



The Stress-Responsive Alternative Sigma Factor SigB of *Bacillus subtilis* and Its Relatives: An Old Friend With New Functions

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Alternative sigma factors have led the core RNA polymerase (RNAP) to recognize different sets of promoters to those recognized by the housekeeping sigma A-directed RNAP. This change in RNAP promoter selectivity allows a rapid and flexible reformulation of the genetic program to face environmental and metabolic stimuli that could compromise bacterial fitness. The model bacterium *Bacillus subtilis* constitutes a matchless living system in the study of the role of alternative sigma factors in gene regulation and physiology. SigB from *B. subtilis* was the first alternative sigma factor described in bacteria. Studies of SigB during the last 40 years have shown that it controls a genetic universe of more than 150 genes playing crucial roles in stress response, adaption, and survival. Activation of SigB relies on three separate pathways that specifically respond to energy, environmental, and low temperature stresses. SigB homologs, present in other Gram-positive bacteria, also play important roles in virulence against mammals. Interestingly, during recent years, other unexpected *B. subtilis* responses were found to be controlled by SigB. In particular, SigB controls the efficiencies of spore and biofilm formation, two important features that play critical roles in adaptation and survival in planktonic and sessile *B. subtilis* communities. In *B. subtilis*, SigB induces the expression of the Spo0E aspartyl-phosphatase, which is responsible for the blockage of sporulation initiation. The upregulated activity of Spo0E connects the two predominant adaptive pathways (i.e., sporulation and stress response) present in *B. subtilis*. In addition, the RsbP serine-phosphatase, belonging to the energy stress arm of the SigB regulatory cascade, controls the expression of the key transcription factor SinR to decide whether cells residing in the biofilm remain in and maintain biofilm growth or scape to colonize new niches through biofilm dispersal. SigB also intervenes in the recognition of and response to surrounding microorganisms, a new SigB role that could have an agronomic impact. SigB is induced when *B. subtilis* is confronted

with phytopathogenic fungi (e.g., *Fusarium verticillioides*) and halts fungal growth to the benefit of plant growth. In this article, we update and review literature on the different regulatory networks that control the activation of SigB and the new roles that have been described the recent years.

Keywords: *Bacillus subtilis*, alternative sigma factors, SigB, general stress response, biofilm fitness, biocontrol, sporulation

SigB AS A MODEL OF ALTERNATIVE SIGMA FACTOR PRESENT IN BACTERIA

Gene expression is a fundamental process that is conserved from bacteria to humans. The first step in gene expression is transcription, which is performed by structurally conserved DNA-dependent RNA polymerases (RNAPs), resulting in the synthesis of an RNA molecule from a DNA template. In bacteria, a single species of RNAP is responsible for transcribing both stable RNA (i.e., t- and rRNA), small RNA (i.e., sRNA), and protein-encoding genes RNA (i.e., mRNA). By contrast, eukaryotic systems use three distinct RNAP species to transcribe the different gene classes (RNAP I transcribes most rRNA, RNAP II transcribes mRNA, and RNAP III transcribes tRNA and 5S rRNA) (Lewis et al., 2008). The prokaryotic core RNA polymerase (RNAP) is a large (~400 kDa) multi-subunit enzyme comprising five ($\alpha_2\beta\beta'\omega$) subunits in a crab-claw-like structure.

Although little sequence homology exists between eubacterial RNAP, archaeal RNAP, and eukaryotic RNAPII, the crab-claw structure is remarkably conserved (Zhang et al., 1999; Cramer et al., 2001; Hirata et al., 2008). The two α subunits act as a scaffold to hold the catalytic β and β' subunits together (Zhang et al., 1999). The exact role of the ω subunit is unclear, but it is related in both structure and sequence to the eukaryotic polymerase subunit Rpb6 (Minakhin et al., 2001). It appears to be responsible for controlling transcription in response to nutrient changes, correct folding of the β' subunit, and its assembly into the core multi-subunit enzyme (Mukherjee et al., 1999; Vrentas et al., 2005; Chatterji et al., 2007).

The channel formed by β and β' is referred to as the primary channel, which contains a deep positively charged cleft housing the enzyme's active site. During transcription, downstream double-stranded DNA separates into a single-stranded DNA template, which enters the primary channel and contacts the active site to allow polymerization of RNA (Borukhov and Nudler, 2008). Due to the crowding of the primary channel by the DNA:RNA hybrid, nucleotide triphosphates (NTPs) must access the active site through an alternative route. They do this through a pore on RNAP called the secondary channel, which allows access to the active site not only for NTPs but also for other regulatory proteins and molecules. The elongating RNA molecule is separated from the DNA template by a wedge-like domain on RNAP to redirect the nascent RNA molecule through a third channel called the RNA exit channel, which then allows upstream DNA to reanneal as it exits RNAP (for a review see Borukhov and Nudler, 2008). Many of the regulatory roles of transcription factors are exerted through interaction with these structural elements (Borukhov et al., 2005). For initiation of transcription to occur, RNAP must first associate with a sixth component, a sigma

factor, to form what is termed the holoenzyme ($\alpha_2\beta\beta'\omega\sigma$) RNAP, which allows it to recognize and bind promoter DNA sequences (Murakami, 2015).

In the model bacteria *Escherichia coli* and *Bacillus subtilis*, the most important housekeeping sigma factors are Sig70 and SigA, respectively. They are present during planktonic and sessile growth (Losick and Pero, 1981; Gitt et al., 1985) and are responsible for initiating transcription from most promoters under optimal physiological conditions. Promoters whose recognition is mediated by Sig70 and SigA exhibit a canonical sequence centered at positions -35 and -10 base pairs from the start point of transcription (Losick and Pero, 1981). In addition, bacteria also have alternative sigma factors (discovered 40 years ago) to redirect the core RNAP to transcribe a minor set of genes required for specific adaptive responses. The availability of different sets of sigma factors in each bacterial species allows a rapid and reversible adaptation to changes in ecological scenarios. The number of alternative sigma factors present in different bacterial genera is variable and may reflect the lifestyle of the bacterium. It can go from only one sigma factor (e.g., in the intracellular pathogen *Mycoplasma genitalium*) to more than 60 sigma factors in the soil and antibiotic producing bacterium *Streptomyces coelicolor* (Gruber and Gross, 2003).

The roles covered by alternative sigma factors are large and diverse. They go from the well characterized roles in stress response, biogenesis of extracellular appendages (e.g., flagella), and developmental programs such as sporulation and biofilm formation; to less characterized processes such as the production of aerial hyphae and photosynthesis regulation in *S. coelicolor* and cyanobacteria, respectively (Helmann, 2002; Gruber and Gross, 2003). For *B. subtilis*, the core RNAP can interact with at least 10 different alternative sigma factors to recognize different promoters under diverse environmental and physiologic conditions (Losick and Pero, 1981; Helmann, 2019). **Table 1** shows an updated list of the alternative sigma factors present in *B. subtilis* and their functions. Within this group, the ones involved in spore formation (SigH, SigF, SigE, SigG, and SigK) (Nicholson et al., 1989; Sun et al., 1991; Predich et al., 1992; Roels et al., 1992; Zheng et al., 1992; Piggot and Hilbert, 2004; Aguilar et al., 2007; Kovács, 2016) and stress adaptation (SigB and SigH) (Boylan et al., 1991; Predich et al., 1992; Méndez et al., 2004; Bartolini et al., 2019a,b; Nadezhdin et al., 2020) are the best characterized.

Bacillus subtilis offers an excellent platform to learn how alternative sigma factors can be controlled. Both SigF are essential to express the early forespore developmental program (Losick and Stragier, 1992; Piggot and Hilbert, 2004) and SigB regulon (Price, 2000, 2002; Hecker et al., 2007). They

TABLE 1 | List of sigma factors (Sig) present in *Bacillus subtilis* and their functions.

Sigma factor	Alternative designation	Coding gene	Detected during vegetative phase, sporulation phase or extracellularly	Attributed roles	References
σ^A	σ^{43} , σ^{55}	<i>sigA</i> , <i>rpoD</i>	Vegetative and sporulation	Housekeeping/early sporulation	Moran et al., 1982
σ^B	σ^{37}	<i>sigB</i>	Vegetative	General stress response Fungal biocontrol Regulation of biofilm aging and dispersal	Boylan et al., 1991; Price, 2002; Hecker et al., 2007; Bartolini et al., 2019a,b
σ^C	σ^{32}	unknown	Vegetative	Postexponential gene expression	Johnson et al., 1983
σ^D	σ^{28}	<i>sigD</i> , <i>flaB</i>	Vegetative	Chemotaxis, autolysis, motility and regulation of flagellar gene expression	Helmann, 1991
σ^H	σ^{30}	<i>sigH</i> , <i>spo0H</i>	Vegetative and sporulation	Postexponential, competence and early sporulation genes expression	Predich et al., 1992
σ^L	σ^{54}	<i>sigL</i>	Vegetative	Degradative enzyme gene expression	Debarbouille et al., 1991
σ^N	ZpdN	<i>sigN</i>	Vegetative	Not yet determined It is found in ancestral strain of <i>B. subtilis</i> , not found in laboratory strains. pBS32-encoded	Burton et al., 2019
σ^E	σ^{29}	<i>sigE</i> , <i>spollGB</i>	Sporulation	Early mother cell gene expression	Roels et al., 1992
σ^F	$\sigma^{spollAC}$	<i>sigF</i> , <i>spollAC</i>	Sporulation	Early forespore gene expression	Sun et al., 1991
σ^G		<i>sigG</i> , <i>spollIG</i>	Sporulation	Late forespore gene expression	Nicholson et al., 1989
σ^K	σ^{27}	<i>sigK</i> , <i>spollVCB</i> , <i>spollIC</i>	Sporulation	Late mother cell gene expression	Zheng et al., 1992
σ^M	YhdM	<i>sigM</i> , <i>yhdM</i>	RNA polymerase ECF-type	Maintenance cell wall integrity in response to environmental and antibiotics stress	Thackray and Moir, 2003; Luo and Helmann, 2012;
σ^V		<i>sigV</i>	RNA polymerase ECF-type	Response against lytic enzymes	Zellmeier et al., 2005
σ^W	YbbL	<i>sigW</i> , <i>ybbL</i>	Extracellular RNA polymerase sigma factor (ECF-type)	Response to cell envelope stress such as antimicrobial peptides and alkaline pH Detoxification of the bacterium	Wiegert et al., 2001; Cao et al., 2002; Pietiaainen et al., 2005
σ^X	YpuM	<i>sigX</i>	ECF-type	Response to cationic antimicrobial peptides. Controlling biofilm architecture	Cao and Helmann, 2004; Murray et al., 2009
σ^Y	YxIB	<i>sigY</i>	ECF-type	Maintenance of the Sp β prophage that contains genes necessary to produce and resist killing by the antibiotic sublancin	Mendez et al., 2012
σ^Z		<i>sigZ</i>	ECF-type	Not yet determined	Sorokin et al., 1997

are governed by analogous phosphorylation-dependent partner switching mechanisms that involve anti-sigma factors with kinase activities, serine-threonine developmental phosphatases, and anti-anti-sigma factors. SigF is regulated by the anti-sigma factor with serine kinase activity SpoIIAB and the anti-anti-sigma factor SpoIIAA, which are cotranscribed in the same operon with the *spolIAC* gene-coding for SigF (i.e., the tricistronic *spolIA* operon: *spolIAAspolIABspolIAC*). Before the formation of the asymmetric septum of the developing sporangium (i.e., forespore plus mother-cell compartments), SpoIIAA remains inactive because it is phosphorylated by the anti-sigma factor SpoIIAB that also captures SigF in an inactive complex. Soon after polar septum formation, SpoIIAA is activated by dephosphorylation in the forespore compartment of the developing sporangium. The compartmentalized activation of SpoIIAA is mediated by the developmental phosphatase SpoIIE that is specifically activated in the forespore compartment (Losick and Stragier, 1992; Piggot and Hilbert, 2004; Pedrido et al., 2013). Once activated in the forespore compartment, SigF directs the expression of *spolIR* that codes for a signaling protein (i.e., SpoIIR), which is responsible for the mother-cell restricted activation of SigE, the first sigma factor of the mother-cell lineage of sporulation genes. SpoIIR acts as a vectorial signal that activates, in the mother cell compartment, the SpoIIGA protease that is responsible for

the proteolytic processing of inactive pro-SigE to active SigE (Piggot and Hilbert, 2004).

