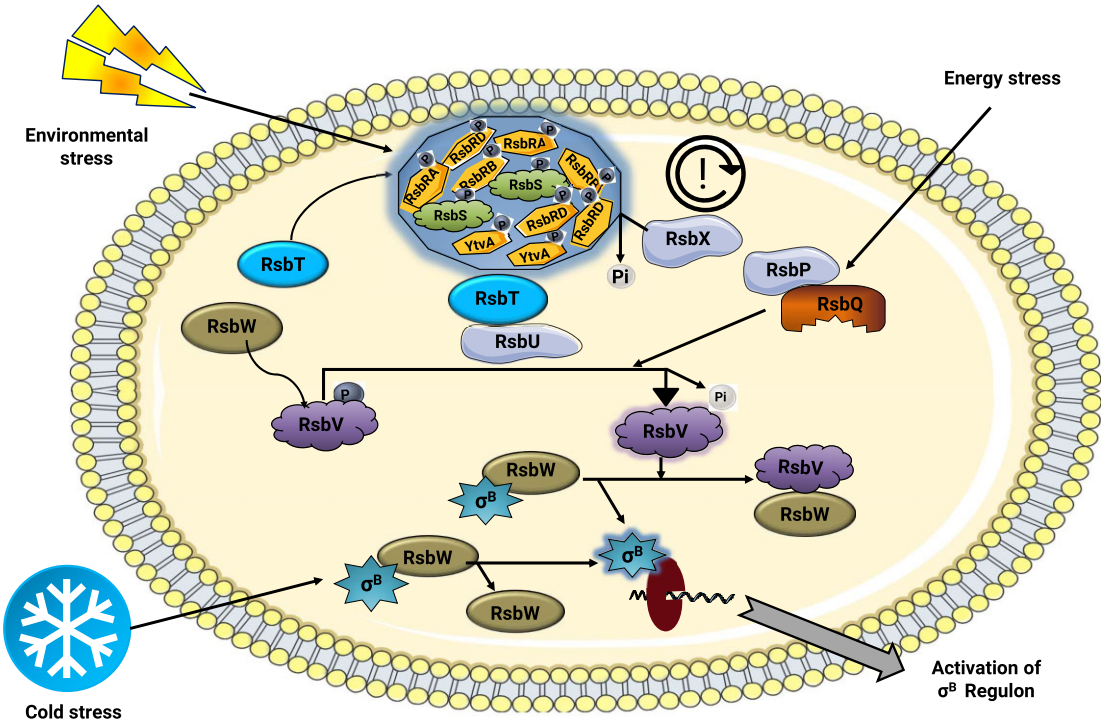

Figure 6. Ser/Thr phosphorylation in the activation of the SigB (σ^B) regulon. In unstressed conditions, the RsbT kinase forms a complex with other Rsb family proteins (RsbS antagonist and RsbR coantagonist with its paralogues). Upon environmental stress, the RsbT kinase phosphorylates RsbS and RsbR with its paralogues at Ser/Thr residues and is released from the complex. RsbT then interacts with the RsbU phosphatase resulting in its activation and dephosphorylation of a downstream antagonist, RsbV, which is further regulated by RsbW-mediated phosphorylation. Unphosphorylated RsbV dissociates the σ^B–RsbW kinase complex by binding to RsbW, thereby releasing σ^B, which in turn associates with RNA Polymerase and activates σ^B regulon gene expression. The RsbX phosphatase resets the entire stressosome machinery by dephosphorylation of RsbS and RsbR. Under energy-deficient conditions, a separate pathway is activated involving dissociation of another complex comprised of the RsbP phosphatase and a stress RR, RsbQ. The released RsbP results in the dephosphorylation of RsbV, which triggers downstream signaling pathways. During cold stress, bacteria respond by triggering another independent pathway involving RsbW. The complex of RsbW and σ^B modulates the expression of genes regulated by σ^B.

Another bacterial GSR involves the activation of sigma factor, SigB (master regulator of GSR), which regulates the expression of over 100 genes in *B. subtilis* during stress conditions (Haldenwang and Losick 1979, Bernhardt et al. 1997, Hecker and Völker 1998). Activation of the SigB regulon is achieved by a signaling cascade that involves Ser/Thr phosphorylation of Rsb (regulator of sigma factor) family proteins comprising TCS such as Ser/Thr kinases (RsbT and RsbW); PP2C-type phosphatases (RsbP, RsbX, and RsbU); antagonists (RsbS and RsbV), and coantagonists (RsbRA, RsbRB, RsbRC, and RsbRD) (Price et al. 2001, Hecker et al. 2007, Rodriguez Ayala et al. 2020).

In normal conditions, Ser/Thr kinase RsbW phosphorylates the antagonist RsbV, causing its inactivation and also acts as a negative regulator of SigB that blocks its binding to the RNA polymerase core enzyme (Alper et al. 1996, Yang et al. 1996, Rodriguez Ayala et al. 2020). Environmental stress conditions (acid/ethanol/salt) trigger dephosphorylation of RsbV by RsbU. RsbU is activated by interacting with RsbT Ser/Thr kinase that acts as a mediator and conveys environmental stress signals to initiate the downstream signaling. In the absence of stress, RsbT is entrapped in a 25S multiprotein oligomeric complex consisting of RsbS (antagonist) and RsbRAs (coantagonist) and its paralogues such as YkoB (RsbRB), YojH (RsbRC), YqhA (RsbRD), and YtvA (Akbar et al. 2001, Chen et al. 2003, Kim et al. 2004a). Under stress conditions, RsbT phosphorylates RsbRA, which facilitates phosphorylation of RsbS. Then RsbT is released from the complex, thus activating RsbU (Chen et al. 2004, Kim et al. 2004b). Activated RsbU dephosphorylates RsbV, which then binds to the RsbW kinase (RsbV–RsbW), thereby dissociating the RsbW–SigB complex. The SigB released from the complex binds to RNA polymerase, thus activating the SigB regulon (Dufour and Haldenwang 1994). This signaling pathway is reset by RsbX STP that dephosphorylates RsbS and RsbRA, and serves as a fine-tuning mechanism resulting in the sequestration of RsbT in the stressosome complex (Yang et al. 1996, Price et al. 2001, Chen et al. 2004). Furthermore, the absence of the RsbX protein in the *B. subtilis* system results in uncontrollable activation of the SigB regulon (Voelker et al. 1997) (Fig. 6). Under energy stress conditions (glucose, oxygen, and phosphate starvation), RsbV dephosphorylation is mediated by a complex of RsbP phosphatase and RsbQ hydrolase, thus activating the SigB regulon (Vijay et al. 2000, Brody et al. 2001). In the case of cold shock, the signaling pathway works independently of RsbP, RsbU, and RsbV. The degree of stability of the RsbW–SigB complex regulates the silencing or transcription of stress-related genes (Brigulla et al. 2003) (Fig. 6).

In *B. cereus*, SigB activation is achieved by a protein complex comprising RsbK (sensor kinase), RsbW (kinase), RsbV (antagonist), and RsbY (phosphatase). Following stress conditions, the sensor kinase RsbK phosphorylates and activates RsbY, which in turn dephosphorylates the RsbV antagonist. Unphosphorylated RsbV sequesters RsbW (a negative regulator of SigB), thus activating the SigB regulon (van Schaik et al. 2005, de Been et al. 2010).

Virulence

While the majority of *Bacillus* species are nonpathogenic, a few of them belonging to the *B. cereus* group are known to cause disease in animals and humans (Ehling-Schulz et al. 2019). The pathogenicity of this group is attributed to two major factors-secreted toxins or capsule. These factors are paramount for the survival of the bacteria inside the host, and thus it is important to study the network that regulates their synthesis for the development of novel prophylactics (Moayeri et al. 2015). In *B. anthracis*, the most studied protein, which is involved in the synthesis of toxins and capsule is the master virulence regulator, AtxA (Anthrax toxin activator) (Fouet 2010, McCall et al. 2019). The *B. anthracis* null mutant of *atxA* is avirulent in the mouse model of infection (Dai et al. 1995). Furthermore, the cellular level of AtxA decides the fate of associated pathways and thereby modulates the pathogenic cycle. High levels of AtxA in the cell are conducive to toxin gene expression while inhibiting sporulation in *B. anthracis* (Dale et al. 2018). The global transcriptional regulators CodY and AbrB regulate the synthesis of AtxA (Strauch et al. 2005, van Schaik et al. 2009, Gangwal et al. 2022), and are themselves targeted by Ser/Thr phosphorylation machinery (Macek et al. 2007, Kobir et al. 2014). This regulation is achieved either by direct binding of the transcriptional regulator to the promoter region of the toxin-producing genes (Dale et al. 2012, Chateau et al. 2013) or by an unknown post-translational mechanism (van Schaik et al. 2009).

In the nonpathogenic *B. subtilis*, phosphorylation of AbrB at a Ser residue inhibits its DNA binding property (Kobir et al. 2014), whereas in *B. anthracis*, CodY phosphorylation at a Ser residue abrogates its DNA binding to the *atxA* promoter, leading to depletion of toxin proteins (Gangwal et al. 2022). In *B. anthracis*, PTS-mediated phosphorylation of AtxA at conserved His residues in the PRD domain (PTS-regulated domain) regulates its activity and affects toxin synthesis (Tsvetanova et al. 2007, Raynor et al. 2018). Interestingly, PTS proteins HPr and Enzyme I were also shown to be required for *atxA* expression and their deletion mutant *B. anthracis* strain showed defective toxin production and attenuated virulence in an animal model of anthrax (Bier et al. 2020). More recently, another STP—PrpN was shown to regulate AtxA synthesis via phosphorylation-mediated regulation of CodY DNA binding activity. CodY was confirmed as a target of the PrkC and PrpN and the null mutant strain of *prpN* demonstrated a significant reduction in anthrax toxins and AtxA. Functional implication of PrpN-mediated dephosphorylation on CodY activity was examined using phosphomimetic and phosphoablative CodY mutants. While phosphorylation of CodY at a Ser residue resulted in complete abolishment of its DNA-binding ability at the *atxA* promoter region, unphosphorylated CodY was reported as a positive regulator of anthrax toxins (Gangwal et al. 2022). This highlights the importance of protein Ser/Thr and His phosphorylation in maintaining the optimum AtxA level for toxin production (Fig. 7).

Toxin biosynthesis is often correlated with carbon sources in pathogenic bacteria (Poncet et al. 2009, Chiang et al. 2011). In *Bacillus sp.*, this is mediated by a carbon catabolite protein (CcpA) and phosphotransferase system (PTS) component, HPr, via the classical carbon catabolite repression (CCR) mechanism (Khan and Banerjee-Bhatnagar 2002, Lorca et al. 2005, Singh et al. 2008). Hpr is phosphorylated by Hpr Kinase (Poncet et al. 2004, Choi et al. 2006) and PrkC (Macek et al. 2007, Pietack et al. 2010). While the functional relevance of phosphorylation by PrkC is still unknown, the phosphorylation by Hpr kinase plays an important role in modulating Hpr activity and binding to CcpA, resulting in differential expression of multiple genes in *B. subtilis* (Blencke et al. 2003, Deutscher et al. 2014) (Fig. 7).

Chain formation is another factor that contributes to the virulence of bacteria including *B. anthracis* by helping the pathogen to evade the host immune response (Moller et al. 2012, Rodriguez et al. 2012, Prashar et al. 2013, Jouvion et al. 2016). In *B. anthracis*, *prkC* deletion results in severe defects in chain formation as compared to wild type strain, indicating the role of PrkC in the regulation of chain length in *B. anthracis* (Dhasmana et al. 2021). Earlier studies have also shown that *B. anthracis* lacking the *prkC/prpC* pair showed impaired survival within macrophages and attenuated virulence in an animal model (Shakir et al. 2010). These studies provide a possible regulatory link between Ser/Thr phosphorylation and virulence via chain formation in *B. anthracis*.

When *B. anthracis* infects its host, it secretes lethal and edema toxins, which target smooth muscle cells and epithelial cells, exacerbating the infection. The BrrAB TCS regulates the expression of these toxins during infection. Deletion of any one of the components of BrrAB causes downregulation of *atxA* and a significant reduction in the toxin-producing ability of the pathogen, thus decreasing the virulence (Vetter and Schlievert 2007). In addition to exotoxins, secreted proteases facilitate the infection via degrading the host tissue and mobilizing the bacteria inside the host (Chung et al. 2006). A novel TCS encoded by BAS2109–2108 regulates the expression of eight intracellular and extracellular proteases (Gupta et al. 2017). Activated BAS2108 (HK) transfers the phosphate group to its cognate RR, BAS2109, which binds to the promoters of many proteases, such as metalloendopeptidase, bacillolysin, and several Ser proteases; few of these proteases are involved in pathogenesis. Additionally, the expression of BAS2109 (RR) increases in the presence of carbon dioxide or nutritional stress (Gupta et al. 2017) (Fig. 7).

The food-borne pathogen *B. cereus* has a TCS, ResDE, which controls the production of hemolysin BL and nonhemolytic enterotoxins under anaerobic conditions, thus controlling levels of virulence (Duport et al. 2006). ResE responds to the acidic environment and oxygen limitation in the gut and helps bacteria to secrete extracellular toxins to establish infection (Duport et al. 2006). YvfTU TCS controls the expression of a major pleiotropic virulence gene regulator, PlcR in *B. cereus* (Gohar et al. 2008, Brillard et al. 2008). Although *plcR* expression is decreased in *yvfTU* mutant bacilli, there are only minor differences reported in the expression of the *plcR* regulon (Brillard et al. 2008).

Cross-talk and cross-phosphorylation

A recent surge in the number of protein–protein interactome and phosphoproteome studies of bacterial systems including that of *Bacillus* have revealed multisite phosphorylation of several proteins by different kinases. These kinases exhibit promiscuous activity and can phosphorylate each other as well as have common substrates, thereby contributing to the biological complexity, adding another regulatory layer to the protein phosphorylation network in the bacterial system (Lin et al. 2009, Chao et al. 2010, Kobir et al. 2011, Baer et al. 2014, Shi et al. 2014a, b, Willett and Crosson 2017, Pinas et al. 2018, Shen et al. 2020). To differentiate these two regulatory networks, we have used the term "cross-talk" to define phosphorylation and dephosphorylation of a substrate at multiple amino acid residues by different kinases/phosphatases and "cross-phosphorylation" to define phosphorylation/dephosphorylation between different kinases/phosphatases (Fig. 8).