This series of early developmental events trigger the compartmentalized expression of forespore and mother-cell genes that will end in the formation of a latent and robust spore cell (Losick and Stragier, 1992; Piggot and Hilbert, 2004). Interestingly, SigB, the first bacterial alternative sigma factor found on the basis of its biochemical properties (Haldenwang and Losick, 1979) is also regulated, like SigF, by proteins with anti-sigma factor, anti-anti-sigma factor and phosphatase activities (see below).

***Bacillus subtilis* GENERAL STRESS RESPONSE CONTROLLED BY SigB FACTOR**

To be able to endure in their natural ecosystems (e.g., soil and host gut) (Hong et al., 2009), *B. subtilis* has developed complex and interconnected molecular pathways to survive starvation and stress conditions. A clear example of this, apart from sporulation (see above), is the biofilm formation (Branda et al., 2001; Lombardia et al., 2006; Pedrido et al., 2013; Vlamakis et al., 2013; Grau et al., 2015; Hölscher et al., 2015; Kovács and Dragoš, 2019).

These responses (i.e., sporulation and biofilm formation) are tightly regulated, time-consuming (e.g., 8–10 h under optimal laboratory conditions), and might be inappropriate to allow a rapid and efficient adaptation of *B. subtilis* cells under unfavorable conditions (Piggot and Hilbert, 2004; Branda et al., 2006; Grau et al., 2015; Kovács and Dragoš, 2019). An almost immediate, but no less sophisticated and efficient, cellular response (i.e., general stress response, GSR) of *B. subtilis* to a wide range of different stresses is the rapid (e.g., 5–15 min after the imposition of the stress) and short-lived induction of more than 150 general stress proteins (GSP), dependent on the transcription factor SigB (Haldenwang and Losick, 1979; Binnie et al., 1986; Price, 2002; Hecker et al., 2007; Losick and Pero, 2018).

The pioneering work of W. Haldenwang and R. Losick discovered SigB and the first gene (*ctc*) coding for a member of the GSR (Haldenwang and Losick, 1979), whilst trying to discover the genes and proteins responsible for a cellular escape from vegetative growth and the start of spore formation. Later, but still in the pre-genome-sequencing era, about fifty genes were individualized as members of the SigB regulon through insertional mutagenesis (using suicide vectors and transposons) and proteomic analysis of wild-type and isogenic *sigB* minus (i.e., $\Delta sigB$) strains under different stress conditions (reviewed in Price, 2002; Hecker et al., 2007). The complete genome sequencing of the *B. subtilis* strain 168 (Kunst et al., 1997) opened the use of postgenome and “omics” strategies that rapidly expanded the SigB regulon to almost 200 genes (Price, 2000, 2002; Hecker et al., 2007; Nicolas et al., 2012).

Comparative proteomic and genetic analysis of wild-type and $\Delta sigB$ *B. subtilis* strains under different culture conditions (i.e., unstressed and stressed) enabled the identification and assignment of a large set of GSP to the SigB regulon, many of which have proven biochemical activities. SigB is transiently induced after the imposition of a particular stress that halts or slows down the rate of growth, and at the end of the logarithmic phase of growth (in the absence of external stresses), before cells stop active growth and enter into a non-growing state. The presence of GSP in resting or non-growing cells might protect them against stresses that would appear in the future and compromise cell survival. Examples of GSP under SigB control are catalases (KatB, KatX), DNA-protecting enzymes (Dps), proteins repairing oxidative damage (OhrB), and disulfide stress (TxrA), proteins involved in osmotic resistance (OpuD, OpuE, YerD), heat stress resistance (ClpC, ClpP), antibiotic resistance (BmrU, BmrR), cold stress resistance (GsiB), cell envelope protection (GtaA, GtaB), accurate protein synthesis under stress (Ctc), sporulation response (Spo0E), etc. (Price, 2000, 2002; Hecker et al., 2007; Nicolas et al., 2012). Many of the SigB-induced genes are also under dual transcriptional control by other regulatory proteins (e.g., PhoP-PhoR, CtsR, Spx, SigH, and ECF sigma factors SigX and SigW) (Price, 2000, 2002; Hecker et al., 2007; Nicolas et al., 2012).

General stress response is conserved among certain Gram-positive bacteria such as *B. subtilis*, *Bacillus licheniformis*, *Bacillus halodurans*, *Bacillus clausii*, *Bacillus cereus*, *Clostridium difficile*, *Oceanobacillus iheyensis*, *Listeria monocytogenes*, *Listeria innocua*, and *Listeria welshimeri*, *Staphylococcus aureus*,

Mycobacterium tuberculosis, *S. coelicolor*, and *Ralstonia eutropha*. However, some facultative anaerobic Gram-positive bacteria have not developed this conserved developmental program (reviewed in Hecker et al., 2007). In Gram-negative bacteria, SigB orthologs are absent but they express other alternative sigma factors, for example, SigS (RpoS) present in *E. coli* and SigE (RpoE) present in *V. cholera*, which shares stress management proficiency with SigB (Kumar et al., 1994; Singh et al., 2017). The alternative transcription factor of SigB and its structural or functional orthologs, are not only the master regulators of the GSR but also control bacterial virulence in pathogens such as *L. monocytogenes* (Chaturongakul et al., 2008), *S. aureus* (Shaw et al., 2006), *B. cereus* (de Been et al., 2010), *Bacillus anthracis* (Fouet et al., 2000), and *Vibrio cholerae* (Singh et al., 2017). **Table 2** shows an updated list of the SigB structural orthologs present in Gram-positive bacteria.

In recent years, independent reports have shown that SigB induction produces a noticeable inhibition of the onset of spore formation (Reder et al., 2012a,b; Rothstein et al., 2017). The blockage of sporulation by specific dephosphorylation and inactivation of Spo0A~Pi (the master regulator of the onset of spore formation) (Piggot and Hilbert, 2004), is driven by the aspartyl phosphatase Spo0E encoded by the gene *spo0E* that possess an active SigB-dependent promoter (Reder et al., 2012b). The proficiency in spore formation is considered a last resort resource because spore formation is a highly energy consuming process and spore germination and outgrowth are also tightly regulated to recover the planktonic growth (Losick and Stragier, 1992; Piggot and Hilbert, 2004; Aguilar et al., 2007; Kovács, 2016). Therefore, it would be beneficial for *B. subtilis*, either living as single planktonic cells or in social biofilm communities, to explore other less extreme and reversible adaptive responses (i.e., GSR) before it selects the last resort choice (i.e., sporulation) to cope with very unfavorable scenarios. The integration of the SigB activity into the decision-making process of sporulation provides a link to interconnect the two dominant and probably mutually exclusive adaptive responses (sporulation and GSR) in the regulatory network that influences the cell fate of *B. subtilis* and its relatives (**Figure 1**; Reder et al., 2012a,b; Rothstein et al., 2017).

The SigB-controlled GSR is activated by diverse stressors including high and low temperature, high salt concentrations, ethanol, antibiotics, starvation for glucose, phosphate, and oxygen; inhibitors that decrease the ATP reservoir as well as blue light (Benson and Haldenwang, 1993b; Boylan et al., 1993; Völker et al., 1995; Price, 2000, 2002; Hecker and Völker, 2001; Hecker et al., 2007; Brigulla et al., 2003; Helmann et al., 2003; Mascher et al., 2003; Méndez et al., 2004; Moore et al., 2004; Zhang and Haldenwang, 2005; Gaidenko et al., 2006; Bonilla, 2020). Each of these stimuli induces one of the three SigB regulatory pathways known today (see below). Once activated, SigB binds to the core RNAP and recognizes a particular promoter structure (Haldenwang, 1995). **Table 3** shows conservation of the SigB promoter sequences from *B. subtilis*, *L. monocytogenes*, *S. aureus*, and other Gram-positive bacteria.

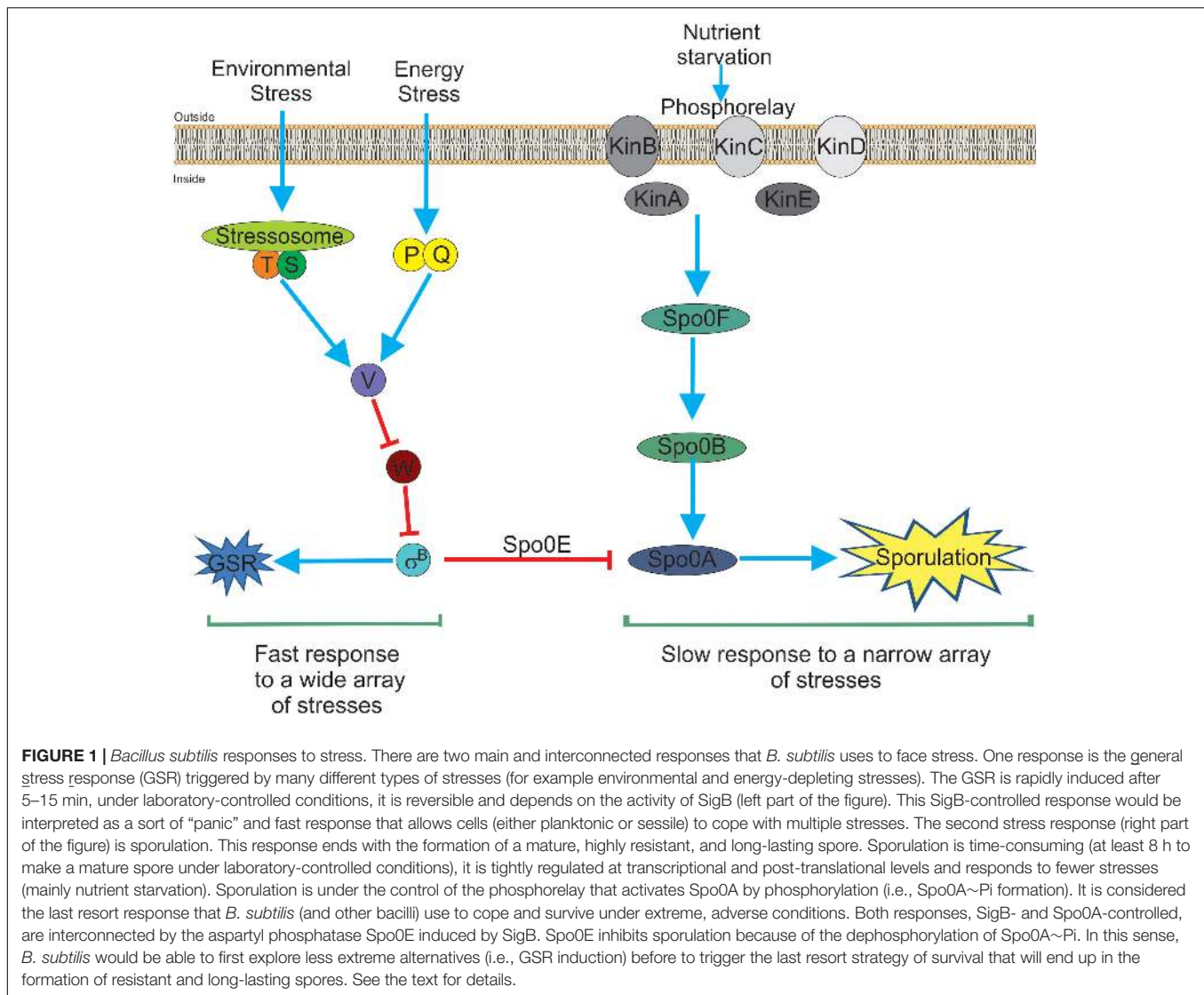
Activation of SigB in response to physical (i.e., environmental) and nutritional (i.e., energy) stresses is separately controlled by two overlapping partner switching

TABLE 2 | List of alternative sigma factors with structural similarity to *B. subtilis* SigB present in different Gram-positive bacteria.

SigB Orthologs				
Module	Microorganism	Name	Functions	References
<i>B. subtilis</i>	<i>B. subtilis</i>	SigB	See Table 1	
	<i>B. licheniformis</i>		General stress sigma factor	Brody et al., 2009
	<i>B. coagulans</i> , <i>B. amyloliquefaciens</i> , <i>B. pumilus</i> , <i>B. clausii</i>		Not reported	de Been et al., 2011
	<i>Oceanobacillus iheyensis</i>		Not reported	de Been et al., 2011
	<i>Listeria monocytogenes</i> , <i>L. innocua</i>		Activated in response to nutritional and environmental stresses. Responsible for swimming motility and invasiveness in the presence of blue light. Involved in the resistance of both planktonic cells and biofilms to the disinfectants benzalkonium chloride and peracetic acid in <i>L. monocytogenes</i> . Deletion of <i>sigB</i> attenuated virulence in <i>L. monocytogenes</i>	Wiedmann et al., 1998; Chaturongakul et al., 2008; Toledo-Arana et al., 2009; van der Veen and Abee, 2010; Ondrusch and Kreft, 2011
<i>B. cereus</i>	<i>L. welshimeri</i>		Not reported	de Been et al., 2011
	<i>B. thuringiensis</i> <i>B. cereus</i> <i>B. anthracis</i> <i>B. weihenstephanensis</i>	SigB	See Table 1	Fouet et al., 2000; van Schaik et al., 2004
<i>S. coelicolor</i>	<i>S. coelicolor</i>	SigF	<i>sigF</i> is needed for spore maturation	Cho et al., 2001; Bentley et al., 2002
		SigH	The <i>sigH</i> operon is controlled by environmental stress (heat, salt, ethanol) and developmental signals. A strain with a mutated <i>sigH</i> allele is reported to have some abnormalities in spore formation and to be slightly osmosensitive.	
<i>S. aureus</i>		SigB	<i>sigB</i> is induced by salt and plays a role in osmoprotection and erection of aerial mycelium	
	<i>S. avermitilis</i> , <i>S. griseus</i>	SigB	Not reported	de Been et al., 2011
	<i>Thermobifida fusca</i>		Not reported	de Been et al., 2011
	<i>Salinispora tropica</i> , <i>S. arenicola</i>		Not reported	de Been et al., 2011
	<i>Frankia alni</i> , <i>Frankia Ccl3</i> , <i>Frankia EAN1pec</i>		Not reported	de Been et al., 2011
<i>Clostridium</i>	<i>C. difficile</i>	SigB	Regulate biosynthesis of staphyloxanthin, a key virulence factor for protecting <i>S. aureus</i> from host-oxidant killing <i>in vivo</i> Its increased expression always is accompanied by enhanced biofilm formation	Wu et al., 1996; Giachino et al., 2001; Bischoff et al., 2004; Chen et al., 2016
		SigB	Responsible for antibiotic resistance	
		SigB	Crucial role in adaptive strategies during gut infection	
<i>M. tuberculosis</i>	<i>C. thermocellum</i> , <i>C. cellulolyticum</i> , <i>C. sticklandii</i> , <i>C. sordellii</i>	SigB	Not reported	Kint et al., 2017, 2019
		SigF	Induced under a variety of stress conditions, most notably antibiotic stress, low oxygen tensions, nutrient depletion, oxidative stress, and during stationary-phase growth Is involved in cell surface modification and virulence factor secretion	DeMaio et al., 1996, 1997; Michele et al., 1999; Geiman et al., 2004
<i>Synechocystis</i>	sp.	SigF	Mutant showed a severe defect in the induction of salt stress proteins Required for the biosynthesis of pili and that specific <i>pilA</i> genes	Bhaya et al., 1999; Huckauf et al., 2000

mechanisms (Alper et al., 1996; Price, 2002). These partner switching mechanisms are composed of four proteins: an input phosphatase (i.e., RsbP or RsbU, for SigB activation under

energy- or environmental-stresses, respectively); a switch kinase with anti-sigma factor activity (RsbW); an antagonist protein or anti-anti-sigma factor (RsbV); and the target protein, the sigma



factor (Alper et al., 1996). Under non-stress conditions, the two SigB activating pathways (i.e., energy- and environmental-stress pathways, see below) do not receive stressor inputs, SigB is inactive and the GSR is not induced. This is because the switch protein RsbW (a kinase with anti-sigma factor activity) has two roles: it phosphorylates and inactivates the anti-anti-sigma factor RsbV and sequesters the target SigB forming an inactive complex, and thus preventing its binding to the core RNAP (Benson and Haldenwang, 1993a; Alper et al., 1996; **Figure 2**).

The energy-stress arm of the SigB regulatory cascade is composed by two members: the protein phosphatase 2C (PP2C)-type RsbP (which dephosphorylates RsbV~Pi); and the agonist RsbQ, which is cotranscribed with RsbP (*P_ArsbPrsbQ*) and forms an active complex with it (Vijay et al., 2000; Brody et al., 2001). In the absence of energy-stress, the RsbQ:RsbP complex is not formed, as RsbP is inactive, RsbV remains phosphorylated, and SigB remains captured in the complex with RsbW (**Figure 2**). The environmental-stress arm of the SigB activation pathway is

structurally more complex than the energetic pathway. Similarly, to the requirement of the agonist protein RsbQ for RsbP phosphatase activity, the environmental-stress responding PP2C-type phosphatase RsbU requires the interaction with the serine-threonine kinase RsbT for its activation (Wise and Price, 1995; Yang et al., 1996). In the absence of environmental stress, RsbT is unable to interact with RsbU because it is bound to the antagonist protein RsbS in a large multiproteic complex: the stressosome (**Figure 2**). The *B. subtilis* stressosome is composed of a family of homologous or paralog proteins (i.e., RsbRs) thought to be sensors and modulators of environmental stimuli. RsbRA (the best characterized RsbR paralog) is transcribed by *rsbRA*, the first gene of an eight-gene operon that harbors *sigB* and other key gene regulators of SigB activity (i.e., the *sigB* operon, see below). The other RsbR paralogs (i.e., RsbRB, RsbRC, RsbRD, and YtvA) are expressed from diverse genes along the *B. subtilis* chromosome (Akbar et al., 2001; Chen et al., 2003; Kim et al., 2004a; Delumeau et al., 2006; Gaidenko et al., 2006, 2012).

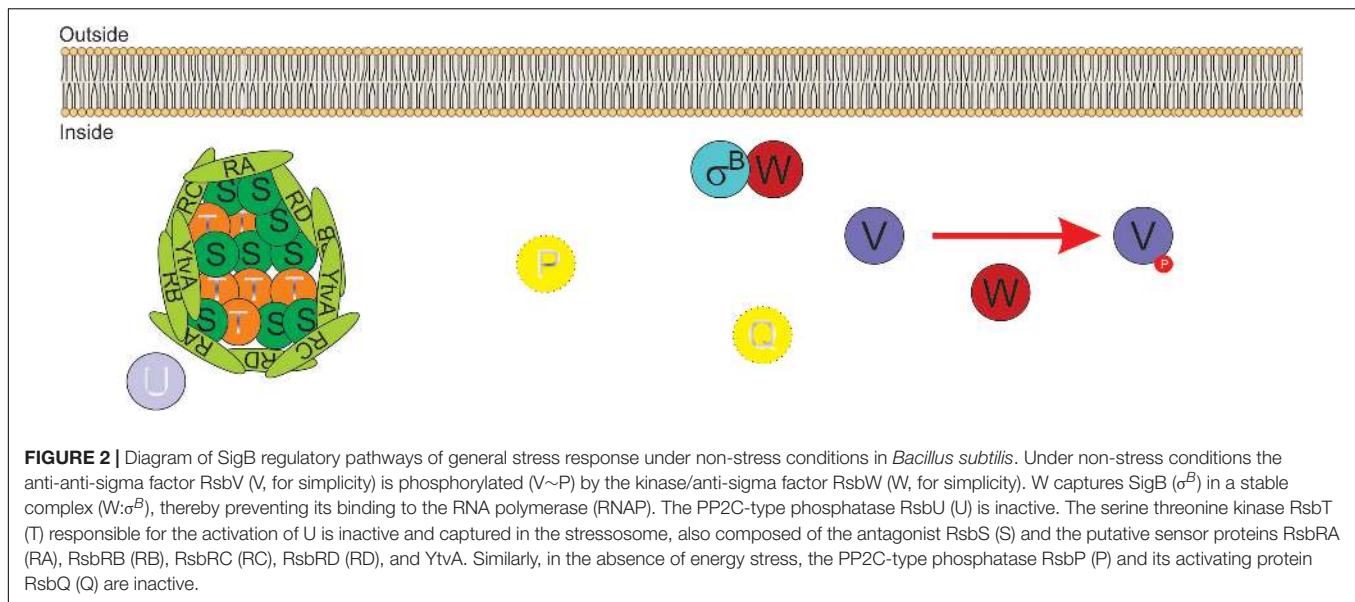
TABLE 3 | Genetic organization of the *sigB* promoter region of representative genes present in *B. subtilis* and selected Gram-positive bacteria.