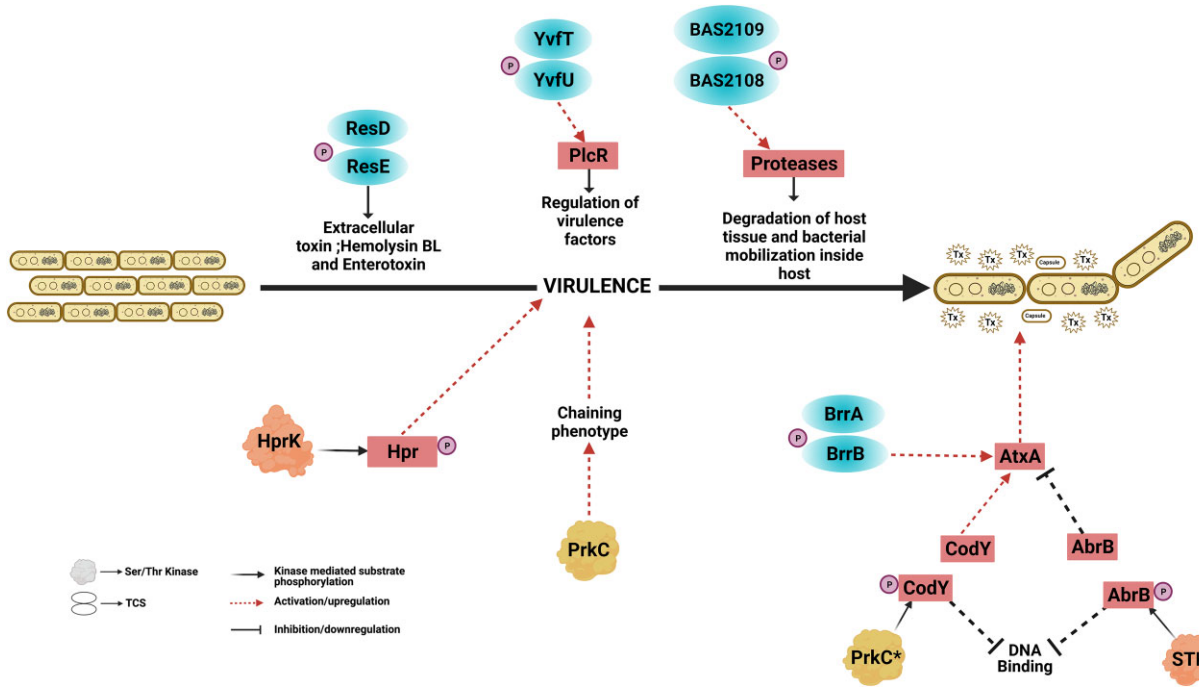

Figure 7. Role of protein kinases in *Bacillus* virulence. The pathways associated with virulence are activated in pathogenic species such as *B. anthracis*. These pathways follow specific signals during the infection stage within the host, which activate a unique set of signaling events emanating usually from surface-bound kinases and phosphatases. The schematic shows these specific proteins and their interacting partners, which carry out the indicated functions. Black solid arrows represent kinase-mediated substrate phosphorylation, red dotted arrows represent activation/upregulation, and black lines with terminal bars represent inhibition/downregulation.

In *B. subtilis*, an interaction network linking different classes of protein kinases/phosphatases was generated using yeast two-hybrid interactome studies and *in-vitro* kinase assays (Shi et al. 2014a, b). A direct interaction was predicted between Ser/Thr kinase-YabT and BY-kinase/PTP PtkB and cognate Phosphatase PtpZ. An indirect interaction was also observed via TkmA (the specific modulator for BY-kinase PtkA) between YabT and another BY-kinase, PtkB. The two BY-kinases, PtkA and PtkB show possible cross-interaction through the PtkA-modulator TkmA. Also, the Tyr phosphatase PtpZ interacts with TkmA and PtkA. RsbT, a two-component-like Ser/Thr kinase regulating the stressosome complex of *B. subtilis* interacts with BY-kinase/phosphatase PtkA/PtpZ and Ser/Thr kinase PrkD. DegS, an HK, was shown to be phosphorylated by Ser/Thr kinases including interacts with the PrkD and YabT/SpoIIE pair (Jers et al. 2011, Shi et al. 2014a, b).

Furthermore, cross phosphorylation among the kinases has been defined by categorizing the kinases into two broad classes. The first class consists of PrkD, PrkC, YabT, and PtkA kinases that can autophosphorylate and phosphorylate other kinases. Among these, PrkC, PrkD, and YabT show intermolecular phosphorylation, while YabT and PtkA can cross-phosphorylate each other. Also, PrkC can phosphorylate PtkA near its autophosphorylating site. The other class includes PtkA and two-component Ser/Thr kinases such as RsbW and SpoIIAB, which are phosphorylated by other Ser/Thr kinases. Additionally, HPr kinase/phosphorylase (PTS component) is phosphorylated by PrkD, PrkC, and PtkA, while RsbT interacts with PrkD, and YabT (Shi et al. 2014a). Another example of cross-phosphorylation involves phosphorylation of TCS proteins DegS (HK) and WalR (RR) by STKs. Phosphorylation of DegS by STKs (PrkD and YabT) at the Ser76 residue enhances the phosphotransfer to DegU (RR) and was found to be important in the regulation of competence development, complex colony formation, and swarming (Macek et al. 2007, Jers et al. 2011). Furthermore, PrkC-mediated phosphorylation of WalR (RR) at Thr101 regulates the expression of genes involved in cell wall metabolism (Libby et al. 2015). These studies suggest different kinase classes work in tandem in a network and illustrate the biological complexity of cross-phosphorylation in *Bacillus* genus (Fig. 8).

Interestingly, in a high-throughput experiment, multisite phosphorylation of substrates was predicted with different classes of kinases/phosphatases. For example, RecA is phosphorylated by YabT, PtkA and Arg kinase (Soufi et al. 2010, Elsholz et al. 2012, Bidnenko et al. 2013, Shi et al. 2014b). Phosphorylation of SalA by PtkA was also validated by an *in-vitro* kinase assay (Derouiche et al. 2015). Another phosphoproteome study reported phosphorylation of known substrates of Tyr kinase PtkA such as aspartate semialdehyde dehydrogenase (Asd), Enolase (Eno), and YjoA at Ser/Thr residues (Jers et al. 2010, Ravikumar et al. 2014). Out of these, Eno was identified as a substrate of the McsB/YwlE pair in an Arg phosphoproteome analysis (Schmidt et al. 2014). In *B. anthracis* Eno was also shown to be phosphorylated at Ser/Thr residues by PrkC (Virmani et al. 2019). This PrkC-mediated multisite phosphorylation of Eno imprints phenotypic memory in *B. anthracis* spores and is important for spore germination. The global transcriptional regulator AbrB is also phosphorylated at Ser and Tyr residues. Phosphorylation at the Ser residue is mediated by STKs (PrkC/PrkD/YabT), while Tyr phosphorylation was detected in the *B. subtilis* phosphoproteome (Kobir et al. 2014, Ravikumar et al. 2014).

Cross-talk among *B. subtilis* phosphorylation pathways involve Ssb (SsbA and SsbB) required for DNA replication that are phosphorylated by PtkA and McsB (Mijakovic et al. 2006, Elsholz et al. 2012). Interestingly, SsbA is also phosphorylated by Ser/Thr kinases (PrkD, PrkC, and most efficiently by YabT), which aids in the cooperative binding of SsbA tetramers to DNA (Derouiche

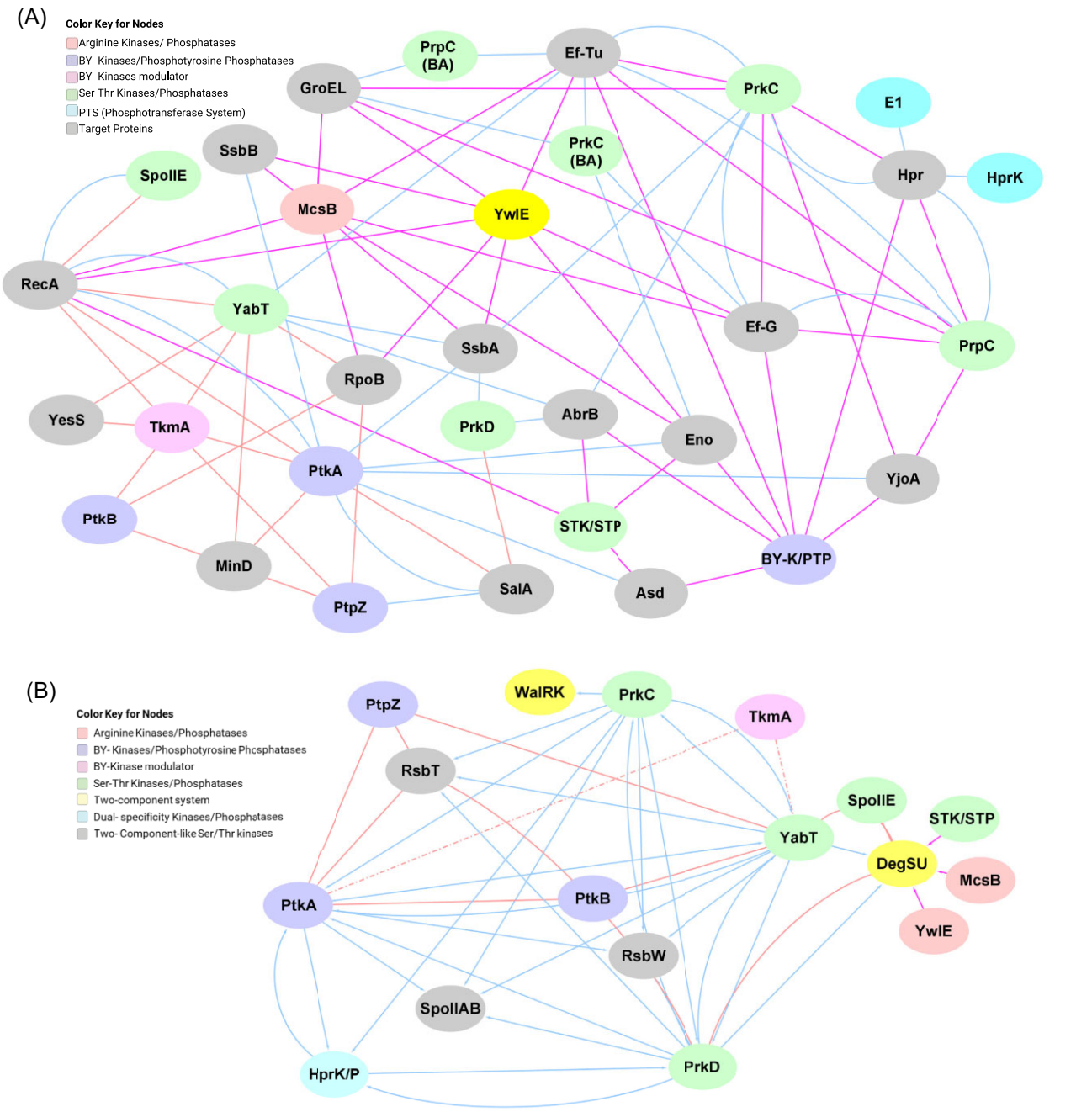

Figure 8. Cross-talk and cross-phosphorylation in *Bacillus*. (A) Cross-talk network representing multisite phosphorylation of proteins by different classes of kinases and phosphatases in *Bacillus*. A protein–protein interaction network was created using Cytoscape 3.8.2 software. Interaction among different kinases/phosphatases and their substrates are represented with colored nodes and colored lines. STK/STP: Ser/Thr kinase and phosphatase, BY-K/PTP: *Bacillus* Tyr kinase and phosphatase, and Hpr K: Hpr kinase. Among the kinases depicted in the figure, only PrkC and PrpC have validated substrates in both *B. anthracis* and *B. subtilis* (labeled BA in parentheses). (B) The cross-phosphorylation network between kinases is shown by a protein–protein interaction network. Different classes of kinases and phosphatases are represented by colored nodes, and interaction among different kinases/phosphatases are determined by colored arrows and lines. Blue arrows represent validated *in-vitro* assays, pink lines indicate phosphoproteome validations, and orange lines denote yeast two-hybrid interactions between bait and prey. Arrows point to the target proteins that are phosphorylated.

et al. 2016). Another example of cross-talk involves phosphorylation of a PTS protein, Hpr, at multiple residues. Phosphorylation at Ser residues is mediated by either a bifunctional ATP dependent Hpr kinase/phosphorylase (Reizer 1989) or Ser/Thr kinase/phosphatase pair PrkC/PrpC (Singh et al. 2007, Pietack et al. 2010), while phosphorylation at His is catalyzed by another PTS protein, Enzyme I (Reizer 1989). Phosphorylation of Hpr at the Ser/Thr/Tyr residues was also observed in phosphoproteome studies of *B. subtilis* (Ravikumar et al. 2014, 2018) (Fig. 8).

Arg phosphoproteome analysis identified several substrates such as GroEL, DegU, and the elongation factors EF-Tu and EF-G in *B. subtilis* that were previously shown to be phosphorylated at other residues (described in above sections), indicating multisite phosphorylation and possible cross-talk among the different

families of kinases (Elsholz et al. 2012, Schmidt et al. 2014, Trentini et al. 2014). EF-Tu and EF-G were also identified as targets of Tyr phosphorylation in the *B. subtilis* germinating spore phosphoproteome (Rosenberg et al. 2015).

Compared to *B. subtilis*, *B. anthracis* has lost some Tyr kinases during evolution. Consequently, two unique DSPKs, PrkD, and PrkG, were identified in *B. anthracis*, belonging to different classes defined on the basis of their target residues. PrkD belongs to "dual-specificity tyrosine phosphorylation-regulated kinase" (DYRK), which autophosphorylates on Ser, Thr, and Tyr but phosphorylates its substrates only on Ser and Thr. PrkG was identified as a bonafide DSPK that can autophosphorylate and mediate substrate phosphorylation on Ser/Thr/Tyr residues. Interestingly, the STP PrpC also exhibits dual specificity (Arora et al. 2012). It can dephosphorylate both PrkD and PrkG, in addition to PrkC (Obuchowski et al. 2000, Shakir et al. 2010).