Microorganism	Gene	Function	Promoter sequence			References
			-35	Spacer (bp)	-10	
<i>B. Subtilis</i>	<i>sigB</i>	see Table 2	GTTT	16	GGGTAT	Petersohn et al., 1999; Reder et al., 2012a
	<i>ctc</i>	General stress protein, similar to ribosomal protein L25	GTTTAA	14	GGGTAT	
	<i>gspA</i>	General stress protein, similar to glycosyl transferase	GTTT	14	GGGTAT	
	<i>trxA</i>	Thioredoxin	GTTT	16	GGGCAT	
	<i>spo0E</i>	Spo0A-P phosphatase, control of the phosphorelay	GTTTAA	14	GGGTAT	
	Consensus		GTTTWW	12-15	GGGWAW	
<i>L. monocytogenes</i>	<i>sigB</i>	See Table 2	GTTT	14	GGGTAA	Kazmierczak et al., 2003
	<i>gadB</i>	Glutamate decarboxylase	GTTT	16	GGGTAT	
	<i>bsh</i>	Bile salt hydrolase	GTTT	14	GGGTAC	
	<i>pdhA</i>	Pyruvate dehydrogenase (E1 alpha subunit)	GTTT	16	GGTAAT	
	<i>inIE</i>	Virulence genes	GTTA	15	GTGATT	
	Consensus		GTTT	13-17	GGGWAT	
<i>B. cereus</i>	<i>rsbV</i>	Stress-induced proteins	ATGTTTA	14	GGGTA	van Schaik et al., 2004
	<i>orf4</i>	Putative bacterioferritin	ATGTTTA	14	GGGTA	
	<i>yflT</i>	Stress-induced proteins	ATGTTTA	15	GTGTA	
	Consensus		AKGKTTA	14-17	GKGTA	
<i>S. coelicolor</i>	<i>catB</i>	Catalase B	GCCT	15	GGGTAC	Lee et al., 2004
	<i>trxC</i>	Thioredoxin C	GTGT	14	GGGTAC	
	<i>SC9C7.23</i>	Putative aminoacylase	GGGT	15	GGGTAC	
	Consensus		GNNTN	14-16	GGGTAC/T	
<i>S. aureus</i>	<i>sar</i>	Staphylococcal accessory regulator A	GTGA	16	GGGTAT	Bischoff et al., 2004
	<i>asp23</i>	Alkaline shock protein 23	GTTT	16	GGGTAT	
	<i>coa</i>	Staphylocoagulase precursor	GTTT	14	GGGCAA	
	Consensus		GTTTWW	12-15	GGGWAW	
<i>M. tuberculosis</i>	<i>usfx</i>	SigF anti-sigma factor	GTTTC	15	GGGTAT	Williams et al., 2007
	<i>phoY1</i>	Probable transcriptional regulatory protein	GGATTG	16	GGGTAT	
	<i>Rv2884</i>	Probable transcriptional regulatory protein	AGTTGG	18	GGGTAC	
	Consensus		NGNTTG	14-18	GGGTAT	
<i>C. difficile</i>	<i>trxA</i>	Thioredoxins	GGGTATA	15	GGGTATA	Kint et al., 2017
	<i>msrAB</i>	Methionine sulfoxide reductase	CAGATT	16	GGGAATA	
	<i>uvrB</i>	Excinuclease ABC subunit B	TAGATT	16	GGGTAAA	
	Consensus		WGWTT	13-17	(G/T)GGTAWA	

The putative promoter sequences are aligned with those of known SigB-dependent promoters. The consensus -35 and -10 promoter regions are highlighted in yellow. B = G/T/C, D = G/A/T, H = A/C/T, K = G/T, M = A/C, N = A/G/C/T, R = G/A, S = G/C, V = G/C/A, Y = C/T, W = A/T.

The stressosome complex adopts a pseudo-icosahedron conformation made of 40 copies of RsbRs, 20 copies of RsbS, and 20 copies of RsbT (Chen et al., 2003; Kwon et al., 2019). Each RsbR paralog contains a variable N-terminal non-heme globin domain (structurally related to globins but lacking conserved histidine residue essential for the incorporation of heme iron), a 13-aminoacid conserved linker domain, and a conserved C-terminal STAS (sulfate

transporter antisigma factor antagonist) domain, except for YtvA, which harbors a LOV (light-oxygen-voltage) sensing domain (Gaidenko et al., 2012). Structural analysis of the stressosome structure by cryo-electron microscopy suggests that the non-conserved N-terminal domain of each RsbR paralog protrudes outward the stressosome and the C-terminal domains face and reside inside the complex bound to RsbS and RsbT (Marles-Wright et al., 2008).



The non-conserved amino acid sequence of the N-terminal domains of RsbR paralogs suggests the existence of a different affinity of each paralog, to perceive stress signals and/or different abilities to interact with RsbT. The second and third genes in the *sigB* operon encode for RsbT and RsbS. This genetic organization opens the possibility that the three proteins (RsbRA, RsbT, and RsbS) could interact with each other soon after their synthesis and enter into the stressosome as a preformed complex (Reeves and Haldenwang, 2006). Once within the stressosome, the RsbRs proteins (RsbRA and its paralogs) seem to be redundant because when many of them are lost, RsbS is unable to retain RsbT in the stressosome. Less characterized stressosome-like complexes are present in other Gram-positive and Gram-negative bacteria, and the *B. subtilis* stressosome represents a model to better understand their functions and molecular organization (Pané-Farré et al., 2005).

Signal Transduction Pathways of SigB Activation

Activation of SigB by Environmental Stress

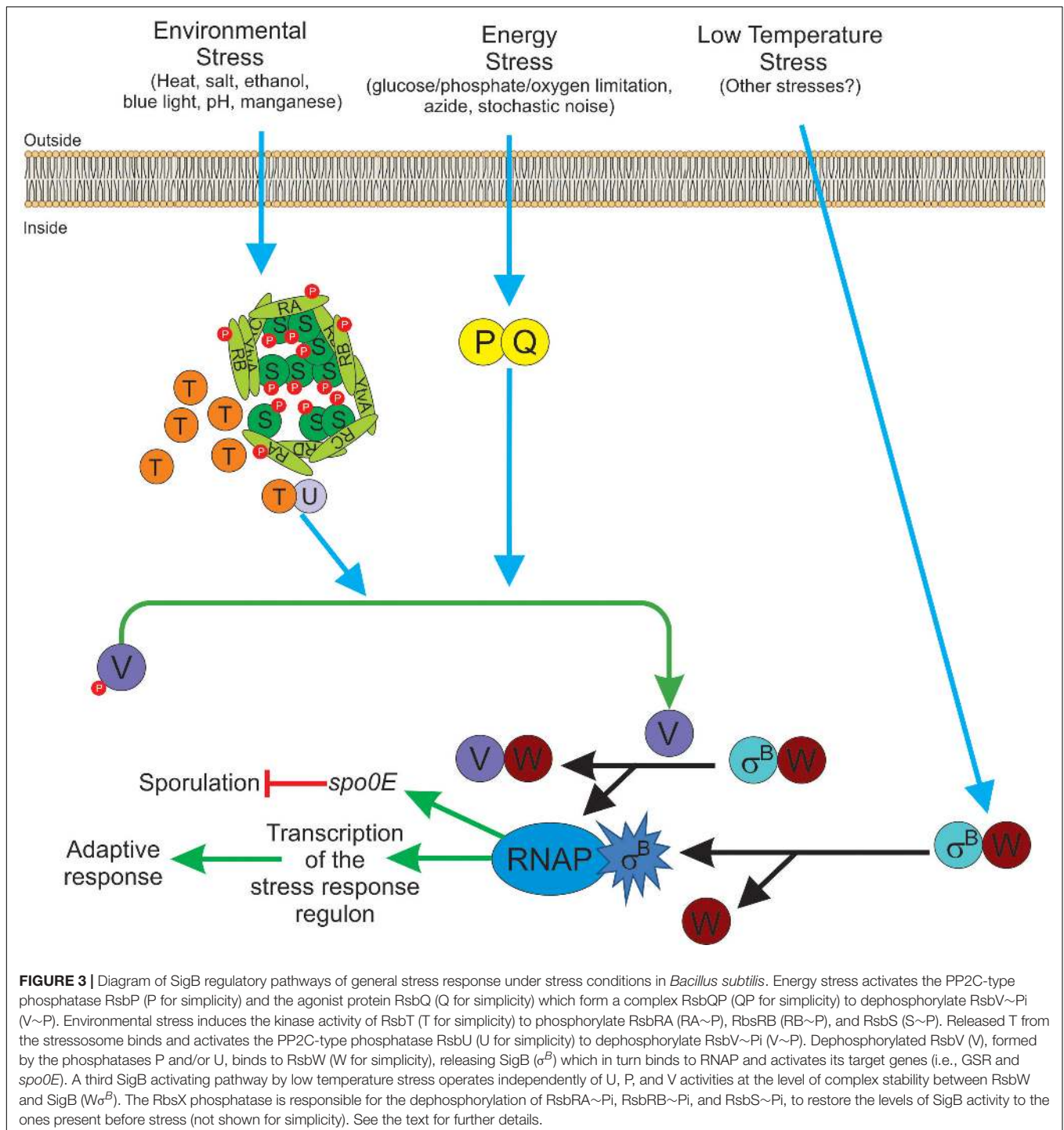
Table 4 shows an updated list of all known SigB regulatory proteins and their functions. In the presence of environmental insults (e.g., acid, heat, alcohol stresses), *B. subtilis* activates the kinase activity of RsbT on RsbRA and RsbS at conserved T171 and S59 residues, respectively (Yang et al., 1996; Kim et al., 2004b; Reeves and Haldenwang, 2006). Diverse genetic, biochemical and modeling studies suggest a progression of events that seem to start when environmental stress increases the kinase activity of RsbT and/or makes RsbS and the RsbR paralogs better suited to become phosphorylated by RsbT (Chen et al., 2003; Kim et al., 2004b; Reeves and Haldenwang, 2006). The phosphorylation of RsbRA at T171 is a prerequisite and facilitates the subsequent phosphorylation of RsbS at S59 by RsbT, and the onset of SigB signaling (Chen et al., 2003; Kim et al., 2004b). RsbS~Pi is unable to retain RsbT, which is

released from the stressosome, and binds to and activates RsbU (Yang et al., 1996). The environmentally activated PP2C-type phosphatase RsbU dephosphorylates the anti-anti-sigma factor, RsbV~Pi at S56 (Hecker et al., 1996; Yang et al., 1996; Vijay et al., 2000). The RsbV is now free to interact with RsbW, releasing active SigB (Figure 3 and Table 4). Interestingly, additional phosphorylation of RsbRA by RsbT at T205 prevents stressosome hyperactivation and thereby limits SigB activation (Eymann et al., 2011; Liebal et al., 2013). This (first) negative feedback loop would be partially responsible for the transient SigB response after stress imposition, reaching the maximum, under laboratory conditions (growth in nutritional rich broth, with shaking at 37°C), 10–40 min after the shift, but thereafter SigB activity

TABLE 4 | List of Rsb (i.e., regulators of sigma B) proteins.

Protein	Function and final outcome	Participation in		
		Environmental route	Energy route	Cold shock route
RsbRA-D YtvA	Antagonist of RsbT $\rightarrow \sigma^B$	✓	X	X
RsbS	Antagonist of RsbT $\rightarrow \sigma^B$	✓	X	X
RsbT	Activator of RsbU $\rightarrow \sigma^B$	✓	X	X
RsbU	Activator of RsbV $\rightarrow \sigma^B$	✓	X	X
RsbV	Antagonist of RsbW $\rightarrow \sigma^B$	✓	✓	X
RsbP	Activator of RsbV $\rightarrow \sigma^B$	X	✓	X
RsbQ	Agonist of RsbP $\rightarrow \sigma^B$	X	✓	X
RsbW	Inhibitor of σ^B and RsbV $\rightarrow \sigma^B$	✓	✓	✓
σ^B	Activator of GSR	✓	✓	✓
RsbX	Antagonist of RsbT $\rightarrow \sigma^B$	✓	✓	X

The participation of each Rsb protein in each SigB regulatory pathway and the assigned Rsb functions are indicated (see text for details). Symbols: ✓, participation; X, non-participation; \rightarrow , activation; \rightarrow , inhibition.



rapidly decreases to a level slightly higher than the pre-shift level (Völker et al., 1995).