Conclusions and future directions

In this review, we assessed the role of protein phosphorylation in different life stages of *Bacillus* species (Table 1). *Bacillus* sp. have the ability to metamorphosize into different forms such as spores, vegetative cells, or biofilms, which is vital for their survival in the environment or in the host. The shift in metabolic needs between these stages is a complex process (Fouet and Mock 2006, Tan and Ramamurthi 2014, Christie and Setlow 2020, Setlow and Christie 2020). Besides basic control of gene expression, PTMs such as phosphorylation, provide an additional layer of regulation. Over the years, the importance of His/Asp and Ser/Thr/Tyr protein phosphorylation has been unraveled in different cellular pathways. More recently, phosphorylation of other residues including Lys, Arg, and Cys have also been discovered. Genetic regulation extends beyond the identification of direct signaling pathways, as many of these phosphorylation events are controlled by more than one phosphorylation scheme, as discussed in the cross-talk section.

Most of the early research on the role of protein phosphorylation was performed using the model organism *B. subtilis* (Cozzone 1988, Perego et al. 1989, Mukai et al. 1990). Over the years, it has been established that protein phosphorylation schemes are ubiquitous throughout the *Bacillus* species, though some members of the *Cereus* clade have lost some Tyr kinases and gained dual-specificity protein kinases that can perform the function of both Ser/Thr and Tyr protein phosphorylation (Arora et al. 2012).

Of the different phosphorylation systems, early discoveries emphasized the role of His/Asp-based TCSs in sensing environmental stress and regulating cellular behavior, while recent advances in this field indicate that Ser/Thr/Tyr/Arg protein phosphorylation may play an even larger role. Though many phosphorylated proteins have been recognized in recent literature, our understanding of upstream sensing stimuli is still limited. Among the Ser/Thr protein kinases, the role of muropeptides as sensory signals to PrkC is well-established (Dworkin and Shah 2010, Squeglia et al. 2011, Pompeo et al. 2018b). Recent studies on the PrkC homolog PknB in *Mycobacterium tuberculosis* indicate that lipid moieties can also be sensory signals (Kaur et al. 2019). Further work is needed to understand if in addition to muropeptides, lipid II, can also act as sensing signals to PrkC. These studies show that protein kinase signaling in *Bacillus* species is highly evolved and regulated by specific sensory signals.

Recent advancement in the MS techniques have facilitated increase in phosphoproteome studies. They range from non-specific simple gel-based studies to more specific and complex MS studies. Improved phosphopeptide enrichment methods coupled with novel MS-based systemic approaches provides a better blueprint of the global phosphoproteome (Liu et al. 2021, Paulo and Schweppe 2021, Pina et al. 2021, Xiao et al. 2023). An in-depth description of these techniques is beyond the scope of this review but has been reported elsewhere (Engholm-Keller and Larsen 2013, Ed Dudley 2014, Yague et al. 2020). Importantly, phosphorylation at specific residues in a protein differs in lability, stoichiometry, and abundance. For instance, compared to phosphorylation at other residues, phospho-Asp/His and Arg are relatively unstable (Gonzalez-Sanchez et al. 2013, Huang et al. 2021). For this reason, enrichment and phosphoproteome studies on Asp/His/Arg phosphorylation are challenging and extensive research is needed in this field.

Unlike protein kinases, there is limited literature on the role of cognate protein phosphatases in cellular processes (Kennelly 2002, Sajid et al. 2015). Also, many regulatory mechanisms of phosphatases have been reported. For example, in *M. tuberculosis*, in a feedback loop system, PstP (homolog of *B. anthracis* PrpC) is phosphorylated by its cognate kinases PknA and PknB, causing its activation, though a similar mode of regulation has not been reported in *Bacillus* (Sajid et al. 2011b). The phosphatases of stressosome complexes are also shown to be regulated by a similar mechanism (Misra et al. 2019). The existing literature also suggests that similar to eukaryotes, a cross-talk and cross-phosphorylation network is also present in the bacterial systems and might provide another layer of regulation for phosphorylation pathways, thus enabling the efficient regulation of principal processes such as growth, DNA repair, cellular metabolism, and stress response.

In conclusion, phosphorylation is the key regulator of every aspect of *Bacillus* life, yet information about what triggers these events and how they maintain control over different pathways and cross-talk with each other is somewhat limited. Having several conserved processes, the *Bacillus* species are one of the best prokaryotic model systems utilized to study various aspects of bacterial physiology (Getz et al. 2019). Continued studies in this field can help to unravel more bacterial signaling systems and networks.

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References

Abriata LA, Albanesi D, Dal Peraro M *et al.* Signal sensing and transduction by histidine kinases as unveiled through studies on a temperature sensor. *Acc Chem Res* 2017;**50**:1359–66.

Absalon C, Hamze K, Blanot D *et al.* The GTPase CpgA is implicated in the deposition of the peptidoglycan sacculus in *Bacillus subtilis*. *J Bacteriol* 2008;**190**:3786–90.

Absalon C, Obuchowski M, Madec E *et al.* CpgA, EF-Tu and the stressosome protein YezB are substrates of the ser/thr kinase/phosphatase couple, PrkC/PrpC, in *Bacillus subtilis*. *Microbiology* 2009;**155**:932–43.

Aggarwal S, Somani VK, Gupta V *et al.* Functional characterization of PhoPR two component system and its implication in regulating

phosphate homeostasis in *Bacillus anthracis*. *Biochim Biophys Gen Sub* 2017;**1861**:2956–70.
Agirrezabala X, Frank J. Elongation in translation as a dynamic interaction among the ribosome, tRNA, and elongation factors EF-G and EF-Tu. *Quart Rev Biophys* 2009;**42**:159–200.
Aguilar C, Vlamakis H, Guzman A *et al.* KinD is a checkpoint protein linking spore formation to extracellular-matrix production in *Bacillus subtilis* biofilms. *mBio* 2010;**1**. https://doi.org/10.1128/mbio.00035-10.
Akbar S, Gaidenko TA, Kang CM *et al.* New family of regulators in the environmental signaling pathway which activates the general stress transcription factor sigma(B) of *Bacillus subtilis*. *J Bacteriol* 2001;**183**:1329–38.
Albanesi D, Mansilla María, De Mendoza D. The membrane fluidity sensor DesK of *Bacillus subtilis* controls the signal decay of its cognate response regulator. *J Bacteriol* 2004;**186**:2655–63.
Alper S, Dufour A, Garsin DA *et al.* Role of adenosine nucleotides in the regulation of a stress-response transcription factor in *Bacillus subtilis*. *J Mol Biol* 1996;**260**:165–77.
Alyahya SA, Alexander R, Costa T *et al.* RodZ, a component of the bacterial core morphogenic apparatus. *Proc Natl Acad Sci USA* 2009;**106**:1239–44.
Angeles DM, Scheffers D-J. The cell wall of *Bacillus subtilis*. *Curr Iss Mol Biol* 2021;**41**:539–96.
Arnaouteli S, Bamford NC, Stanley-Wall NR *et al.* *Bacillus subtilis* biofilm formation and social interactions. *Nat Rev Micro* 2021;**19**:600–14.
Arora G, Bothra A, Prosser G *et al.* Role of post-translational modifications in the acquisition of drug resistance in *Mycobacterium tuberculosis*. *FEBS J* 2021;**288**:3375–93.
Arora G, Misra R, Sajid A. Model systems for pulmonary infectious diseases: paradigms of anthrax and tuberculosis. *CTMC* 2017a;**17**:2077–99.
Arora G, Sajid A, Arulanandh MD *et al.* Unveiling the novel dual specificity protein kinases in *Bacillus anthracis*: identification of the first prokaryotic dual specificity tyrosine phosphorylation-regulated kinase (DYRK)-like kinase. *J Biol Chem* 2012;**287**:26749–63.
Arora G, Sajid A, Arulanandh MD *et al.* Zinc regulates the activity of kinase-phosphatase pair (BasPrkC/BasPrpC) in *Bacillus anthracis*. *Biometals* 2013;**26**:715–30.
Arora G, Sajid A, Gupta M *et al.* Understanding the role of PknJ in *Mycobacterium tuberculosis*: biochemical characterization and identification of novel substrate pyruvate kinase A. *PLoS ONE* 2010;**5**:e10772.
Arora G, Sajid A, Singhal A *et al.* Identification of ser/thr kinase and forkhead associated domains in *Mycobacterium ulcerans*: characterization of novel association between protein kinase Q and MupFHA. *PLoS Negl Trop Dis* 2014;**8**:e3315.
Arora G, Sajid A, Virmani R *et al.* Ser/thr protein kinase PrkC-mediated regulation of GroEL is critical for biofilm formation in *Bacillus anthracis*. *npj Biofilms Microbiomes* 2017b;**3**:7.
Ash C, Farrow JAE, Wallbanks S *et al.* Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative-analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* 1991;**13**:202–6.
Baer CE, Iavarone AT, Alber T *et al.* Biochemical and spatial coincidence in the provisional ser/thr protein kinase interaction network of *Mycobacterium tuberculosis*. *J Biol Chem* 2014;**289**:20422–33.
Bange G, Bedrunka P. Physiology of guanosine-based second messenger signaling in *Bacillus subtilis*. *Biol Chem* 2020;**401**:1307–22.
Banse AV, Hobbs EC, Losick R. Phosphorylation of Spo0A by the histidine kinase KinD requires the lipoprotein med in *Bacillus subtilis*. *J Bacteriol* 2011;**193**:3949–55.
Barbieri G, Albertini AM, Ferrari E *et al.* Interplay of CodY and ScoC in the regulation of major extracellular protease genes of *Bacillus subtilis*. *J Bacteriol* 2016;**198**:907–20.
Baros SS, Blackburn JM, Soares NC. Phosphoproteomic approaches to discover novel substrates of mycobacterial Ser/Thr protein kinases. *Mol Cell Proteomics* 2020;**19**:233–44.
Bernhardt JR, Lker UV, Lker AV *et al.* Specific and general stress proteins in *Bacillus subtilis*–a two-deimensional protein electrophoresis study. *Microbiology* 1997;**143**:999–1017.
Bhandari V, Ahmod NZ, Shah HN *et al.* Molecular signatures for *Bacillus* species: demarcation of the *Bacillus subtilis* and *Bacillus cereus* clades in molecular terms and proposal to limit the placement of new species into the genus *Bacillus*. *Int J Syst Evol Microbiol* 2013;**63**:2712–26.
Bidnenko V, Shi L, Kobir A *et al.* *Bacillus subtilis* serine/threonine protein kinase YabT is involved in spore development via phosphorylation of a bacterial recombinase. *Mol Microbiol* 2013;**88**:921–35.
Bier N, Hammerstrom TG, Koehler TM. Influence of the phosphoenolpyruvate:carbohydrate phosphotransferase system on toxin gene expression and virulence in *Bacillus anthracis*. *Mol Microbiol* 2020;**113**:237–52.
Bijlsma JJ, Groisman EA. Making informed decisions: regulatory interactions between two-component systems. *Trends Microbiol* 2003;**11**:359–66.
Birk MS, Charpentier E, Frese CK. Automated phosphopeptide enrichment for Gram-positive bacteria. *J Proteome Res* 2021;**20**:4886–92.
Birkey S, Zhang X, Duggan MF *et al.* Pho signal transduction network reveals direct transcriptional regulation of one two-component system by another two-component regulator: *Bacillus subtilis* PhoP directly regulates production of ResD. *Mol Microbiol* 1998;**30**:943–53.
Blencke HM, Homuth G, Ludwig H *et al.* Transcriptional profiling of gene expression in response to glucose in *Bacillus subtilis*: regulation of the central metabolic pathways. *Metab Eng* 2003;**5**:133–49.
Bonne Køhler J, Jers C, Senissar M *et al.* Importance of protein Ser/Thr/Tyr phosphorylation for bacterial pathogenesis. *FEBS Lett* 2020;**594**:2339–69.
Borriss R, Danchin A, Harwood CR *et al.* *Bacillus subtilis*, the model Gram-positive bacterium: 20 years of annotation refinement. *Microb Biotechnol* 2018;**11**:3–17.
Bortolotti A, Vazquez DB, Almada JC *et al.* A transmembrane histidine kinase functions as a pH sensor. *Biomolecules* 2020;**10**:1183.
Bourne HR, Sanders DA, Mccormick F. The GTPase superfamily: conserved structure and molecular mechanism. *Nature* 1991;**349**:117–27.
Bozue J, Moody KL, Cote CK *et al.* *Bacillus anthracis* spores of the bclA mutant exhibit increased adherence to epithelial cells, fibroblasts, and endothelial cells but not to macrophages. *Infect Immun* 2007;**75**:4498–505.
Bressuire-Isoard C, Broussolle V, Carlin F. Sporulation environment influences spore properties in *Bacillus*: evidence and insights on underlying molecular and physiological mechanisms. *FEMS Microbiol Rev* 2018;**42**:614–26.
Brigulla M, Hoffmann T, Krisp A *et al.* Chill induction of the SigB-dependent general stress response in *Bacillus subtilis* and its contribution to low-temperature adaptation. *J Bacteriol* 2003;**185**:4305–14.
Brillard J, Susanna K, Michaud C *et al.* The YvfTU two-component system is involved in plcR expression in *Bacillus cereus*. *BMC Microbiol* 2008;**8**:183.
Brody MS, Vijay K, W. Price C. Catalytic function of an alpha/beta hydrolase is required for energy stress activation of the sigma(B) transcription factor in *Bacillus subtilis*. *J Bacteriol* 2001;**183**:6422–8.

Brunsing RL, La Clair C, Tang S *et al.* Characterization of sporulation histidine kinases of *Bacillus anthracis*. *J Bacteriol* 2005;**187**:6972–81.
Burbulys D, Trach KA, Hoch JA. Initiation of sporulation in *B. subtilis* is controlled by a multicomponent phosphorelay. *Cell* 1991;**64**:545–52.
Capra EJ, Laub MT. Evolution of two-component signal transduction systems. *Annu Rev Microbiol* 2012;**66**:325–47.
Carballido-López R. The bacterial actin-like cytoskeleton. *Microbiol Mol Biol Rev* 2006;**70**:888–909.
Chandramohan L, Ahn J-S, Weaver KE *et al.* An overlap between the control of programmed cell death in *Bacillus anthracis* and sporulation. *J Bacteriol* 2009;**191**:4103–10.
Chao JD, Papavinasasundaram KG, Zheng X *et al.* Convergence of Ser/Thr and two-component signaling to coordinate expression of the dormancy regulon in *Mycobacterium tuberculosis*. *J Biol Chem* 2010;**285**:29239–46.
Chao JD, Wong D, Av-Gay Y. Microbial protein-tyrosine kinases. *J Biol Chem* 2014;**289**:9463–72.
Chateau A, Van der Verren SE, Remaut H *et al.* The *Bacillus anthracis* cell envelope: composition, physiological role, and clinical relevance. *Microorganisms* 2020;**8**:864.
Chateau A, van Schaik W, Joseph P *et al.* Identification of CodY targets in *Bacillus anthracis* by genome-wide in vitro binding analysis. *J Bacteriol* 2013;**195**:1204–13.
Chaudhary R, Kota S, Misra HS. DivIVA phosphorylation affects its dynamics and cell cycle in radioresistant *Deinococcus radiodurans*. *Microbiol Spectr* 2023;**11**:e0314122.
Chen CC, Lewis RJ, Harris R *et al.* A supramolecular complex in the environmental stress signalling pathway of *Bacillus subtilis*. *Mol Microbiol* 2003;**49**:1657–69.
Chen CC, Yudkin MD, Delumeau O. Phosphorylation and RsbX-dependent dephosphorylation of RsbR in the RsbR-RsbS complex of *Bacillus subtilis*. *J Bacteriol* 2004;**186**:6830–6.
Chen Y, Cao S, Chai Y *et al.* A *Bacillus subtilis* sensor kinase involved in triggering biofilm formation on the roots of tomato plants. *Mol Microbiol* 2012;**85**:418–30.
Chen Z, Srivastava P, Zarazua-Osorio B *et al. Bacillus subtilis* histidine kinase KinC activates biofilm formation by controlling heterogeneity of single-cell responses. *mBio* 2022;**13**:e0169421.
Cheng-Guang H, Gualerzi CO. The ribosome as a switchboard for bacterial stress response. *Front Microbiol* 2020;**11**:619038.
Chiang C, Bongiorni C, Perego M. Glucose-dependent activation of *Bacillus anthracis* toxin gene expression and virulence requires the carbon catabolite protein CcpA. *J Bacteriol* 2011;**193**:52–62.
Choi ID, Kim KN, Yun CW *et al.* Cloning and characterization of the hpr kinase/phosphorylase gene from *Bacillus stearothermophilus* No. 236. *Biosci Biotechnol Biochem* 2006;**70**:1089–101.
Christie G, Setlow P. *Bacillus* spore germination: knowns, unknowns and what we need to learn. *Cell Signal* 2020;**74**:109729.
Chung MC, Popova TG, Millis BA *et al.* Secreted neutral metalloproteases of *Bacillus anthracis* as candidate pathogenic factors. *J Biol Chem* 2006;**281**:31408–18.
Cladiere L, Hamze K, Madec E *et al.* The GTPase, CpgA(YloQ), a putative translation factor, is implicated in morphogenesis in *Bacillus subtilis*. *Mol Genet Genomics* 2006;**275**:409–20.
Clarkson J, Campbell ID, Yudkin MD. Efficient regulation of sigmaF, the first sporulation-specific sigma factor in *B. subtilis*. *J Mol Biol* 2004;**342**:1187–95.
Cozzone AJ. Protein phosphorylation in prokaryotes. *Annu Rev Microbiol* 1988;**42**:97–125.
Cui W, Han L, Suo F *et al.* Exploitation of *Bacillus subtilis* as a robust workhorse for production of heterologous proteins and beyond. *World J Microbiol Biotechnol* 2018;**34**:145.
Cybulski LE, del Solar G, Craig PO *et al. Bacillus subtilis* DesR functions as a phosphorylation-activated switch to control membrane lipid fluidity. *J Biol Chem* 2004;**279**:39340–7.
Dahl MK, Msadek T, Kunst F *et al.* The phosphorylation state of the DegU response regulator acts as a molecular switch allowing either degradative enzyme synthesis or expression of genetic competence in *Bacillus subtilis*. *J Biol Chem* 1992;**267**:14509–14.
Dai Z, Sirard JC, Mock M *et al.* The atxA gene product activates transcription of the anthrax toxin genes and is essential for virulence. *Mol Microbiol* 1995;**16**:1171–81.
Dale JL, Raynor MJ, Dwivedi P *et al.* cis-acting elements that control expression of the master virulence regulatory gene atxA in *Bacillus anthracis*. *J Bacteriol* 2012;**194**:4069–79.
Dale JL, Raynor MJ, Ty MC *et al.* A dual role for the *Bacillus anthracis* master virulence regulator AtxA: control of sporulation and anthrax toxin production. *Front Microbiol* 2018;**9**:482.
Darmon E, Noone D, Masson A *et al.* A novel class of heat and secretion stress-responsive genes is controlled by the autoregulated CssRS two-component system of *Bacillus subtilis*. *J Bacteriol* 2002;**184**:5661–71.
de Been M, Tempelaars MH, van Schaik W *et al.* A novel hybrid kinase is essential for regulating the sigma(B)-mediated stress response of *Bacillus cereus*. *Environ Microbiol* 2010;**12**:730–45.
Derouiche A, Bidnenko V, Grenha R *et al.* Interaction of bacterial fatty-acid-displaced regulators with DNA is interrupted by tyrosine phosphorylation in the helix-turn-helix domain. *Nucleic Acids Res* 2013;**41**:9371–81.
Derouiche A, Petranovic D, Macek B *et al. Bacillus subtilis* single-stranded DNA-binding protein SsbA is phosphorylated at threonine 38 by the serine/threonine kinase YabT. *Periodicum Biologorum* 2016;**118**:399–404.
Derouiche A, Shi L, Bidnenko V *et al. Bacillus subtilis* SalA is a phosphorylation-dependent transcription regulator that represses scoC and activates the production of the exoprotease AprE. *Mol Microbiol* 2015;**97**:1195–208.
Deutscher J, Ake FM, Derkaoui M *et al.* The bacterial phosphoenolpyruvate:carbohydrate phosphotransferase system: regulation by protein phosphorylation and phosphorylation-dependent protein-protein interactions. *Microbiol Mol Biol Rev* 2014;**78**:231–56.
Devi SN, Vishnoi M, Kiehler B *et al.* In vivo functional characterization of the transmembrane histidine kinase KinC in *Bacillus subtilis*. *Microbiology* 2015;**161**:1092–104.
Dhasmana N, Kumar N, Gangwal A *et al. Bacillus anthracis* chain length, a virulence determinant, is regulated by membrane localized serine/threonine protein kinase PrkC. *J Bacteriol* 2021;**203**:e00582–00520.
Dhiman A, Bhatnagar S, Kulshreshtha P *et al.* Functional characterization of WalRK: a two-component signal transduction system from *Bacillus anthracis*. *FEBS Open Bio* 2014;**4**:65–76.
Dhiman A, Gopalani M, Bhatnagar R. WalRK two component system of *Bacillus anthracis* responds to temperature and antibiotic stress. *Biochem Biophys Res Commun* 2015;**459**:623–8.
Di Gregorio Barletta G, Vittoria M, Lanzilli M *et al.* CotG controls spore surface formation in response to the temperature of growth in *Bacillus subtilis*. *Environ Microbiol* 2022;**24**:2078–88.
Dintner S, Heermann R, Fang C *et al.* A sensory complex consisting of an ATP-binding cassette transporter and a two-component regulatory system controls bacitracin resistance in *Bacillus subtilis*. *J Biol Chem* 2014;**289**:27899–910.
Diomande SE, Chamot S, Antolinos V *et al.* The CasKR two-component system is required for the growth of mesophilic and psychrotolerant *Bacillus cereus* strains at low temperatures. *Appl Environ Microb* 2014;**80**:2493–503.

Diomande SE, Doublet B, Vasai F *et al.* Expression of the genes encoding the CasK/R two-component system and the DesA desaturase during *Bacillus cereus* cold adaptation. *FEMS Microbiol Lett* 2016;**363**:fnw174.

Diomande SE, Nguyen-the C, Abee T *et al.* Involvement of the CasK/R two-component system in optimal unsaturation of the *Bacillus cereus* fatty acids during low-temperature growth. *Int J Food Microbiol* 2015;**213**:110–7.

Dobihal GS, Brunet YR, Flores-Kim J *et al.* Homeostatic control of cell wall hydrolysis by the WalRK two-component signaling pathway in *Bacillus subtilis*. *eLife* 2019;**8**:e52088.

Dobihal GS, Flores-Kim J, Roney IJ *et al.* The WalR-WalK signaling pathway modulates the activities of both CwlO and LytE through control of the peptidoglycan deacetylase PdaC in *Bacillus subtilis*. *J Bacteriol* 2022;**204**:e0053321.

Dominguez-Cuevas P, Mercier R, Leaver M *et al.* The rod to L-form transition of *Bacillus subtilis* is limited by a requirement for the protoplast to escape from the cell wall sacculus. *Mol Microbiol* 2012;**83**:52–66.

Dubnau D, Hahn J, Roggiani M *et al.* Two-component regulators and genetic competence in *Bacillus subtilis*. *Res Microbiol* 1994;**145**:403–11.

Dufour A, Haldenwang WG. Interactions between a *Bacillus subtilis* anti-sigma factor (RsbW) and its antagonist (RsbV). *J Bacteriol* 1994;**176**:1813–20.

Duncan L, Alper S, Arigoni F *et al.* Activation of cell-specific transcription by a serine phosphatase at the site of asymmetric division. *Science* 1995;**270**:641–4.

Duncan L, Losick R. SpoIIAB is an anti-sigma factor that binds to and inhibits transcription by regulatory protein sigma F from *Bacillus subtilis*. *Proc Natl Acad Sci USA* 1993;**90**:2325–9.

Duport C, Zigha A, Rosenfeld E *et al.* Control of enterotoxin gene expression in *Bacillus cereus* F4430/73 involves the redox-sensitive ResDE signal transduction system. *J Bacteriol* 2006;**188**:6640–51.

Dworkin J, Shah IM. Exit from dormancy in microbial organisms. *Nat Rev Micro* 2010;**8**:890–6.

Dworkin J. Ser/thr phosphorylation as a regulatory mechanism in bacteria. *Curr Opin Microbiol* 2015;**24**:47–52.

Ed Dudley AEB. *Advances in Protein Chemistry and Structural Biology*. Cambridge: Academic Press, 2014.

Ehling-Schulz M, Lereclus D, Koehler TM. The *Bacillus cereus* group: *Bacillus* species with pathogenic potential. *Microbiol Spectr* 2019;**7**:10.

Elhawy MI, Huc-Brandt S, Patzold L *et al.* The phosphoarginine phosphatase PtpB from *Staphylococcus aureus* is involved in bacterial stress adaptation during infection. *Cells* 2021;**10**:645.

Elsholz AK, Hempel K, Michalik S *et al.* Activity control of the ClpC adaptor McsB in *Bacillus subtilis*. *J Bacteriol* 2011;**193**:3887–93.

Elsholz AK, Turgay K, Michalik S *et al.* Global impact of protein arginine phosphorylation on the physiology of *Bacillus subtilis*. *Proc Natl Acad Sci USA* 2012;**109**:7451–6.

Engholm-Keller K, Larsen MR. Technologies and challenges in large-scale phosphoproteomics. *Proteomics* 2013;**13**:910–31.

Enosi Tuipulotu D, Mathur A, Ngo C *et al. Bacillus cereus*: epidemiology, virulence factors, and host-pathogen interactions. *Trends Microbiol* 2021;**29**:458–71.

Errington J, Aart LTV. Microbe profile: *Bacillus subtilis*: model organism for cellular development, and industrial workhorse. *Microbiology* 2020;**166**:425–7.

Errington J, Wu LJ. Cell cycle machinery in *Bacillus subtilis*. *Subcell Biochem* 2017;**84**:67–101.

Errington J. Regulation of endospore formation in *Bacillus subtilis*. *Nat Rev Micro* 2003;**1**:117–26.

Eymann C, Becher D, Bernhardt J *et al.* Dynamics of protein phosphorylation on Ser/Thr/Tyr in *Bacillus subtilis*. *Proteomics* 2007;**7**:3509–26.

Fabret C, Feher VA, Hoch JA. Two-component signal transduction in *Bacillus subtilis*: how one organism sees its world. *J Bacteriol* 1999;**181**:1975–83.

Fajardo-Cavazos P, Waters SM, Schuerger AC *et al.* Evolution of *Bacillus subtilis* to enhanced growth at low pressure: up-regulated transcription of des-desKR, encoding the fatty acid desaturase system. *Astrobiology* 2012;**12**:258–70.

Fioravanti A, Mathelie-Guinlet M, Dufrene YF *et al.* The *Bacillus anthracis* S-layer is an exoskeleton-like structure that imparts mechanical and osmotic stabilization to the cell wall. *PNAS Nexus* 2022;**1**:pgac121.

Fischer C, Geourjon C, Bourson C *et al.* Cloning and characterization of the *Bacillus subtilis* prkA gene encoding a novel serine protein kinase. *Gene* 1996;**168**:55–60.

Fleurie A, Manuse S, Zhao C *et al.* Interplay of the serine/threonine-kinase StkP and the paralogs DivIVA and GpsB in pneumococcal cell elongation and division. *PLoS Genet* 2014;**10**:e1004275.

Formstone A, Errington J. A magnesium-dependent mreB null mutant: implications for the role of mreB in *Bacillus subtilis*. *Mol Microbiol* 2005;**55**:1646–57.

Fouet A, Mock M. Regulatory networks for virulence and persistence of *Bacillus anthracis*. *Curr Opin Microbiol* 2006;**9**:160–6.

Fouet A. AtxA, a *Bacillus anthracis* global virulence regulator. *Res Microbiol* 2010;**161**:735–42.

Foulquier E, Pompeo F, Bernadac A *et al.* The YvcK protein is required for morphogenesis via localization of PBP1 under gluconeogenic growth conditions in *Bacillus subtilis*. *Mol Microbiol* 2011;**80**:309–18.

Foulquier E, Pompeo F, Freton C *et al.* PrkC-mediated phosphorylation of overexpressed YvcK protein regulates PBP1 protein localization in *Bacillus subtilis* mreB mutant cells. *J Biol Chem* 2014;**289**:23662–9.

Freitas C, Plannic J, Isticato R *et al.* A protein phosphorylation module patterns the *Bacillus subtilis* spore outer coat. *Mol Microbiol* 2020;**114**:934–51.

Fry B, Zhu T, Domach MM *et al.* Characterization of growth and acid formation in a *Bacillus subtilis* pyruvate kinase mutant. *Appl Environ Microb* 2000;**66**:4045–9.

Fuhrmann J, Mierzwa B, Trentini DB *et al.* Structural basis for recognizing phosphoarginine and evolving residue-specific protein phosphatases in Gram-positive bacteria. *Cell Rep* 2013;**3**:1832–9.

Fuhrmann J, Schmidt A, Spiess S *et al.* McsB is a protein arginine kinase that phosphorylates and inhibits the heat-shock regulator CtsR. *Science* 2009;**324**:1323–7.