To reinforce the transient and controlled SigB response, there is a serine-threonine phosphatase, RsbX, responsible for the dephosphorylation of RsbS~Pi and RsbRA~Pi at their S59 and T171 residues, respectively. These dephosphorylations are part of a fine tuning device that allows RsbT to be again sequestered in the stressosome and ensures the transient and

controlled nature (second negative feedback regulatory loop) of SigB activation (Yang et al., 1996; Price, 2002). Supporting this model, it has been shown that the phosphatase activity of RsbX is low under non-stress conditions (Chen et al., 2004). Under these conditions, the predominant components of the stressosome are unphosphorylated RsbS and RsbRA. After the imposition of initial stress, the kinase activity of RsbT is activated, and RsbRA and RsbS become phosphorylated by RsbT at T171

and S59 positions, respectively. These environmentally controlled phosphorylations trigger a cascade of biochemical reactions that ends with the liberation of SigB from RsbW and its activation (**Figure 3**). Because of the positive feedback loop of SigB on its coding operon, the levels of RsbX will increase to compensate for the activation level of RsbT, leading to dephosphorylation of RsbS~Pi and RsbRA~Pi at T171 residue, and downregulation of the SigB-dependent response. As expected, in the absence of RsbX, the activity of SigB increases to very high and uncontrolled levels (Benson and Haldenwang, 1992; Boylan et al., 1992; Völker et al., 1997).

How the environmental-related signals are transduced to control the kinase and phosphatase activities of RsbT and RsbX is unknown, and opens the possibility that the stressosome might not constitute the most upstream component of the environmental signaling cascade controlling SigB activation (Kuo et al., 2008). It has been indicated that the ribosomal protein L11 and the essential GTP-binding protein Obg, encoded by the same operon as the phosphorelay gene *spo0B*, were required for stress activation of SigB (Trach and Hoch, 1989; Scott and Haldenwang, 1999; Zhang et al., 2001). *In vitro* analysis showed that Obg co-fractionated with ribosomal subunits and the stressosome components RsbR, RsbT, and RsbS (Scott and Haldenwang, 1999). Even though the physiological role of ribosomal subunits and Obg on the environmental-stress arm of the SigB regulatory cascade is unknown, it allows the possibility of a link between the protein synthesis machinery (i.e., the ribosome) and cell-cycle signals (putative mediated by Obg) with the stressosome. Other clues related to the unnoticed existence of upstream participants of the stressosome are found in the Gram-positive pathogen *L. monocytogenes* where an integral membrane protein required for SigB activation might be the environmental sensor interacting with the stressosome *in vivo* (Impens et al., 2017).

Activation of SigB by Energy Stress

The input phosphatase RsbP is another PP2C-type specific phosphatase responsible for sensing a depletion of energy (ATP levels). The PP2C-type domain of RsbP is located on its C-terminal region, and the N-terminal region of RsbP contains a Per-Arnt-Sim (PAS) domain that would be involved in the sensing of redox potential, oxygen levels, and protein-protein interactions (Price, 2000, 2002; Hecker et al., 2007). RsbP is cotranscribed and works together with the RsbQ protein (**Table 4**; Brigulla et al., 2003; Kaneko et al., 2005). RsbPQ dephosphorylates RsbV~Pi and RsbV activate SigB as described above (**Figure 3**). Interestingly, in the absence of energy stress, RsbP and RsbQ were responsible for the formation of discrete stochastic pulses of SigB activation (Locke et al., 2011). The significance of the RsbPQ-dependent noise activation of SigB is unclear but it might be advantageous for the survival and persistence of the bacterial population to always have (also in the absence of apparent stress) a minor proportion of cells with active SigB to face sudden and threatening conditions of population survival (i.e., bet-hedging) (Acar et al., 2005; Kussell and Leibler, 2005). Since many bacteria share transcription factors similar to SigB and its regulatory partner switching pathways (**Figure 3**

and **Tables 2, 4**), it is tempting to think that the stochastic pulse modulation of the GSR as an important trait that “always” guarantees bacterial survival and persistence.

Soon after the liberation of active SigB and the formation of the RsbW-RsbV complex induced by the environmental and/or energy stresses, RsbW phosphorylates and inactivates RsbV, and released RsbW is now able to be captured and inactivates SigB again (Dufour and Haldenwang, 1994). This event represents a third negative feedback loop responsible for the controlled and transient nature of the SigB response that operates together with the double phosphorylation of RsbRA, and the dephosphorylation of RsbS~Pi by RsbT kinase and RsbX phosphatase, respectively.

Activation of SigB by Cold Shock Stress

An alternative pathway of SigB activation has been described, which operates during growth at low temperatures (between 17 and 20°C) or after a growth temperature downshift from 37 to 20°C (Brigulla et al., 2003; Méndez et al., 2004). One feature of this pathway is the high and persistent levels of SigB induction (Brigulla et al., 2003; Méndez et al., 2004), compared to the less dramatic and transient nature of the SigB induction observed after bacterial exposition to environmental or energy stresses (Benson and Haldenwang, 1993b; Boylan et al., 1993; Völker et al., 1995; Price, 2000, 2002; Hecker and Völker, 2001; Helmann et al., 2003; Mascher et al., 2003; Moore et al., 2004; Zhang and Haldenwang, 2005; Gaidenko et al., 2006).

Another feature of this induction pathway at low temperature, compared to the environmental and energy pathways, is that SigB cold-shock induction does not need for RsbP, RsbU, and RsbV (**Figure 3** and **Table 4**). The nature of this RsbP-, RsbU-, and RsbV-independent activation pathway is not known but there are some hypotheses proposed by Brigulla et al. (2003). Among these is the idea that at low temperatures an unknown protein or metabolite disrupts the inhibitory RsbW-SigB complex, or it might be the case that the physical interaction between SigB and RsbW could be decreased or impaired at low temperatures (Brigulla et al., 2003). In any of these two hypothetical situations, SigB activity should become high, persistent, and independent of RsbX to improve general stress resistance at low temperature (Brigulla et al., 2003; Méndez et al., 2004).

Interestingly, a recent report showed that *B. subtilis* survival after oxidative stress (e.g., by addition of sodium nitroprusside) required SigB activation independent from RsbP but also surprisingly, independent of RsbT (Tran et al., 2019). This finding opens the possibility that the RsbT- and RsbP-independent, cold-shock-dependent pathway of SigB activation could recognize a wider spectrum of stresses (**Figure 3**).

Despite the high SigB activity of *B. subtilis* growing at low temperature, the sporulation proficiency is extremely low, less than 1% for cultures grown at 17–20°C, compared to a nearly 100% of sporulation efficiency for cultures grown at 37°C (Brigulla et al., 2003; Méndez et al., 2004). Even though an explanation for the low sporulation efficiency at low growth temperatures has not yet been established, we hypothesize that it could result from the high and persistent levels of SigB

activity found at low growth temperature, which could trigger the upregulation of the SigB-controlled aspartyl phosphatase Spo0E, responsible for the dephosphorylation of Spo0A~Pi, which in turn blocks the onset of sporulation (Reder et al., 2012a,b). In any case, why *B. subtilis* chooses activation of SigB instead Spo0A activation at low temperatures remains a mystery (Figure 3).

Besides the posttranslational regulation imposed by environmental, energy and low temperature stresses, SigB upregulation is also modulated at the transcriptional level (Wise and Price, 1995). SigB is encoded by the seventh genetic unit of the eight-gene *sigB* operon, and the components of the environmental stress pathway are encoded by the first four genes of this operon. The expression of the *sigB* operon is under the control of a SigA-dependent promoter (P_A) that is responsible for the constitutive and low expression of the operon. There is an extra promoter (P_B) under SigB control, which induces the expression of the last four genes of the *sigB* operon (i.e., P_A *rsbR-rsbS-rsbT-rsbU-P_B* *rsbV-rsbW-sigB-rsbX*) (Boylan et al., 1992; Völker et al., 1997; Price, 2002; Hecker et al., 2007). The SigA-dependent expression of the entire *sigB* operon (and the stochastic noise, see above) ensures that even under non-stress conditions, SigB will always be present in *B. subtilis* cells to allow a rapid response after sudden and unexpected stress. Thus, any single stress (energy, environmental or low temperature insult) will release SigB from the inhibitory complex formed with RsbW (Figure 3) and upregulate its expression from the P_B -dependent promoter. In this sense, active SigB is now able to specifically activate genes expression needed to cope with the inducing stress and also expression of the GSR, thus providing a multiple, unspecific and preventive cell stress adaptation (Hecker and Völker, 2001; Price, 2002; Hecker et al., 2007). However, although most of the genes involved in the GSR are induced after specific or unique stress, the activation level of each of the more than 150 genes that integrate the GSR, will depend on the specific imposed stress (i.e., the transcription of not all the SigB-controlled genes respond in the same magnitude to different types of stresses).

The lack of RsbP and RsbQ orthologs does not preclude the ability to induce SigB in response to energy stress. In *L. monocytogenes*, the stressosome is activated not only after environmental stress but also after energy stress (Martinez et al., 2010). For the opportunistic pathogen *S. aureus*, and other cocci, it has been observed that there is an absence of RsbP and RsbQ orthologs, but also that stressosome components (i.e., RsbRs, RsbS, and RsbT) are missing (Pané-Farré et al., 2009). Therefore, the components of the SigB regulon in *S. aureus* are restricted to four members (i.e., RsbU, RsbV, RsbW, and SigB). It seems that only the transcriptional regulation of the *S. aureus sigB* operon (P_A *rsbUP_B* *rsbVrsbWrsigB*) would be responsible for SigB regulation. Transcriptional control of *rsbU* is sufficient to activate the SigB-dependent stress-response, but surprisingly, the ratio of RsbV to RsbV~Pi does not increase (as it is the case in *B. subtilis*) after an environmental insult.

Furthermore, in *B. cereus*, *B. anthracis*, and *Bacillus thuringiensis* the energy- and environmental-stress dependent routes of SigB activation are absent, and the control of the

phosphorylation level and kinase activity of the RsbV and RsbW orthologs, respectively, might be performed using other protein modules. In *B. cereus*, the hybrid and membrane-bound histidine kinase RsbK autophosphorylates under diverse stress conditions at a conserved histidine residue within the H-box domain. RsbK~Pi is now able to phosphorylate the RsbY protein at its N-terminal receptor domain at a conserved aspartyl residue. Phosphorylation of RsbY activates its C-terminal PP2C-type phosphatase domain to dephosphorylate RsbV~Pi and so allows SigB activation (de Been et al., 2011).

These observations in *L. monocytogenes*, *S. aureus*, and other bacilli, strongly suggest the existence of new and uncharacterized SigB control mechanisms that are absent in *B. subtilis* but present in other Gram-positive bacteria that expand the significance and complexity of the SigB regulatory network on bacterial GSR for adaptation and survival (van Schaik and Abee, 2005; Hecker et al., 2007; O'Byrne and Karatzas, 2008; Pané-Farré et al., 2009; Martinez et al., 2010; de Been et al., 2011).

NEW ROLE FOR THE TRANSCRIPTION FACTOR SigB IN BIOFILM GROWTH, AGING, AND DISPERSAL

Biofilms are well organized bacteria ecosystems, in which sessile cells form multicellular aggregations in a self-secreted extracellular matrix with protective and adhesive functions (Vlamakis et al., 2013). Biofilms have the property to adhere to living or non-living surfaces and can be prevalent in natural, industrial, and hospital settings. The proficiency in biofilm formation represents a key feature that many bacterial pathogens share that enables them to resist the action of microbicides and antibiotics, giving rise to the failure of medical therapy and the persistence and dissemination of the pathogen infection (Flemming et al., 2016). Wild-type *B. subtilis* isolates are predominantly known to form architecturally complex colonies and wrinkled pellicles that serve as models of solid (colony) and liquid (pellicle) bacterial biofilms (Branda et al., 2001; Lombardia et al., 2006; Pedrido et al., 2013; Vlamakis et al., 2013; Grau et al., 2015; Hölscher et al., 2015; Kovács and Dragoš, 2019).

Although most biofilms are studied in pathogenic bacteria and therefore are associated with chronic and persistent infections (such are the cases for *S. aureus* and *Pseudomonas aeruginosa* biofilms), we now know that some bacteria produce beneficial biofilms. The clearest example of this is the *B. subtilis* biofilm formed either on the roots or leaves of plants where they promote plant growth (Beauregard et al., 2013), and biofilms formed in the intestinal mucosa of the eukaryotic host, where they produce an improvement in the host immunity (Donato et al., 2017; Smolentseva et al., 2017). It has recently been shown that *B. subtilis* can prevent the formation of *S. aureus* biofilm in both mice and human intestines through a molecular mechanism of quorum-sensing interference that produces the competitive exclusion of the pathogen from the host gut colonized by *B. subtilis* (Piewngam et al., 2018). By contrast, the co-culture of *B. subtilis* under biofilm-supporting conditions favored the

growth and survival of probiotic lactic acid bacteria (LAB), protected in the extracellular matrix of the biofilm formed by *B. subtilis*. Otherwise, LABs would perish upon exposure to the acidic pH of the stomach and the microbicide action of intestinal bile (Yahav et al., 2018). More recently, it has been shown in animal models that the *B. subtilis* biofilm is important in delaying neurodegenerative diseases (i.e., Alzheimer's and Parkinson's diseases) (Cogliati et al., 2019; Goya et al., 2020). Therefore, the importance of *B. subtilis* biofilm in basic and applied research cannot be questioned.

Interestingly, two recent publications (Bartolini et al., 2019a; Nadezhdin et al., 2020) have demonstrated that SigB was induced during *B. subtilis* biofilm development. These findings expanded the significance of the expression of the SigB-dependent GSR from the self-sufficient and planktonic life style of individual bacteria to the collective and social life style found in biofilm communities (Branda et al., 2001). Bartolini et al. (2019a) reported that the activity of SigB in *B. subtilis* pellicles (liquid biofilm) during the first hours of cultivation (when the biofilm was juvenile) was not detected. However, the induction of SigB began at the moment that the biofilm decreased its rate of growth, with the highest levels of SigB activity observed when the biofilm grasped its plateau of growth (mature biofilm; Bartolini et al., 2019a). The analysis of biofilm formation at different temperatures in *B. subtilis* strains lacking key components for SigB activation during environmental (Δ rsbU), metabolic (Δ rsbP), or both types of stresses (Δ rsbUP) showed interesting results (Bartolini et al., 2019a). During biofilm development, neither the environmentally related (RsbU) nor the low temperature-related route of SigB activation was expressed, and only the energy stress related (RsbPQ) route was responsible for activation of SigB inside the biofilm (Figure 4A).

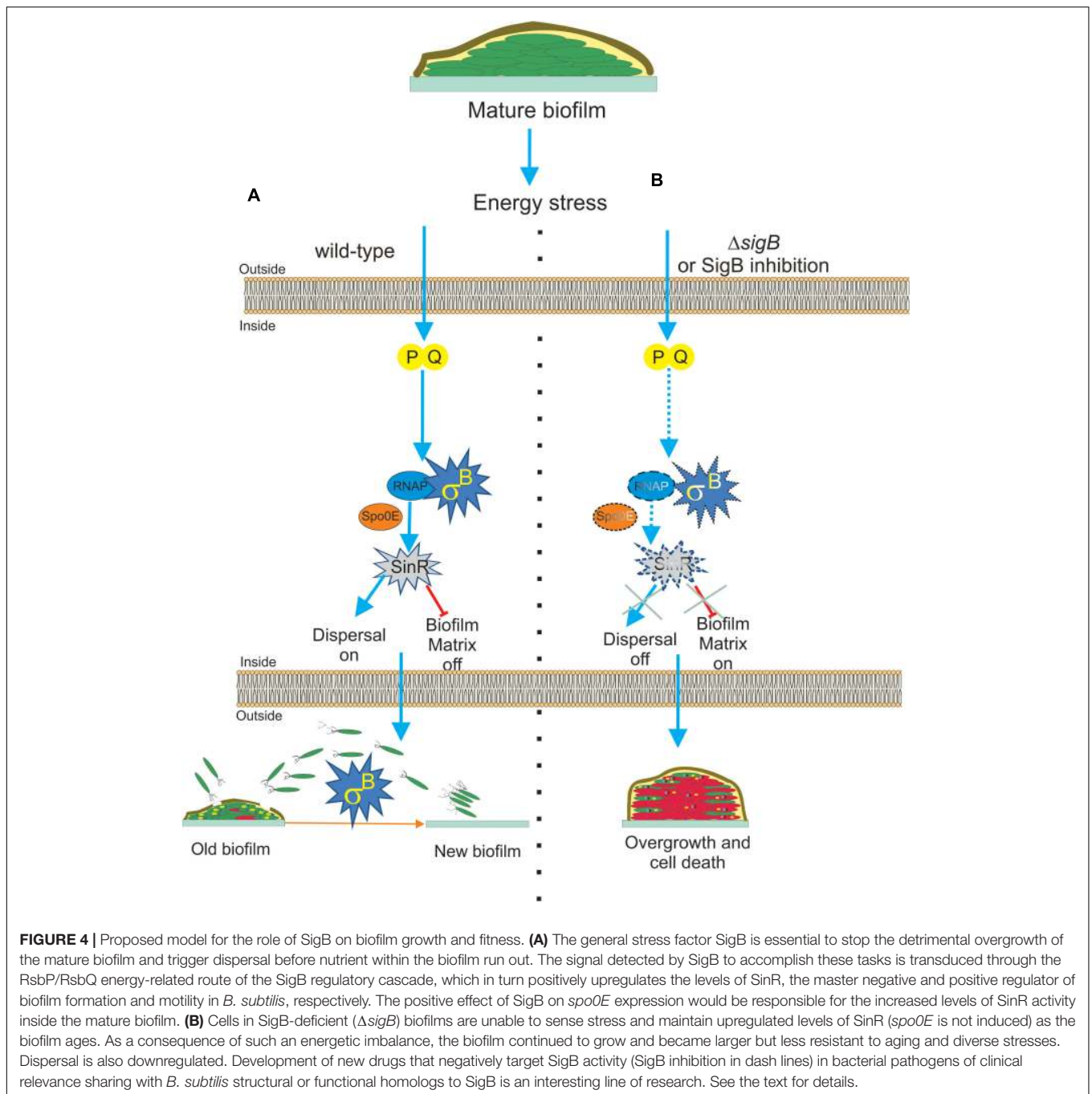
Nadezhdin et al. (2020) also reported SigB activation in *B. subtilis* solid biofilm (wrinkle colonies). Using different state of the art techniques, such as time-lapse imaging and quantitative microscopy, they observed that stochastic pulses of SigB activation in the individual cells of the developing biofilm depend on the functionality of the energy stress pathway of SigB activation, as previously observed by Locke et al. (2011) in *B. subtilis* planktonic cultures. Therefore, stochastic RsbPQ-mediated SigB activation is a common phenomenon that occurs in planktonic and sessile *B. subtilis* communities (Locke et al., 2011; Nadezhdin et al., 2020). The stochastic activation of SigB was orchestrated together with the activation of the other main survival pathways present in *B. subtilis*, the sporulation program (Piggot and Hilbert, 2004; Aguilar et al., 2007; Kovács, 2016). The activities of both adaptive and survival pathways (SigB regulon and sporulation) were expressed as a gradient inside the biofilm, with the peak of expression of SigB located at the top of the biofilm and the sporulation pathway activity restricted to the middle of the biofilm because of the Spo0E-dependent inhibitory effect of SigB on spore formation (see Figure 1 and Nadezhdin et al., 2020). Under non-stress conditions, Δ sigB *B. subtilis* produced more biofilm compared to the wild-type cells, but these cells died more rapidly because of their increased vulnerability to different stress conditions (Figure 4B). According to these results, it is reasonable to conclude that SigB could prevent

undesirable biofilm overgrowth and maintain the fitness of old biofilms (Figure 4A).

SigB might regulate biofilm growth and fitness, as it has been shown that SigB activity was required to maintain expression of *sinR* in the mature biofilm (Bartolini et al., 2019a, Figure 4A). SinR is an inhibitor of biofilm formation because it represses the operons *epsA-O* and *tapA-sigW-tasA* in charge of the synthesis of the exopolysaccharide and the amyloid fiber TasA of the extracellular matrix of the biofilm (Kearns et al., 2005; Chu et al., 2006). Therefore, the biofilm overgrowth observed in Δ sigB sessile cultures of *B. subtilis* was due to the downregulation of the biofilm inhibitor SinR (Figure 4B). The activity of SinR is also essential for flagellum-mediated *B. subtilis* motility (swimming and swarming), and *sinR* mutant strains are non-motile (Fredrick and Helmann, 1996; Kearns and Losick, 2003; Gottig et al., 2005; Grau et al., 2015). Swimming and swarming were reduced in Δ sigB cells, supporting the positive role of SigB on SinR function (Bartolini et al., 2019a). During the biofilm cycle, a phenomenon known as dispersal takes place, where some motile cells detach from the aged biofilm and escape from it to avoid famine (Jefferson, 2004; Liu et al., 2015). According to the requirement of cellular motility for dispersal proficiency (Kaplan, 2010; Dalton et al., 2011; McDougald et al., 2011; Stacy et al., 2014; Barraud et al., 2015; Guilhen et al., 2017), and the motility control by SinR in *B. subtilis*, it was shown that the biofilm formed by Δ sigB cells had reduced dispersal (Bartolini et al., 2019a), Figure 4B. These findings open the possibility to explore novel strategies for blocking the expression or activity of the SigB homologs present in bacterial pathogenesis as a way to downregulate dispersal and make disease-related biofilms more susceptible to traditional microbicides. However, the mechanism by which SigB controls *sinR* expression is still unknown, because the *sinR* promoter lacks a SigB consensus sequence (Nicolas et al., 2012). One possible mechanism is that SigB activates *sinR* expression indirectly. In line with this, the aspartyl phosphatase Spo0E is a possible candidate because SigB induces expression of *spo0E* (Reder et al., 2012b). The Spo0E aspartyl phosphatase inactivates Spo0A~Pi by dephosphorylation and the decreased levels of Spo0A~Pi releases *sinR* from its repression by Spo0A~Pi. Therefore, SigB could be able to increase *sinR* expression indirectly through Spo0E activity (Figure 4A).

A NOVEL POSITIVE ROLE FOR THE TRANSCRIPTION FACTOR SigB IN THE ANTIFUNGAL PROFICIENCY OF *Bacillus subtilis*

Pesticides are extensively used to control plant diseases (Pingali, 2012). However, a negative impact on human health and the environment has been observed (Tago et al., 2014; Kim et al., 2017). Numerous species of Bacilli have been identified as eco-friendly plant-growth promoting bacteria (PGPB) and/or biocontrol agents. PGPB employ direct and indirect mechanisms to enhance plant growth. These direct mechanisms involve phytohormone production, the acquisition of nutrients, and the control of pathogens through the synthesis of hydrolytic



enzymes and biopesticides (Ongena et al., 2005; Raja, 2013; Luo et al., 2015). The indirect mechanisms include the triggering of specific defense-related pathways, particularly the induction of systemic resistance (ISR) (Zerriouh et al., 2014; Debois et al., 2015; Aleti et al., 2016), and the release of volatile organic compounds (VOCs) against pathogens (Ongena and Jacques, 2008; Raaijmakers et al., 2010; Cawoy et al., 2014; Cawoy et al., 2015). Most of the characterized plant-beneficial *B. subtilis* strains display direct and indirect mechanisms to benefit plant growth and can form robust root-associated biofilms.