Fuhrmann J, Subramanian V, Kojetin DJ *et al.* Activity-based profiling reveals a regulatory link between oxidative stress and protein arginine phosphorylation. *Cell Chem Biol* 2016;**23**:967–77.

Fujita M, Losick R. Evidence that entry into sporulation in *Bacillus subtilis* is governed by a gradual increase in the level and activity of the master regulator Spo0A. *Genes Dev* 2005;**19**:2236–44.

Fujita M, Losick R. The master regulator for entry into sporulation in *Bacillus subtilis* becomes a cell-specific transcription factor after asymmetric division. *Genes Dev* 2003;**17**:1166–74.

Gaidenko TA, Kim TJ, Price CW. The PrpC serine-threonine phosphatase and PrkC kinase have opposing physiological roles in stationary-phase *Bacillus subtilis* cells. *J Bacteriol* 2002;**184**:6109–14.

Gangwal A, Sangwan N, Dhasmana N *et al.* Role of serine/threonine protein phosphatase PrpN in the life cycle of *Bacillus anthracis*. *PLoS Pathog* 2022;**18**:e1010729.

Garcia Garcia T, Ventroux M, Derouiche A *et al.* Phosphorylation of the *Bacillus subtilis* replication controller YabA plays a role in

regulation of sporulation and biofilm formation. *Front Microbiol* 2018;**9**:486.
Garcia PS, Simorre JP, Brochier-Armanet C *et al.* Cell division of *Streptococcus pneumoniae*: think positive!. *Curr Opin Microbiol* 2016;**34**:18–23.
Garcia-Garcia T, Poncet S, Derouiche A *et al.* Role of protein phosphorylation in the regulation of cell cycle and DNA-related processes in bacteria. *Front Microbiol* 2016;**7**:184.
Geng H, Zhu Y, Mullen K *et al.* Characterization of ResDE-dependent fnr transcription in *Bacillus subtilis*. *J Bacteriol* 2007;**189**:1745–55.
Gerwig J, Kiley TB, Gunka K *et al.* The protein tyrosine kinases EpsB and PtkA differentially affect biofilm formation in *Bacillus subtilis*. *Microbiology* 2014;**160**:682–91.
Getz LJ, Runte CS, Rainey JK& *et al.* Tyrosine phosphorylation as a widespread regulatory mechanism in prokaryotes. *J Bacteriol* 2019;**201**:e00205–19.
Gohar M, Faegri K, Perchat S *et al.* The PlcR virulence regulon of *Bacillus cereus*. *PLoS ONE* 2008;**3**:e2793.
Gonzalez-Sanchez MB, Lanucara F, Helm M *et al.* Attempting to rewrite history: challenges with the analysis of histidine-phosphorylated peptides. *Biochem Soc Trans* 2013;**41**:1089–95.
Gopalani M, Dhiman A, Rahi A *et al.* Identification, functional characterization and regulon prediction of a novel two component system comprising BAS0540-BAS0541 of *Bacillus anthracis*. *PLoS ONE* 2016;**11**:e0158895.
Gorke B, Foulquier E, Galinier A. YvcK of *Bacillus subtilis* is required for a normal cell shape and for growth on Krebs cycle intermediates and substrates of the pentose phosphate pathway. *Microbiology* 2005;**151**:3777–91.
Gourse RL, Chen AY, Gopalkrishnan S *et al.* Transcriptional responses to ppGpp and DksA. *Annu Rev Microbiol* 2018;**72**:163–84.
Graham-Smith GS. The longevity of dry spores of *B. anthracis*. *J Hyg* 1930;**30**:213–5.
Grangeasse C, Nessler S, Mijakovic I. Bacterial tyrosine kinases: evolution, biological function and structural insights. *Phil Trans R Soc B* 2012;**367**:2640–55.
Groisman EA. Feedback control of two-component regulatory systems. *Annu Rev Microbiol* 2016;**70**:103–24.
Gupta V, Chaudhary N, Aggarwal S *et al.* Functional analysis of BAS2108-2109 two component system: evidence for protease regulation in *Bacillus anthracis*. *Int J Biochem Cell Biol* 2017;**89**:71–84.
Gupta V, Jain K, Garg R *et al.* Characterization of a two component system, Bas1213-1214, important for oxidative stress in *Bacillus anthracis*. *J Cell Biochem* 2018;**119**:5761–74.
Guttenplan SB, Kearns DB. Regulation of flagellar motility during biofilm formation. *FEMS Microbiol Rev* 2013;**37**:849–71.
Hajdusits B, Suskiewicz MJ, Hundt N *et al.* McsB forms a gated kinase chamber to mark aberrant bacterial proteins for degradation. *eLife* 2021;**10**:e63505.
Haldenwang WG, Losick R. A modified RNA polymerase transcribes a cloned gene under sporulation control in *Bacillus subtilis*. *Nature* 1979;**282**:256–60.
Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol Rev* 2017;**41**:276–301.
Hamoen LW, Venema G, Kuipers OP. Controlling competence in *Bacillus subtilis*: shared use of regulators. *Microbiology* 2003;**149**:9–17.
Hamon MA, Lazazzera BA. The sporulation transcription factor Spo0A is required for biofilm development in *Bacillus subtilis*. *Mol Microbiol* 2001;**42**:1199–209.
Hardt P, Engels I, Rausch M *et al.* The cell wall precursor lipid II acts as a molecular signal for the ser/thr kinase PknB of *Staphylococcus aureus*. *Int J Med Microbiol* 2017;**307**:1–10.
Harvey KL, Jarocki VM, Charles IG *et al.* The diverse functional roles of elongation factor tu (EF-Tu) in microbial pathogenesis. *Front Microbiol* 2019;**10**:2351.
Harwood CR, Mouillon JM, Pohl S *et al.* Secondary metabolite production and the safety of industrially important members of the *Bacillus subtilis* group. *FEMS Microbiol Rev* 2018;**42**:721–38.
Hecker M, Pane-Farre J, Völker U. SigB-dependent general stress response in *Bacillus subtilis* and related Gram-positive bacteria. *Annu Rev Microbiol* 2007;**61**:215–36.
Hecker M, Völker U. Non-specific, general and multiple stress resistance of growth-restricted *Bacillus subtilis* cells by the expression of the sigmaB regulon. *Mol Microbiol* 1998;**29**:1129–36.
Higgins D, Dworkin J. Recent progress in *Bacillus subtilis* sporulation. *FEMS Microbiol Rev* 2012;**36**:131–48.
Hobley L, Harkins C, MacPhee CE& *et al.* Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. *FEMS Microbiol Rev* 2015;**39**:649–69.
Hoch JA. A life in *Bacillus subtilis* signal transduction. *Annu Rev Microbiol* 2017;**71**:1–19.
Hoch JA. Two-component and phosphorelay signal transduction. *Curr Opin Microbiol* 2000;**3**:165–70.
Hoiby N, Bjarnsholt T, Givskov M *et al.* Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* 2010;**35**:322–32.
Houry A, Briandet R, Aymerich S *et al.* Involvement of motility and flagella in *Bacillus cereus* biofilm formation. *Microbiology* 2010;**156**:1009–18.
Howell A, Dubrac S, Andersen KK *et al.* Genes controlled by the essential YycG/YycF two-component system of *Bacillus subtilis* revealed through a novel hybrid regulator approach. *Mol Microbiol* 2003;**49**:1639–55.
Huang B, Zhao Z, Zhao Y *et al.* Protein arginine phosphorylation in organisms. *Int J Biol Macromol* 2021;**171**:414–22.
Huang Y, Flint SH&, Palmer JS. *Bacillus cereus* spores and toxins – the potential role of biofilms. *Food Microbiol* 2020;**90**:103493.
Isticato R, Lanzilli M, Petrillo C *et al.* *Bacillus subtilis* builds structurally and functionally different spores in response to the temperature of growth. *Environ Microbiol* 2020;**22**:170–82.
Iwanicki A, Herman-Antosiewicz A, Pierechod M *et al.* PrpE, a PPP protein phosphatase from *Bacillus subtilis* with unusual substrate specificity. *Biochem J* 2002;**366**:929–36.
Jakobsen OM, Brautaset T, Degnes KF *et al.* Overexpression of wild-type aspartokinase increases L-lysine production in the thermotolerant methylotrophic bacterium *Bacillus methanolicus*. *Appl Environ Microb* 2009;**75**:652–61.
Jers C, Kobir A, Sondergaard EO *et al.* *Bacillus subtilis* two-component system sensory kinase DegS is regulated by serine phosphorylation in its input domain. *PLoS ONE* 2011;**6**:e14653.
Jers C, Pedersen MM, Paspaliari DK *et al.* *Bacillus subtilis* BY-kinase PtkA controls enzyme activity and localization of its protein substrates. *Mol Microbiol* 2010;**77**:287–99.
Jezewska-Frackowiak J, Seroczynska K, Banaszczyk J *et al.* The promises and risks of probiotic *Bacillus* species. *Acta Biochim Pol* 2018;**65**:509–19.
Jiang M, Shao W, Perego M *et al.* Multiple histidine kinases regulate entry into stationary phase and sporulation in *Bacillus subtilis*. *Mol Microbiol* 2000;**38**:535–42.
Jiang M, Tzeng YL, Feher VA *et al.* Alanine mutants of the Spo0F response regulator modifying specificity for sensor kinases in sporulation initiation. *Mol Microbiol* 1999;**33**:389–95.
Jones LJ, Carballido-Lopez R, Errington J. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* 2001;**104**:913–22.

Joon S, Gopalani M, Rahi A *et al.* Biochemical characterization of the GTP-sensing protein, CodY of *Bacillus anthracis*. *Pathog Dis* 2017;**75**:28472295.

Jordan S, Junker A, Helmann JD *et al.* Regulation of LiaRS-dependent gene expression in *Bacillus subtilis*: identification of inhibitor proteins, regulator binding sites, and target genes of a conserved cell envelope stress-sensing two-component system. *J Bacteriol* 2006;**188**:5153–66.

Jordan S, Rietkotter E, Strauch MA *et al.* LiaRS-dependent gene expression is embedded in transition state regulation in *Bacillus subtilis*. *Microbiology* 2007;**153**:2530–40.

Joseph P, Ratnayake-Lecamwasam M, Sonenshein AL. A region of *Bacillus subtilis* CodY protein required for interaction with DNA. *J Bacteriol* 2005;**187**:4127–39.

Jouvion G, Corre JP, Khun H *et al.* Physical sequestration of *Bacillus anthracis* in the pulmonary capillaries in terminal infection. *J Infect Dis* 2016;**214**:281–7.

Kalantari A, Derouiche A, Shi L *et al.* Serine/threonine/tyrosine phosphorylation regulates DNA binding of bacterial transcriptional regulators. *Microbiology* 2015;**161**:1720–9.

Karst JC, Foucher AE, Campbell TL *et al.* The atpase activity of an 'essential' *Bacillus subtilis* enzyme, YdiB, is required for its cellular function and is modulated by oligomerization. *Microbiology* 2009;**155**:944–56.

Kaur P, Rausch M, Malakar B *et al.* LipidII interaction with specific residues of *Mycobacterium tuberculosis* PknB extracytoplasmic domain governs its optimal activation. *Nat Commun* 2019;**10**:1231.

Kawai Y, Daniel RA, Errington J. Regulation of cell wall morphogenesis in *Bacillus subtilis* by recruitment of PBP1 to the MreB helix. *Mol Microbiol* 2009;**71**:1131–44.

Keijser BJ, Ter Beek A, Rauwerda H *et al.* Analysis of temporal gene expression during *Bacillus subtilis* spore germination and outgrowth. *J Bacteriol* 2007;**189**:3624–34.

Kennelly PJ. Protein kinases and protein phosphatases in prokaryotes: a genomic perspective. *FEMS Microbiol Lett* 2002;**206**:1–8.

Kesel S, Mader A, Hofler C *et al.* Immediate and heterogeneous response of the LiaFSR two-component system of *Bacillus subtilis* to the peptide antibiotic bacitracin. *PLoS ONE* 2013;**8**:e53457.

Khan MZ, Kaur P, Nandicoori VK. Targeting the messengers: serine/threonine protein kinases as potential targets for antimycobacterial drug development. *IUBMB Life* 2018;**70**:889–904.

Khan SR, Banerjee-Bhatnagar N. Loss of catabolite repression function of hpr, the phosphocarrier protein of the bacterial phosphotransferase system, affects expression of the cry4A toxin gene in *Bacillus thuringiensis* subsp. *Israelensis*. *J Bacteriol* 2002;**184**:5410–7.

Khanna K, Lopez-Garrido J, Pogliano K. Shaping an endospore: architectural transformations during *Bacillus subtilis* sporulation. *Annu Rev Microbiol* 2020;**74**:361–86.

Kiley TB, Stanley-Wall NR. Post-translational control of *Bacillus subtilis* biofilm formation mediated by tyrosine phosphorylation. *Mol Microbiol* 2010;**78**:947–63.

Kim TJ, Gaidenko TA, Price CW. A multicomponent protein complex mediates environmental stress signaling in *Bacillus subtilis*. *J Mol Biol* 2004a;**341**:135–50.

Kim TJ, Gaidenko TA, Price CW. In vivo phosphorylation of partner switching regulators correlates with stress transmission in the environmental signaling pathway of *Bacillus subtilis*. *J Bacteriol* 2004b;**186**:6124–32.

Kirstein J, Dougan DA, Gerth U *et al.* The tyrosine kinase McsB is a regulated adaptor protein for ClpCP. *EMBO J* 2007;**26**:2061–70.

Kirstein J, Zuhlke D, Gerth U *et al.* A tyrosine kinase and its activator control the activity of the CtsR heat shock repressor in *B. subtilis*. *EMBO J* 2005;**24**:3435–45.

Kobayashi K, Ikemoto Y. Biofilm-associated toxin and extracellular protease cooperatively suppress competitors in *Bacillus subtilis* biofilms. *PLoS Genet* 2019;**15**:e1008232.

Kobayashi K. Gradual activation of the response regulator DegU controls serial expression of genes for flagellum formation and biofilm formation in *Bacillus subtilis*. *Mol Microbiol* 2007;**66**:395–409.

Kobir A, Poncet S, Bidnenko V *et al.* Phosphorylation of *Bacillus subtilis* gene regulator AbrB modulates its DNA-binding properties. *Mol Microbiol* 2014;**92**:1129–41.

Kobir A, Shi L, Boskovic A *et al.* Protein phosphorylation in bacterial signal transduction. *Biochim Biophys Gen Sub* 2011;**1810**:989–94.