In a recent study by Bartolini et al. (2019b), it has been shown that SigB and its GSR were largely activated when *B. subtilis* interacts antagonistically with the phytopathogen *Fusarium verticillioides* (Figure 5). Specifically, the RsbPQ-route sensing energy stress was required for SigB activation and the antifungal control (Figure 5) when *B. subtilis* cells were cultured in the presence of live or dead *F. verticillioides* micelia or cell-free supernatants of the fungi (Bartolini et al., 2019b). In the light of these results, it is assumed that an unknown *F. verticillioides* metabolite is the primary cause of the energy depletion in

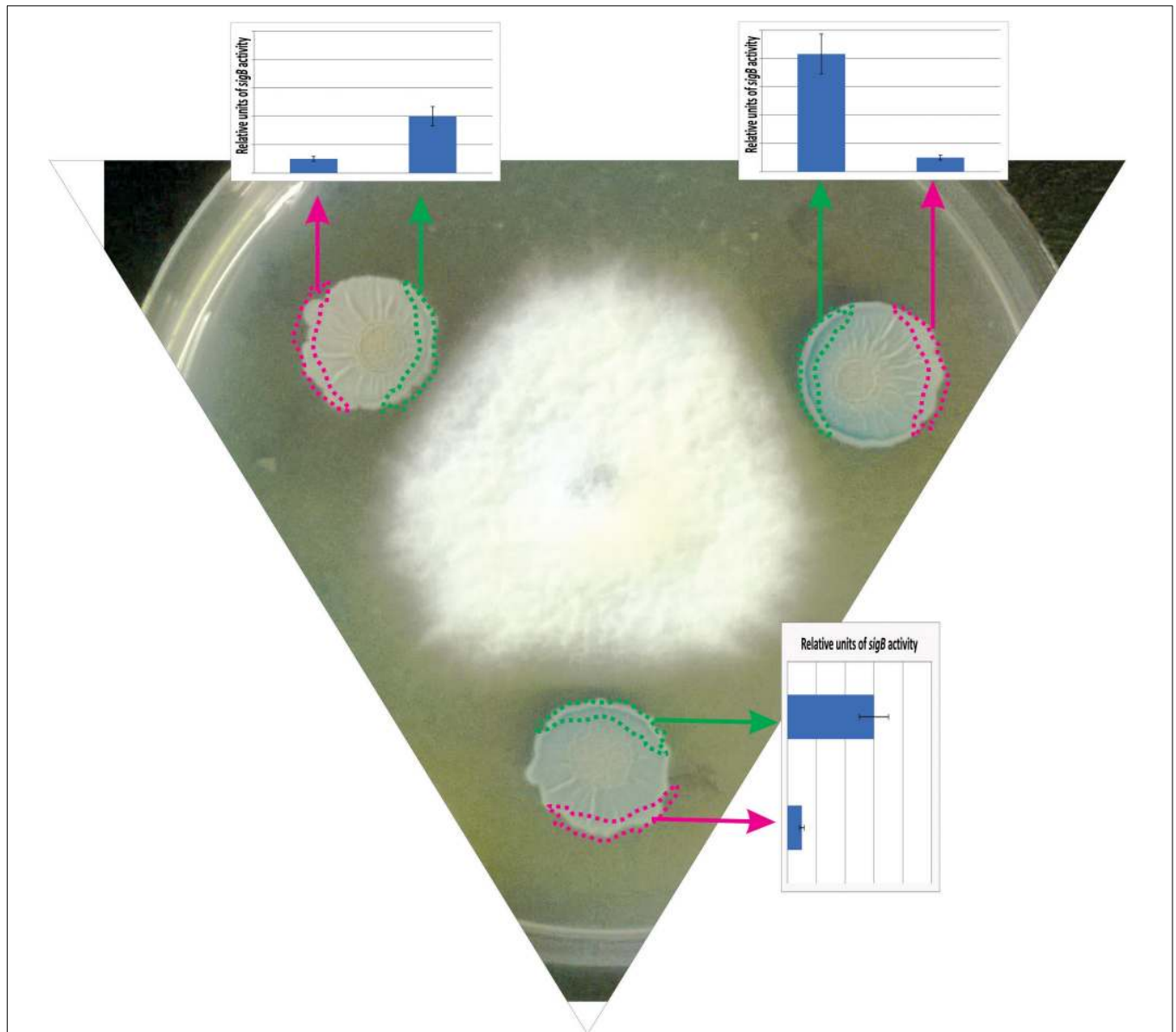


FIGURE 5 | Antagonist response of *B. subtilis* confronted with the phytopathogen *Fusarium verticillioides*. The co-culture of a wild-type *B. subtilis* strain harboring a SigB-reporter gene fusion (*ctc-lacZ*) with *F. verticillioides* allows the observation of the antagonistic fungus-bacterium interaction. At the start of the co-incubation, 5×10^5 colony forming units (CFU) of fungal mycelia were poured at the center of the Petri dish, and 1×10^5 colony forming units (CFU) of a stationary phase culture of *B. subtilis* was placed at three different positions surrounding the fungus to better observe the antagonistic interaction. *F. verticillioides*-induced SigB activation is evidenced by blue color (derived from the expression of the *ctc-lacZ* fusion) in the borders of the *B. subtilis* biofilms (colonies) closer to the fungus. The areas represented by the green and pink rectangles correspond to the biofilm areas used to quantify the β -galactosidase activity (derived from the expressed *ctc-lacZ* fusion, blue color, inside the biofilm). The relative quantification of the β -galactosidase activity is shown as bar graphs. Microorganisms (*B. subtilis* and fungi) were co-incubated in PDA (Potato Dextrose Agar) plates at 28°C during 96 h before the photograph is taken.

B. subtilis. Another interesting finding of Bartolini et al. (2019b), is that biocontrol of *F. verticillioides* by *B. subtilis* depended on the production of the antifungal lipopeptide surfactin given that $\Delta sigB$ cultures produced fewer quantities of surfactin compared to wild-type *B. subtilis*, and $\Delta srfA$ *B. subtilis* cultures showed poor antifungal activity (Figure 6).

A recent report, using fluorescent reporter gene fusions to *sigB* promoter (P_{sig} -YFP), conclusively demonstrated the

existence of stochastic pulsing of SigB activation, absent in $\Delta sigB$ cells, during biofilm formation at the interface with the roots of the model plant *Arabidopsis thaliana* (Nadezhdin et al., 2020). Interestingly, the *sigB* expression pattern was not affected when the environmental-related stress pathway was inactivated ($\Delta rsbRU$ strain) (Nadezhdin et al., 2020). In contrast, *sigB* expression was severely downregulated when the energy-related pathway ($\Delta rbsPQ$) was inactivated. These

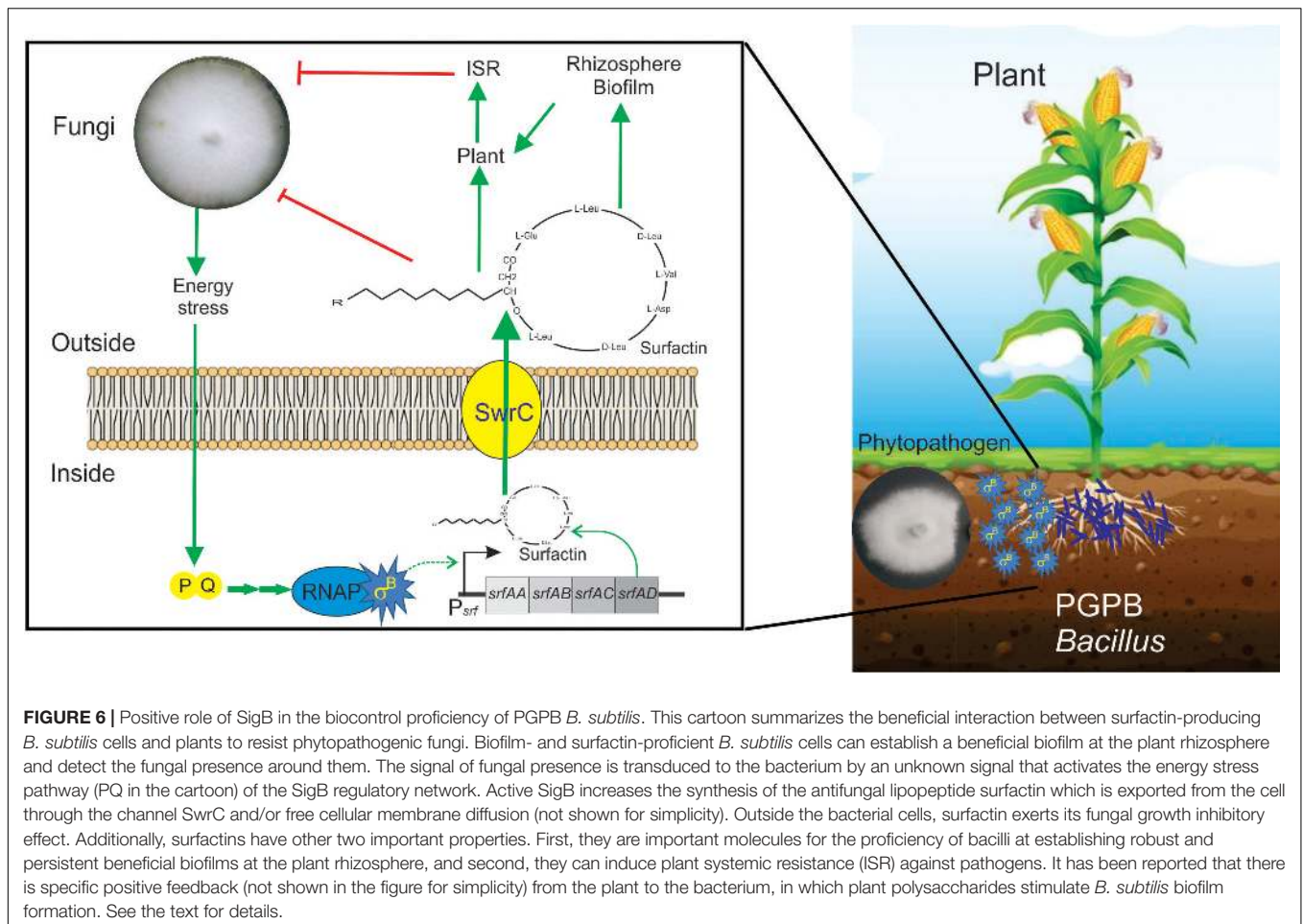


FIGURE 6 | Positive role of SigB in the biocontrol proficiency of PGPB *B. subtilis*. This cartoon summarizes the beneficial interaction between surfactin-producing *B. subtilis* cells and plants to resist phytopathogenic fungi. Biofilm- and surfactin-proficient *B. subtilis* cells can establish a beneficial biofilm at the plant rhizosphere and detect the fungal presence around them. The signal of fungal presence is transduced to the bacterium by an unknown signal that activates the energy stress pathway (PQ in the cartoon) of the SigB regulatory network. Active SigB increases the synthesis of the antifungal lipopeptide surfactin which is exported from the cell through the channel SwrC and/or free cellular membrane diffusion (not shown for simplicity). Outside the bacterial cells, surfactin exerts its fungal growth inhibitory effect. Additionally, surfactins have other two important properties. First, they are important molecules for the proficiency of bacilli at establishing robust and persistent beneficial biofilms at the plant rhizosphere, and second, they can induce plant systemic resistance (ISR) against pathogens. It has been reported that there is specific positive feedback (not shown in the figure for simplicity) from the plant to the bacterium, in which plant polysaccharides stimulate *B. subtilis* biofilm formation. See the text for details.

observations (Nadezhdin et al., 2020) agree with the results of Bartolini et al. (2019b), showing that only *B. subtilis* strains proficient in surfactin production and RsbPQ-dependent SigB activation formed beneficial biofilms at the roots of maize plants to protect them from the fungi assault. Overall these results (Bartolini et al., 2019b; Nadezhdin et al., 2020) point to the importance of the energy stress route of SigB activation in the environmentally friendly biofilm established at the plane of *B. subtilis*-plant interaction against phytopathogenic fungi of agronomic impact (Figure 6).