Koh A, Gibbon MJ, Van der Kamp MW *et al.* Conformation control of the histidine kinase BceS of *Bacillus subtilis* by its cognate ABC-transporter facilitates need-based activation of antibiotic resistance. *Mol Microbiol* 2021;**115**:157–74.

Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb Perspect Med* 2013;**3**:a010306.

Krab IM, Parmeggiani A. Mechanisms of EF-Tu, a pioneer GTPase. *Prog Nucleic Acid Res Mol Biol* 2002;**71**:513–51.

Kroos L, Zhang B, Ichikawa H *et al.* Control of sigma factor activity during *Bacillus subtilis* sporulation. *Mol Microbiol* 1999;**31**:1285–94.

Landmann JJ, Werner S, Hillen W *et al.* Carbon source control of the phosphorylation state of the *Bacillus subtilis* carbon-flux regulator Crh in vivo. *FEMS Microbiol Lett* 2012;**327**:47–53.

Laut CLPW, Metzger AL, Weiss A *et al.* *Bacillus anthracis* responds to Targocil-induced envelope damage through EdsRS activation of Cardiolipin synthesis. *mBio* 2020;**11**:e03375–19.

LeDeaux JR, Grossman AD. Isolation and characterization of kinC, a gene that encodes a sensor kinase homologous to the sporulation sensor kinases KinA and KinB in *Bacillus subtilis*. *J Bacteriol* 1995;**177**:166–75.

LeDeaux JR, Yu N, Grossman AD. Different roles for KinA, KinB, and KinC in the initiation of sporulation in *Bacillus subtilis*. *J Bacteriol* 1995;**177**:861–3.

Lee JJ, Kan CM, Lee JH *et al.* Phosphorylation-dependent interaction between a serine/threonine kinase PknA and a putative cell division protein Wag31 in *Mycobacterium tuberculosis*. *New Microbiol* 2014;**37**:525–33.

Levine A, Vannier F, Absalon C *et al.* Analysis of the dynamic *Bacillus subtilis* Ser/Thr/Tyr phosphoproteome implicated in a wide variety of cellular processes. *Proteomics* 2006;**6**:2157–73.

Li Y, Chen N, Wu Q *et al.* A flagella hook coding gene flgE positively affects biofilm formation and cereulide production in emetic *Bacillus cereus*. *Front Microbiol* 2022;**13**:897836.

Liaqat I, Mirza SA, Iqbal R *et al.* Flagellar motility plays important role in biofilm formation of *Bacillus cereus* and *Yersinia enterocolitica*. *Pak J Pharm Sci* 2018;**31**:2047–52.

Libby EA, Goss LA, Dworkin J. The eukaryotic-like ser/thr kinase PrkC regulates the essential WalRK two-component system in *Bacillus subtilis*. *PLoS Genet* 2015;**11**:e1005275.

Libby EA, Reuveni S, Dworkin J. Multisite phosphorylation drives phenotypic variation in (p)ppGpp synthetase-dependent antibiotic tolerance. *Nat Commun* 2019;**10**:5133.

Lilge L, Reder A, Tippmann F *et al.* The involvement of the McsB arginine kinase in clp-dependent degradation of the MgsR regulator in *Bacillus subtilis*. *Front Microbiol* 2020;**11**:900.

Lima A, Duran R, Schujman GE *et al.* Serine/threonine protein kinase PrkA of the human pathogen *Listeria monocytogenes*: biochemical characterization and identification of interacting partners through proteomic approaches. *J Proteomics* 2011;**74**:1720–34.

Lin WJ, Walthers D, Connelly JE *et al.* Threonine phosphorylation prevents promoter DNA binding of the Group B *Streptococcus* response regulator CovR. *Mol Microbiol* 2009;**71**:1477–95.

Lindner C, Nijland R, van Hartskamp M *et al.* Differential expression of two paralogous genes of *Bacillus subtilis* encoding single-stranded DNA binding protein. *J Bacteriol* 2004;**186**:1097–105.

Liu X, Fields R, Schweppe DK *et al.* Strategies for mass spectrometry-based phosphoproteomics using isobaric tagging. *Expert Rev Proteomics* 2021;**18**:795–807.

Liu YC, Han LL, Chen TY *et al.* Characterization of a protease hyperproductive mutant of *Bacillus pumilus* by comparative genomic and transcriptomic analysis. *Curr Microbiol* 2020;**77**:3612–22.

Lopez D, Fischbach MA, Chu F *et al.* Structurally diverse natural products that cause potassium leakage trigger multicellularity in *Bacillus subtilis*. *Proc Natl Acad Sci USA* 2009;**106**:280–5.

Lorca GL, Chung YJ, Barabote RD *et al.* Catabolite repression and activation in *Bacillus subtilis*: dependency on CcpA, hpr, and HprK. *J Bacteriol* 2005;**187**:7826–39.

McCall RM, Sievers ME, Fattah R *et al. Bacillus anthracis* virulence regulator AtxA binds specifically to the pagA promoter region. *J Bacteriol* 2019;**201**:e00569–19.

McPherson SA, Li M, Kearney JF *et al.* ExsB, an unusually highly phosphorylated protein required for the stable attachment of the exosporium of *Bacillus anthracis*. *Mol Microbiol* 2010;**76**:1527–38.

Macek B, Forchhammer K, Hardouin J *et al.* Protein post-translational modifications in bacteria. *Nat Rev Micro* 2019;**17**:651–64.

Macek B, Mijakovic I, Olsen JV *et al.* The serine/threonine/tyrosine phosphoproteome of the model bacterium *Bacillus subtilis*. *Mol Cell Proteomics* 2007;**6**:697–707.

Madec E, Laszkiewicz A, Iwanicki A *et al.* Characterization of a membrane-linked ser/thr protein kinase in *Bacillus subtilis*, implicated in developmental processes. *Mol Microbiol* 2002;**46**:571–86.

Maier B. Competence and transformation in *Bacillus subtilis*. *Curr Iss Mol Biol* 2020;**37**:57–76.

Manuse S, Fleurie A, Zucchini L *et al.* Role of eukaryotic-like serine/threonine kinases in bacterial cell division and morphogenesis. *FEMS Microbiol Rev* 2016;**40**:41–56.

Maracci C, Rodnina MV. Review: translational GTPases. *Biopolymers* 2016;**105**:463–75.

Marles-Wright J&, Lewis RJ. Stress responses of bacteria. *Curr Opin Struct Biol* 2007;**17**:755–60.

Mascher T, Zimmer SL, Smith TA *et al.* Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*. *Antimicrob Agents Chemother* 2004;**48**:2888–96.

Mattoo AR, Arora A, Maiti S *et al.* Identification, characterization and activation mechanism of a tyrosine kinase of *Bacillus anthracis*. *FEBS J* 2008;**275**:6237–47.

Maurya GK, Modi K, Banerjee M *et al.* Phosphorylation of FtsZ and FtsA by a DNA damage-responsive Ser/Thr protein kinase affects their functional interactions in *Deinococcus radiodurans*. *mSphere* 2018;**3**:e00325–18.

Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci* 2005;**62**:670–84.

Mielich-Suss B, Lopez D. Molecular mechanisms involved in *Bacillus subtilis* biofilm formation. *Environ Microbiol* 2015;**17**:555–65.

Mijakovic I, Deutscher J. Protein-tyrosine phosphorylation in *Bacillus subtilis*: a 10-year retrospective. *Front Microbiol* 2015;**6**:18.

Mijakovic I, Grangeasse C, Turgay K. Exploring the diversity of protein modifications: special bacterial phosphorylation systems. *FEMS Microbiol Rev* 2016;**40**:398–417.

Mijakovic I, Musumeci L, Tautz L *et al.* In vitro characterization of the *Bacillus subtilis* protein tyrosine phosphatase YwqE. *J Bacteriol* 2005;**187**:3384–90.

Mijakovic I, Petranovic D, Deutscher J. How tyrosine phosphorylation affects the UDP-glucose dehydrogenase activity of *Bacillus subtilis* YwqF. *J Mol Microbiol Biotechnol* 2004;**8**:19–25.

Mijakovic I, Petranovic D, Macek B *et al.* Bacterial single-stranded DNA-binding proteins are phosphorylated on tyrosine. *Nucleic Acids Res* 2006;**34**:1588–96.

Mijakovic I, Poncet S, Boel G *et al.* Transmembrane modulator-dependent bacterial tyrosine kinase activates UDP-glucose dehydrogenases. *EMBO J* 2003;**22**:4709–18.

Mike LA, Choby JE, Brinkman PR *et al.* Two-component system cross-regulation integrates *Bacillus anthracis* response to heme and cell envelope stress. *PLoS Pathog* 2014;**10**:e1004044.

Miller ML, Soufi B, Jers C *et al.* NetPhosBac – a predictor for Ser/Thr phosphorylation sites in bacterial proteins. *Proteomics* 2009;**9**:116–25.

Million-Weaver S, Samadpour AN, Merrikh H. Replication restart after replication-transcription conflicts requires RecA in *Bacillus subtilis*. *J Bacteriol* 2015;**197**:2374–82.

Min KT, Hilditch CM, Diederich B *et al.* 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* 1993;**74**:735–42.

Misra R, Menon D, Arora G *et al.* Tuning the *Mycobacterium tuberculosis* alternative sigma factor SigF through the multidomain regulator Rv1364c and osmosensory kinase protein kinase D. *J Bacteriol* 2019;**201**:e00725–18.

Moayeri M, Leppla SH, Vrentas C *et al.* Anthrax pathogenesis. *Annu Rev Microbiol* 2015;**69**:185–208.

Molle V, Fujita M, Jensen ST *et al.* The Spo0A regulon of *Bacillus subtilis*. *Mol Microbiol* 2003;**50**:1683–701.

Moller J, Luehmann T, Hall H& *et al.* The race to the pole: how high-aspect ratio shape and heterogeneous environments limit phagocytosis of filamentous *Escherichia coli* bacteria by macrophages. *Nano Lett* 2012;**12**:2901–5.

Moszer I. The complete genome of *Bacillus subtilis*: from sequence annotation to data management and analysis. *FEBS Lett* 1998;**430**:28–36.

Mukai K, Kawata M, Tanaka T. Isolation and phosphorylation of the *Bacillus subtilis* degS and degU gene products. *J Biol Chem* 1990;**265**:20000–6.

Murray EJ, Kiley TB, Stanley-Wall NR. A pivotal role for the response regulator DegU in controlling multicellular behaviour. *Microbiology* 2009;**155**:1–8.

Musumeci L, Bongiorni C, Tautz L *et al.* Low-molecular-weight protein tyrosine phosphatases of *Bacillus subtilis*. *J Bacteriol* 2005;**187**:4945–56.

Myers CL, Li FK, Koo BM *et al.* Identification of two phosphate starvation-induced wall teichoic acid hydrolases provides first insights into the degradative pathway of a key bacterial cell wall component. *J Biol Chem* 2016;**291**:26066–82.

Naclerio G, Baccigalupi L, Zilhao R *et al. Bacillus subtilis* spore coat assembly requires cotH gene expression. *J Bacteriol* 1996;**178**:4375–80.

Naerdal I, Netzer R, Ellingsen TE *et al.* Analysis and manipulation of aspartate pathway genes for L-lysine overproduction from methanol by *Bacillus methanolicus*. *Appl Environ Microb* 2011;**77**:6020–6.

Nahrstedt H, Schroder C, Meinhardt F. Evidence for two recA genes mediating DNA repair in *Bacillus megaterium*. *Microbiology* 2005;**151**:775–87.

Nanamiya H, Kasai K, Nozawa A *et al.* Identification and functional analysis of novel (p)ppGpp synthetase genes in *Bacillus subtilis*. *Mol Microbiol* 2008;**67**:291–304.
Nguyen HA, El Khoury T, Guiral S *et al.* Expanding the kinome world: a new protein kinase Family widely conserved in bacteria. *J Mol Biol* 2017;**429**:3056–74.
Nguyen KB, Sreelatha A, Durrant ES *et al.* Phosphorylation of spore coat proteins by a family of atypical protein kinases. *Proc Natl Acad Sci USA* 2016;**113**:E3482–3491.
Nicholson WL, Munakata N, Horneck G *et al.* Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol Mol Biol Rev* 2000;**64**:548–72.
Nicholson WL. Roles of *Bacillus* endospores in the environment. *Cell Mol Life Sci* 2002;**59**:410–6.
Noirot-Gros MF, Velten M, Yoshimura M *et al.* Functional dissection of YabA, a negative regulator of DNA replication initiation in *Bacillus subtilis*. *Proc Natl Acad Sci USA* 2006;**103**:2368–73.
Noone D, Botella E, Butler C *et al.* Signal perception by the secretion stress-responsive CssRS two-component system in *Bacillus subtilis*. *J Bacteriol* 2012;**194**:1800–14.
O'Connell LM, Kelleher P, van Rijswijck IMH *et al.* Natural transformation in Gram-positive bacteria and its biotechnological relevance to lactic acid bacteria. *Annu Rev Food Sci Technol* 2022;**13**:409–31.
Obuchowski M, Madec E, Delattre D *et al.* Characterization of PrpC from *Bacillus subtilis*, a member of the PPM phosphatase family. *J Bacteriol* 2000;**182**:5634–8.
Ogura M, Matsuzawa A, Yoshikawa H *et al.* *Bacillus subtilis* SalA (YbaL) negatively regulates expression of scoC, which encodes the repressor for the alkaline exoprotease gene, aprE. *J Bacteriol* 2004;**186**:3056–64.
Ogura M. Glucose-mediated protein arginine phosphorylation/dephosphorylation regulates ylxR encoding nucleoid-associated protein and cell growth in *Bacillus subtilis*. *Front Microbiol* 2020;**11**:590828.
Ohki R, Giyanto TK, Masuyama W *et al.* The BceRS two-component regulatory system induces expression of the bacitracin transporter, BceAB, in *Bacillus subtilis*. *Mol Microbiol* 2003;**49**:1135–44.
Ohlsen KL, Grimsley JK, Hoch JA. Deactivation of the sporulation transcription factor Spo0A by the Spo0E protein phosphatase. *Proc Natl Acad Sci USA* 1994;**91**:1756–60.
Ojha A, Anand M, Bhatt A *et al.* GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. *Cell* 2005;**123**:861–73.
Pan Z, Wang B, Zhang Y *et al.* dbPSP: a curated database for protein phosphorylation sites in prokaryotes. *Database* 2015;**2015**:bav031.
Parte AC. LPSN – list of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. *Int J Syst Evol Microbiol* 2018;**68**:1825–9.
Paschalis V, Le Chatelier E, Green M *et al.* Interactions of the *Bacillus subtilis* DnaE polymerase with replisomal proteins modulate its activity and fidelity. *Open Biol* 2017;**7**:170146.
Paulo JA, Schweppe DK. Advances in quantitative high-throughput phosphoproteomics with sample multiplexing. *Proteomics* 2021;**21**:2000140.
Peake P, Winter N, Britton W. Phosphorylation of *Mycobacterium leprae* heat-shock 70 protein at threonine 175 alters its substrate binding characteristics. *Biochim Biophy Acta Prot Struct Mol Enzymol* 1998;**1387**:387–94.
Peng Q, Wu J, Chen X *et al.* Disruption of two-component system LytSR affects forespore engulfment in *Bacillus thuringiensis*. *Front Cell Infect Microbiol* 2017;**7**:468.
Perchat S, Dubois T, Zouhir S *et al.* A cell-cell communication system regulates protease production during sporulation in bacteria of the *Bacillus cereus* group. *Mol Microbiol* 2011;**82**:619–33.
Perego M, Cole SP, Burbulys D *et al.* Characterization of the gene for a protein kinase which phosphorylates the sporulation-regulatory proteins Spo0A and Spo0F of *Bacillus subtilis*. *J Bacteriol* 1989;**171**:6187–96.
Perego M, Hanstein C, Welsh KM *et al.* Multiple protein-aspartate phosphatases provide a mechanism for the integration of diverse signals in the control of development in *B. subtilis*. *Cell* 1994;**79**:1047–55.
Perego M. A new family of aspartyl phosphate phosphatases targeting the sporulation transcription factor Spo0A of *Bacillus subtilis*. *Mol Microbiol* 2001;**42**:133–43.
Pereira SF, Goss L, Dworkin J. Eukaryote-like serine/threonine kinases and phosphatases in bacteria. *Microbiol Mol Biol Rev* 2011;**75**:192–212.
Pereira SFF, Gonzalez RL, Dworkin J *et al.* Protein synthesis during cellular quiescence is inhibited by phosphorylation of a translational elongation factor. *Proc Natl Acad Sci USA* 2015;**112**:E3274–3281.
Petranovic D, Grangeasse C, Macek B *et al.* Activation of *Bacillus subtilis* ugd by the BY-kinase PtkA proceeds via phosphorylation of its residue tyrosine 70. *J Mol Microbiol Biotechnol* 2009;**17**:83–89.
Petranovic D, Michelsen O, Zahradka K *et al.* *Bacillus subtilis* strain deficient for the protein-tyrosine kinase PtkA exhibits impaired DNA replication. *Mol Microbiol* 2007;**63**:1797–805.
Pi H, Chu ML, Ivan SJ *et al.* Directed evolution reveals the mechanism of HitRS signaling transduction in *Bacillus anthracis*. *PLoS Pathog* 2020;**16**:e1009148.
Pietack N, Becher D, Schmidl SR *et al.* In vitro phosphorylation of key metabolic enzymes from *Bacillus subtilis*: prkC phosphorylates enzymes from different branches of basic metabolism. *J Mol Microbiol Biotechnol* 2010;**18**:129–40.
Piggot PJ, Hilbert DW. Sporulation of *Bacillus subtilis*. *Curr Opin Microbiol* 2004;**7**:579–86.
Pina AS, Batalha IL, Dias A *et al.* Affinity tags in protein purification and peptide enrichment: an overview. *Methods Mol Biol* 2021;**2178**:107–32.
Pinas GE, Reinoso-Vizcaino NM, Yandar Barahona NY *et al.* Crosstalk between the serine/threonine kinase StkP and the response regulator ComE controls the stress response and intracellular survival of *Streptococcus pneumoniae*. *PLoS Pathog* 2018;**14**:e1007118.
Pompeo F, Byrne D, Mengin-Lecreulx D *et al.* Dual regulation of activity and intracellular localization of the PASTA kinase PrkC during *Bacillus subtilis* growth. *Sci Rep* 2018b;**8**:1660.
Pompeo F, Foulquier E, Galinier A. Impact of serine/threonine protein kinases on the regulation of sporulation in *Bacillus subtilis*. *Front Microbiol* 2016;**7**:568.
Pompeo F, Freton C, Wicker-Planquart C *et al.* Phosphorylation of CpgA protein enhances both its GTPase activity and its affinity for ribosome and is crucial for *Bacillus subtilis* growth and morphology. *J Biol Chem* 2012;**287**:20830–8.
Pompeo F, Rismondo J, Grundling A *et al.* Investigation of the phosphorylation of *Bacillus subtilis* LTA synthases by the serine/threonine kinase PrkC. *Sci Rep* 2018a;**8**:17344.
Poncet S, Mijakovic I, Nessler S *et al.* HPr kinase/phosphorylase, a Walker motif A-containing bifunctional sensor enzyme controlling catabolite repression in Gram-positive bacteria. *Biochim Biophys Acta Proteins Proteomics* 2004;**1697**:123–35.
Poncet S, Milohanic E, Maze A *et al.* Correlations between carbon metabolism and virulence in bacteria. *Contrib Microbiol* 2009;**16**:88–102.

Potrykus K, Cashel M. (p)ppGpp: still magical?. *Annu Rev Microbiol* 2008;**62**:35–51.
Pragai Z, Allenby NE, O'Connor N *et al.* Transcriptional regulation of the phoPR operon in *Bacillus subtilis*. *J Bacteriol* 2004;**186**:1182–90.
Prashar A, Bhatia S, Gigliozzi D *et al.* Filamentous morphology of bacteria delays the timing of phagosome morphogenesis in macrophages. *J Cell Biol* 2013;**203**:1081–97.
Price CW, Fawcett P, Ceremonie H *et al.* Genome-wide analysis of the general stress response in *Bacillus subtilis*. *Mol Microbiol* 2001;**41**:757–74.
Rajagopalan K, Dworkin J, Nagle E. Identification and biochemical characterization of a novel protein phosphatase 2C-like Ser/Thr phosphatase in *Escherichia coli*. *J Bacteriol* 2018;**200**:e00225–18.
Rajpurohit YS, Sharma DK, Misra HS. Involvement of serine /threonine protein kinases in DNA damage response and cell division in bacteria. *Res Microbiol* 2022;**173**:103883.
Ramirez-Guadiana FH, Barajas-Ornelas Rdel C, Corona-Bautista SU *et al.* The RecA-dependent SOS response is active and required for processing of DNA damage during *Bacillus subtilis* sporulation. *PLoS ONE* 2016;**11**:e0150348.
Ravikumar V, Nalpas NC, Anselm V *et al.* In-depth analysis of *Bacillus subtilis* proteome identifies new ORFs and traces the evolutionary history of modified proteins. *Sci Rep* 2018;**8**:17246.
Ravikumar V, Shi L, Krug K *et al.* Quantitative phosphoproteome analysis of *Bacillus subtilis* reveals novel substrates of the kinase PrkC and phosphatase PrpC. *Mol Cell Proteomics* 2014;**13**:1965–78.
Raynor MJ, Roh JH, Widen SG *et al.* Regulons and protein-protein interactions of PRD-containing *Bacillus anthracis* virulence regulators reveal overlapping but distinct functions. *Mol Microbiol* 2018;**109**:1–22.
Reizer J. Regulation of sugar uptake and efflux in Gram-positive bacteria. *FEMS Microbiol Rev* 1989;**5**:149–56.
Repizo GD, Blancato VS, Sender PD *et al.* Catabolite repression of the citST two-component system in *Bacillus subtilis*. *FEMS Microbiol Lett* 2006;**260**:224–31.
Richts B, Rosenberg J, Commichau FM. A survey of pyridoxal 5'-phosphate-dependent proteins in the Gram-positive model bacterium *Bacillus subtilis*. *Front Mol Biosci* 2019;**6**:32.
Rigo MM, Borges TJ, Lang BJ *et al.* Host expression system modulates recombinant Hsp70 activity through post-translational modifications. *FEBS J* 2020;**287**:4902–16.
Rodnina MV, Savelsbergh A, Katunin VI *et al.* Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. *Nature* 1997;**385**:37–41.
Rodriguez Ayala F, Bartolini M, Grau R. The stress-responsive alternative sigma factor SigB of *Bacillus subtilis* and its relatives: an old friend with new functions. *Front Microbiol* 2020;**11**:1761.
Rodriguez JL, Dalia AB, Weiser JN. Increased chain length promotes pneumococcal adherence and colonization. *Infect Immun* 2012;**80**:3454–9.
Roncarati D, Scarlato V. Regulation of heat-shock genes in bacteria: from signal sensing to gene expression output. *FEMS Microbiol Rev* 2017;**41**:549–74.
Rooney AP, Price NP, Ehrhardt C *et al.* Phylogeny and molecular taxonomy of the *Bacillus subtilis* species complex and description of *Bacillus subtilis* subsp. *Inaquosorum* subsp. nov. *Int J Syst Evol Microbiol* 2009;**59**:2429–36.
Rosenberg A, Soufi B, Ravikumar V *et al.* Phosphoproteome dynamics mediate revival of bacterial spores. *BMC Biol* 2015;**13**:76.
Sajid A, Arora G, Gupta M *et al.* Interaction of *Mycobacterium* tuberculosis elongation factor Tu with GTP is regulated by phosphorylation. *J Bacteriol* 2011a;**193**:5347–58.
Sajid A, Arora G, Gupta M *et al.* Phosphorylation of *Mycobacterium tuberculosis* Ser/Thr phosphatase by PknA and PknB. *PLoS ONE* 2011b;**6**:e17871.
Sajid A, Arora G, Singhal A *et al.* Protein phosphatases of pathogenic bacteria: role in physiology and virulence. *Annu Rev Microbiol* 2015;**69**:527–47.
Salzberg LI, Powell L, Hokamp K *et al.* The WalRK (YycFG) and sigma(I) RsgI regulators cooperate to control CwlO and LytE expression in exponentially growing and stressed *Bacillus subtilis* cells. *Mol Microbiol* 2013;**87**:180–95.
Satomura T, Shimura D, Asai K *et al.* Enhancement of glutamine utilization in *Bacillus subtilis* through the GlnK-GlnL two-component regulatory system. *J Bacteriol* 2005;**187**:4813–21.
Schenk K, Hervas AB, Rosch TC *et al.* Rapid turnover of DnaA at replication origin regions contributes to initiation control of DNA replication. *PLoS Genet* 2017;**13**:e1006561.
Schilling O, Frick O, Herzberg C *et al.* Transcriptional and metabolic responses of *Bacillus subtilis* to the availability of organic acids: transcription regulation is important but not sufficient to account for metabolic adaptation. *Appl Environ Microb* 2007;**73**:499–507.
Schmeing TM, Voorhees RM, Kelley AC *et al.* The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science* 2009;**326**:688–94.
Schmidt A, Trentini DB, Spiess S *et al.* Quantitative phosphoproteomics reveals the role of protein arginine phosphorylation in the bacterial stress response. *Mol Cell Proteomics* 2014;**13**:537–50.
Schmidt RM, Carter MM, Chu ML *et al.* Heme sensing in *Bacillus thuringiensis*: a supplementary HssRS-regulated heme resistance system. *FEMS Microbiol Lett* 2016;**363**:fnw076.
Scott JD, Newton AC. Bacterial spore coat protein kinases: a new twist to an old story. *Proc Natl Acad Sci USA* 2016;**113**:6811–2.
Seeger M, Osorio G, Jerez CA. Phosphorylation of GroEL, DnaK and other proteins from *Thiobacillus ferrooxidans* grown under different conditions. *FEMS Microbiol Lett* 1996;**138**:129–34.
Sella SR, Vandenberghe LP, Soccol CR. Life cycle and spore resistance of spore-forming *Bacillus atrophaeus*. *Microbiol Res* 2014;**169**:931–9.
Setlow P, Christie G. Bacterial spore mRNA – what's up with that?. *Front Microbiol* 2020;**11**:596092.
Shafikhani SH, Leighton T. AbrB and Spo0E control the proper timing of sporulation in *Bacillus subtilis*. *Curr Microbiol* 2004;**48**:262–9.
Shah IM, Dworkin J. Induction and regulation of a secreted peptidoglycan hydrolase by a membrane Ser/Thr kinase that detects muropeptides. *Mol Microbiol* 2010;**75**:1232–43.
Shah IM, Laaberki MH, Popham DL *et al.* A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* 2008;**135**:486–96.
Shakir SM, Bryant KM, Larabee JL *et al.* Regulatory interactions of a virulence-associated serine/threonine phosphatase-kinase pair in *Bacillus anthracis*. *J Bacteriol* 2010;**192**:400–9.
Sharma AK, Dhasmana N, Dubey N *et al.* Bacterial virulence factors: secreted for survival. *Ind J Microbiol* 2017;**57**:1–10.
Shemesh M, Chai Y. A combination of glycerol and manganese promotes biofilm formation in *Bacillus subtilis* via histidine kinase KinD signaling. *J Bacteriol* 2013;**195**:2747–54.
Shemesh M, Kolter R, Losick R. The biocide chlorine dioxide stimulates biofilm formation in *Bacillus subtilis* by activation of the histidine kinase KinC. *J Bacteriol* 2010;**192**:6352–6.
Shemesh M, Ostrov I. Role of *Bacillus* species in biofilm persistence and emerging antibiofilm strategies in the dairy industry. *J Sci Food Agric* 2020;**100**:2327–36.

Shen D, Perpich JD, Stocke KS *et al.* Role of the RprY response regulator in *P. gingivalis* community development and virulence. *Mol Oral Microbiol* 2020;**35**:231–9.

Sherman MY, Goldberg AL. Heat shock of *Escherichia coli* increases binding of dnaK (the hsp70 homolog) to polypeptides by promoting its phosphorylation. *Proc Natl Acad Sci USA* 1993;**90**:8648–52.

Shi L, Pigeonneau N, Ravikumar V *et al.* Cross-phosphorylation of bacterial serine/threonine and tyrosine protein kinases on key regulatory residues. *Front Microbiol* 2014a;**5**:495.

Shi L, Pigeonneau N, Ventroux M *et al.* Protein-tyrosine phosphorylation interaction network in *Bacillus subtilis* reveals new substrates, kinase activators and kinase cross-talk. *Front Microbiol* 2014b;**5**:538.

Shi L, Ravikumar V, Derouiche A *et al.* Tyrosine 601 of *Bacillus subtilis* DnaK undergoes phosphorylation and is crucial for chaperone activity and heat shock survival. *Front Microbiol* 2016;**7**:533.

Shi Y, Zhang Y, Lin S *et al.* dbPSP 2.0, an updated database of protein phosphorylation sites in prokaryotes. *Sci Data* 2020;**7**:164.

Sinai L, Rosenberg A, Smith Y *et al.* The molecular timeline of a reviving bacterial spore. *Mol Cell* 2015;**57**:695–707.

Singh KD, Halbedel S, Gorke B *et al.* Control of the phosphorylation state of the HPr protein of the phosphotransferase system in *Bacillus subtilis*: implication of the protein phosphatase PrpC. *J Mol Microbiol Biotechnol* 2007;**13**:165–71.

Singh KD, Schmalisch MH, Stülke J *et al.* Carbon catabolite repression in *Bacillus subtilis*: quantitative analysis of repression exerted by different carbon sources. *J Bacteriol* 2008;**190**:7275–84.

Singh LK, Dhasmana N, Sajid A *et al.* clpC operon regulates cell architecture and sporulation in *Bacillus anthracis*. *Environ Microbiol* 2015;**17**:855–65.

Singhal A, Virmani R, Naz S *et al.* Methylation of two-component response regulator MtrA in mycobacteria negatively modulates its DNA binding and transcriptional activation. *Biochem J* 2020;**477**:4473–89.

Soufi B, Kumar C, Gnad F *et al.* Stable isotope labeling by amino acids in cell culture (SILAC) applied to quantitative proteomics of *Bacillus subtilis*. *J Proteome Res* 2010;**9**:3638–46.

Soule MH. Identity of *Bacillus subtilis*, Cohn 1872. *J Infect Dis* 1932;**51**:191–215.

Squeglia F, Marchetti R, Ruggiero A *et al.* Chemical basis of peptidoglycan discrimination by PrkC, a key kinase involved in bacterial resuscitation from dormancy. *J Am Chem Soc* 2011;**133**:20676–9.

Stancik IA, Sestak MS, Ji B *et al.* Serine/threonine protein kinases from bacteria, Archaea and Eukarya share a common evolutionary origin deeply rooted in the tree of life. *J Mol Biol* 2018;**430**:27–32.

Stannek L, Gunka K, Care RA *et al.* Factors that mediate and prevent degradation of the inactive and unstable GudB protein in *Bacillus subtilis*. *Front Microbiol* 2014;**5**:758.

Stauff DL, Skaar EP. *Bacillus anthracis* HssRS signalling to HrtAB regulates haem resistance during infection. *Mol Microbiol* 2009;**72**:763–78.

Steinberg N, Keren-Paz A, Hou Q *et al.* The extracellular matrix protein TasA is a developmental cue that maintains a motile subpopulation within *Bacillus subtilis* biofilms. *Sci Signal* 2020;**13**:eaaw8905.

Steinchen W, Bange G. The magic dance of the alarmones (p)ppGpp. *Mol Microbiol* 2016;**101**:531–44.

Stephenson K, Hoch JA. Evolution of signalling in the sporulation phosphorelay. *Mol Microbiol* 2002;**46**:297–304.

Stewart GC. The exosporium layer of bacterial spores: a connection to the environment and the infected host. *Microbiol Mol Biol Rev* 2015;**79**:437–57.

Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. *Annu Rev Biochem* 2000;**69**:183–215.

Strauch M, Webb V, Spiegelman G *et al.* The SpoOA protein of *Bacillus subtilis* is a repressor of the abrB gene. *Proc Natl Acad Sci USA* 1990;**87**:1801–5.

Strauch MA, Ballar P, Rowshan AJ *et al.* The DNA-binding specificity of the *Bacillus anthracis* AbrB protein. *Microbiology* 2005;**151**:1751–9.

Stubbendieck RM, Straight PD. Linearmycins activate a two-component signaling system involved in bacterial competition and biofilm morphology. *J Bacteriol* 2017;**199**:e00186–17.

Su Y, Liu C, Fang H *et al.* *Bacillus subtilis*: a universal cell factory for industry, agriculture, biomaterials and medicine. *Microb Cell Fact* 2020;**19**:173.

Suskiewicz MJ, Hajdusits B, Beveridge R *et al.* Structure of McsB, a protein kinase for regulated arginine phosphorylation. *Nat Chem Biol* 2019;**15**:510–8.

Takada H, Shiwa Y, Takino Y *et al.* Essentiality of WalRK for growth in *Bacillus subtilis* and its role during heat stress. *Microbiology* 2018;**164**:670–84.

Talavera A, Hendrix J, Versees W *et al.* Phosphorylation decelerates conformational dynamics in bacterial translation elongation factors. *Sci Adv* 2018;**4**:eaap9714.

Tan IS, Ramamurthi KS. Spore formation in *Bacillus subtilis*. *Environ Microbiol Rep* 2014;**6**:212–25.

Tanaka K, Kobayashi K, Ogasawara N. The *Bacillus subtilis* YufLM two-component system regulates the expression of the malate transporters MaeN (YufR) and YflS, and is essential for utilization of malate in minimal medium. *Microbiology* 2003;**149**:2317–29.

Tao L, Chattoraj P, Biswas I. CtsR regulation in mcsAB-deficient Gram-positive bacteria. *J Bacteriol* 2012;**194**:1361–8.

Torres R, Serrano E&, Alonso JC. *Bacillus subtilis* RecA interacts with and loads RadA/Sms to unwind recombination intermediates during natural chromosomal transformation. *Nucleic Acids Res* 2019;**47**:9198–215.

Trentini DB, Fuhrmann J, Mechtler K *et al.* Chasing phosphoarginine proteins: development of a selective enrichment method using a phosphatase trap. *Mol Cell Proteomics* 2014;**13**:1953–64.

Trentini DB, Suskiewicz MJ, Heuck A *et al.* Arginine phosphorylation marks proteins for degradation by a Clp protease. *Nature* 2016;**539**:48–53.

Tsvetanova B, Wilson AC, Bongiorni C *et al.* Opposing effects of histidine phosphorylation regulate the AtxA virulence transcription factor in *Bacillus anthracis*. *Mol Microbiol* 2007;**63**:644–55.

Ultee E, Ramijan K, Dame RT *et al.* Stress-induced adaptive morphogenesis in bacteria. *Adv Microb Physiol* 2019;**74**:97–141.

van Beilen J, Blohmke CJ, Folkerts H *et al.* RodZ and PgsA play intertwined roles in membrane homeostasis of *Bacillus subtilis* and resistance to weak organic acid stress. *Front Microbiol* 2016;**7**:1633.

van den Esker MH, Kovacs AT, Kuipers OP. YsbA and LytST are essential for pyruvate utilization in *Bacillus subtilis*. *Environ Microbiol* 2017;**19**:83–94.

van Schaik W, Chateau A, Dillies MA *et al.* The global regulator CodY regulates toxin gene expression in *Bacillus anthracis* and is required for full virulence. *Infect Immun* 2009;**77**:4437–45.

van Schaik W, Tempelaars MH, Zwietering MH *et al.* Analysis of the role of RsbV, RsbW, and RsbY in regulating sigmaB activity in *Bacillus cereus*. *J Bacteriol* 2005;**187**:5846–51.

Verhamme DT, Kiley TB, Stanley-Wall NR. DegU co-ordinates multicellular behaviour exhibited by *Bacillus subtilis*. *Mol Microbiol* 2007;**65**:554–68.

Vermassen A, Leroy S, Talon R *et al.* Cell wall hydrolases in bacteria: insight on the diversity of cell wall amidases, glycosidases and peptidases toward peptidoglycan. *Front Microbiol* 2019;**10**:331.

Vetter SM, Schlievert PM. The two-component system *Bacillus* respiratory response A and B (BrrA-BrrB) is a virulence factor regulator in *Bacillus anthracis*. *Biochemistry* 2007;**46**:7343–52.

Vijay K, Brody MS, Fredlund E *et al.* A PP2C phosphatase containing a PAS domain is required to convey signals of energy stress to the SigmaB transcription factor of *Bacillus subtilis*. *Mol Microbiol* 2000;**35**:180–8.

Virmani R, Pradhan P, Joshi J *et al.* Phosphorylation-mediated regulation of the *Bacillus anthracis* phosphoglycerate mutase by the Ser/Thr protein kinase PrkC. *Biochem Biophys Res Commun* 2023;**665**:88–97.

Virmani R, Sajid A, Singhal A *et al.* The Ser/Thr protein kinase PrkC imprints phenotypic memory in *Bacillus anthracis* spores by phosphorylating the glycolytic enzyme enolase. *J Biol Chem* 2019;**294**:8930–41.

Vlamakis H, Chai Y, Beauregard P *et al.* Sticking together: building A biofilm the *Bacillus subtilis* way. *Nat Rev Micro* 2013;**11**:157–68.

Voelker U, Luo T, Smirnova N *et al.* Stress activation of *Bacillus subtilis* sigma B can occur in the absence of the sigma B negative regulator RsbX. *J Bacteriol* 1997;**179**:1980–4.

Wang Y, Jenkins SA, Gu C *et al. Bacillus anthracis* spore surface protein BclA mediates complement factor H binding to spores and promotes spore persistence. *PLoS Pathog* 2016;**12**:e1005678.

Wang ZA, Cole PA. The chemical biology of reversible lysine post-translational modifications. *Cell Chem Biol* 2020;**27**:953–69.

Watabe K. [Overview of study on *Bacillus subtilis* spores]. *Yakugaku Zasshi* 2013;**133**:783–97.

Weinrauch Y, Penchev R, Dubnau E *et al.* A *Bacillus subtilis* regulatory gene product for genetic competence and sporulation resembles sensor protein members of the bacterial two-component signal-transduction systems. *Genes Dev* 1990;**4**:860–72.

Westers H, Westers L, Darmon E *et al.* The CssRS two-component regulatory system controls a general secretion stress response in *Bacillus subtilis*. *FEBS J* 2006;**273**:3816–27.

White AK, Hoch JA, Grynberg M *et al.* Sensor domains encoded in *Bacillus anthracis* virulence plasmids prevent sporulation by hijacking a sporulation sensor histidine kinase. *J Bacteriol* 2006;**188**:6354–60.

Willett JW, Crosson S. Atypical modes of bacterial histidine kinase signaling. *Mol Microbiol* 2017;**103**:197–202.

Wu X, Song Q, Han A. Interacting proteins of the essential two-component system YycFG in *Bacillus subtilis*. *J Basic Microbiol* 2019;**59**:950–9.

Xiao D, Chen C, Yang P. Computational systems approach towards phosphoproteomics and their downstream regulation. *Proteomics* 2023;**23**:2200068.

Xing Y, Harper WF. *Bacillus* spore awakening: recent discoveries and technological developments. *Curr Opin Biotechnol* 2020;**64**:110–5.

Xu B, Liu L, Song G. Functions and regulation of translation elongation factors. *Front Mol Biosci* 2021;**8**:816398.

Yadav T, Carrasco B, Hejna J *et al. Bacillus subtilis* DprA recruits RecA onto single-stranded DNA and mediates annealing of complementary strands coated by SsbB and SsbA. *J Biol Chem* 2013;**288**:22437–50.

Yadav T, Carrasco B, Serrano E *et al.* Roles of *Bacillus subtilis* DprA and SsbA in RecA-mediated genetic recombination. *J Biol Chem* 2014;**289**:27640–52.

Yague P, Gonzalez-Quinonez N, Fernandez-Garcia G *et al.* Correction: Yague, P., et al. Goals and challenges in bacterial phosphoproteomics. *Int. J. Mol. Sci.* 2019, 20, 5678. *Int J Mol Sci* 2020;**21**:9381.

Yamaguchi H, Furuhata K, Fukushima T *et al.* Characterization of a new *Bacillus subtilis* peptidoglycan hydrolase gene, yvcE (named cwlO), and the enzymatic properties of its encoded protein. *J Biosci Bioeng* 2004;**98**:174–81.

Yan J, Zou W, Fang J *et al.* Eukaryote-like Ser/Thr protein kinase PrkA modulates sporulation via regulating the transcriptional factor sigma(K) in *Bacillus subtilis*. *Front Microbiol* 2015;**06**:382.

Yang X, Kang CM, Brody MS *et al.* Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. *Genes Dev* 1996;**10**:2265–75.

Yin W, Wang Y, Liu L *et al.* Biofilms: the microbial "protective clothing" in extreme environments. *Int J Mol Sci* 2019;**20**:3423.

Zhang A, Lebrun R, Espinosa L *et al.* PrkA is an ATP-dependent protease that regulates sporulation in *Bacillus subtilis*. *J Biol Chem* 2022;**298**:102436.

Zhang A, Pompeo F, Galinier A. Overview of protein phosphorylation in bacteria with a main focus on unusual protein kinases in *Bacillus subtilis*. *Res Microbiol* 2021;**172**:103871.

Zhang QB, Yu K, Liu Z *et al.* Prediction of prkC-mediated protein serine/threonine phosphorylation sites for bacteria. *PLoS ONE* 2018;**13**:e0203840.

Zhang S, Hu Y, Fan Q *et al.* Two-component system YvqEC-dependent bacterial resistance against vancomycin in *Bacillus thuringiensis*. *Antonie Van Leeuwenhoek* 2015;**108**:365–76.

Zhang S, Li X, Wang X *et al.* The two-component signal transduction system YvcPQ regulates the bacterial resistance to bacitracin in *Bacillus thuringiensis*. *Arch Microbiol* 2016;**198**:773–84.

Zhou B, Semanjski M, Orlovetskie N *et al.* Arginine dephosphorylation propels spore germination in bacteria. *Proc Natl Acad Sci USA* 2019;**116**:14228–37.

Zhou C, Zhang H, Fang H *et al.* Transcriptome based functional identification and application of regulator AbrB on alkaline protease synthesis in *Bacillus licheniformis* 2709. *Int J Biol Macromol* 2021;**166**:1491–8.

Zhou X, Zhang N, Xia L *et al.* ResDE two-component regulatory system mediates oxygen limitation-induced biofilm formation by *Bacillus amyloliquefaciens* SQR9. *Appl Environ Microb* 2018;**84**:e02744–17.

Zolfaghari Emameh R, Kazokaite J, Yakhchali B. Bioinformatics analysis of extracellular subtilisin E from *Bacillus subtilis*. *J Biomol Struct Dyn* 2022;**40**:7183–90.

Zwick JV, Noble S, Ellaicy YK *et al.* AhpA is a peroxidase expressed during biofilm formation in *Bacillus subtilis*. *Microbiologyopen* 2017;**6**:e00403.

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