CONCLUDING REMARKS

SigB was the first alternative sigma factor characterized in bacteria, initially hypothesized 40 years ago as a specific transcription factor responsible for the onset of sporulation. Over the years, we have established that SigB plays much broader roles that go beyond spore formation to general stress response, adaptation, and survival. Even though early studies involved bacteria living a planktonic lifestyle, recent advances have shown the clear participation of SigB in the regulation of bacterial multicellular lifestyles, such as social biofilms and interaction with other microorganisms. SigB activation is

now reported to be casuistically or stochastically established by environmental and energy stresses or noise modulation, respectively. Much work is still needed to establish all the functions regulated by SigB, which is conserved in *B. subtilis* and many other bacteria.

AUTHOR CONTRIBUTIONS

FR and RG wrote the main text. FR and MB designed the tables and figures. All authors contributed to the article and approved the submitted version.

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REFERENCES

- Acar, M., Becskei, A., and van Oudenaarden, A. (2005). Enhancement of cellular memory by reducing stochastic transitions. *Nature* 435, 228–232. doi: 10.1038/nature03524
- Aguilar, C., Vlamakis, H., Losick, R., and Kolter, R. (2007). Thinking about *Bacillus subtilis* as a multicellular organism. *Curr. Opin. Microbiol.* 10, 638–643. doi: 10.1016/j.mib.2007.09.006
- Akbar, S., Gaidenko, T. A., Kang, C. M., O'Reilly, M., Devine, K. M., and Price, C. W. (2001). New family of regulators in the environmental signaling pathway which activates the general stress transcription factor sigma (B) of *Bacillus subtilis*. *J. Bacteriol.* 183, 1329–1338. doi: 10.1128/jb.183.4.1329-1338.2001
- Aleti, G., Lehner, S., Bacher, M., Compant, S., Nikolic, B., Plesko, M., et al. (2016). Surfactant variants mediate species, specific biofilm formation and root colonization in *Bacillus*: surfactins mediate species-specific signaling. *Environ. Microbiol.* 18, 2634–2645. doi: 10.1111/1462-2920.13405
- Alper, S., Dufour, A., Garsin, D. A., Duncan, L., and Losick, R. (1996). Role of adenosine nucleotides in the regulation of a stress-response transcription factor in *Bacillus subtilis*. *J. Mol. Biol.* 260, 165–177. doi: 10.1006/jmbi.1996.0390
- Barraud, N., Kjelleberg, S., and Rice, S. A. (2015). Dispersal from microbial biofilms. *Microbiol. Spectrum* 3:MB-0015-2014. doi: 10.1128/microbiolspec.MB-0015-2014
- Bartolini, M., Cogliati, S., Vileta, D., Bauman, C., Rateni, L., Leñini, C., et al. (2019a). Regulation of biofilm aging and dispersal in *Bacillus subtilis* by the alternative sigma factor SigB. *J. Bacteriol.* 201:e00473-18. doi: 10.1128/JB.00473-18
- Bartolini, M., Cogliati, S., Vileta, D., Bauman, C., Ramirez, W., and Grau, R. (2019b). Stress-responsive alternative sigma factor sigb plays a positive role in the antifungal proficiency of *Bacillus subtilis*. *Appl. Environ. Microbiol.* 85:e00178-19. doi: 10.1128/AEM.00178-19
- Beauregard, P. B., Chaib, Y., Vlamakis, H., Losick, R., and Kolter, R. (2013). *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1621–1630.
- Benson, A. K., and Haldenwang, W. G. (1992). Characterization of a regulatory network that controls sigma B expression in *Bacillus subtilis*. *J. Bacteriol.* 174, 749–757. doi: 10.1128/jb.174.3.749-757.1992
- Benson, A. K., and Haldenwang, W. G. (1993a). *Bacillus subtilis*σB is regulated by a binding protein (RsbW) that blocks its association with core RNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* 90, 2330–2334. doi: 10.1073/pnas.90.6.2330
- Benson, A. K., and Haldenwang, W. G. (1993b). The σB dependent promoter of the *Bacillus subtilis* sigB operon is induced by heat shock. *J. Bacteriol.* 175, 1929–1935. doi: 10.1128/jb.175.7.1929-1935.1993
- Bentley, S. D., Chater, K. F., Cerdeño-Tarraga, A. M., Challis, G. L., Thomson, N. R., James, K. D., et al. (2002). Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* 417, 141–147.
- Bhaya, D., Watanabe, N., Ogawa, T., and Grossman, A. R. (1999). The role of an alternative sigma factor in motility and pilus formation in the cyanobacterium *Synechocystis* sp. strain PCC6803. *Proc. Natl. Acad. Sci. U.S.A.* 96, 3188–3193. doi: 10.1073/pnas.96.6.3188
- Binnie, C., Lampe, M., and Losick, R. (1986). Gene encoding the s37 species of RNA polymerase s factor from *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.S.A.* 83, 5943–5947. doi: 10.1073/pnas.83.16.5943
- Bischoff, M., Dunman, P., Kormanec, J., Macapagal, D., Murphy, E., Mounts, W., et al. (2004). Microarray-based analysis of the *Staphylococcus aureus* sigma B regulon. *J. Bacteriol.* 186, 4085–4099. doi: 10.1128/jb.186.13.4085-4099.2004
- Bonilla, C. Y. (2020). Generally stressed out bacteria: environmental stress response mechanisms in Gram-positive bacteria [published online ahead of print, 2020 Feb 11]. *Integr. Comp. Biol.* 2020:icaa002.
- Borukhov, S., Lee, J., and Laptchenko, O. (2005). Bacterial transcription elongation factors: new insights into molecular mechanism of action. *Mol. Microbiol.* 55, 1315–1324. doi: 10.1111/j.1365-2958.2004.04481.x
- Borukhov, S., and Nudler, E. (2008). RNA polymerase: the vehicle of transcription. *Trends Microbiol.* 16, 126–134. doi: 10.1016/j.tim.2007.12.006
- Boylan, S. A., Redfield, A. R., Brody, M. S., and Price, C. W. (1993). Stress-induced activation of the σB transcription factor of *Bacillus subtilis*. *J. Bacteriol.* 175, 7931–7937. doi: 10.1128/jb.175.24.7931-7937.1993
- Boylan, S. A., Rutherford, A., Thomas, S. M., and Price, C. W. (1992). Activation of *Bacillus subtilis* transcription factor sigma B by a regulatory pathway responsive to stationary-phase signals. *J. Bacteriol.* 174, 3695–3706. doi: 10.1128/jb.174.11.3695-3706.1992
- Boylan, S. A., Thomas, M. D., and Price, C. W. (1991). Genetic method to identify regulons controlled by nonessential elements: isolation of a gene dependent on alternate transcription factor sB of *Bacillus subtilis*. *J. Bacteriol.* 173, 7856–7866. doi: 10.1128/jb.173.24.7856-7866.1991
- Branda, S. S., Chu, F., Kearns, D. B., Losick, R., and Kolter, R. (2006). A major protein component of the *Bacillus subtilis* biofilm matrix. *Mol. Microbiol.* 59, 1229–1238. doi: 10.1111/j.1365-2958.2005.05020.x
- Branda, S. S., González-Pastor, J. E., Ben-Yehuda, S., Losick, R., and Kolter, R. (2001). Fruiting body formation by *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.S.A.* 98, 11621–11626. doi: 10.1073/pnas.191384198
- Brigulla, M., Hoffmann, T., Krisp, A., Volker, A., Bremer, E., and Volker, U. (2003). Chill induction of the SigB-dependent general stress response in *Bacillus subtilis* and its contribution to low-temperature adaptation. *J. Bacteriol.* 185, 4305–4314. doi: 10.1128/jb.185.15.4305-4314.2003
- Brody, M. S., Stewart, V., and Price, C. W. (2009). Bypass suppression analysis maps the signalling pathway within a multidomain protein: the RsbP energy stress phosphatase 2C from *Bacillus subtilis*. *Mol. Microbiol.* 72, 1221–1234. doi: 10.1111/j.1365-2958.2009.06722.x
- Brody, M. S., Vijay, K., and Price, C. W. (2001). Catalytic function of an alpha/beta hydrolase is required for energy stress activation of the sigma (B) transcription factor in *Bacillus subtilis*. *J. Bacteriol.* 183, 6422–6428. doi: 10.1128/jb.183.21.6422-6428.2001
- Burton, A. T., DeLoughery, A., Li, G. W., and Kearns, D. B. (2019). Transcriptional regulation and mechanism of SigN (ZpdN), a pBS32-encoded sigma factor in *Bacillus subtilis*. *mBio* 10:e01899-19. doi: 10.1128/mBio.01899-19
- Cao, M., and Helmann, J. D. (2004). The *Bacillus subtilis* extracytoplasmic-function sigma X factor regulates modification of the cell envelope and resistance to cationic antimicrobial peptides. *J. Bacteriol.* 186, 1136–1146. doi: 10.1128/jb.186.4.1136-1146.2004
- Cao, M., Wang, T., Ye, R., and Helmann, J. D. (2002). Antibiotics that inhibit cell wall biosynthesis induce expression of the *Bacillus subtilis* sigma W and sigma M regulons. *Mol. Microbiol.* 45, 1267–1276. doi: 10.1046/j.1365-2958.2002.03050.x
- Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., and Ongena, M. (2015). Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis*/amyloliquefaciens: lipopeptides as inhibitors of phytopathogens. *Mol. Microbiol.* 8, 281–295. doi: 10.1111/1751-7915.12238
- Cawoy, H., Mariutto, M., Henry, G., Fisher, C., Vasilyeva, N., Thonart, P., et al. (2014). Plant defense stimulation by natural isolates of *Bacillus* depends on efficient surfactin production. *Mol. Plant Microbe Interact.* 27, 87–100. doi: 10.1094/mpmi-09-13-0262-r
- Chatterji, D., Ogawa, Y., Shimada, T., and Ishihama, A. (2007). The role of the omega subunit of RNA polymerase in expression of the *relA* gene in *Escherichia coli*. *FEMS Microbiol. Lett.* 267, 51–55. doi: 10.1111/j.1574-6968.2006.00532.x
- Chaturongakul, S., Raengpradub, S., Wiedmann, M., and Boor, K. J. (2008). Modulation of stress and virulence in *Listeria monocytogenes*. *Trends Microbiol.* 16, 388–396. doi: 10.1016/j.tim.2008.05.006
- Chen, C. C., Lewis, R. J., Harris, R., Yudkin, M. D., and Delumeau, O. (2003). A supramolecular complex in the environmental stress signalling pathway of *Bacillus subtilis*. *Mol. Microbiol.* 49, 1657–1669. doi: 10.1046/j.1365-2958.2003.03663.x
- Chen, F., Di, H., Wang, Y., Cao, Q., Xu, B., Zhang, X., et al. (2016). Small-molecule targeting of a diaphytoene desaturase inhibits *S. aureus* virulence. *Nat. Chem. Biol.* 12, 174–179. doi: 10.1038/nchembio.2003
- Chen, C. C., Yudkin, M. D., and Delumeau, O. (2004). Phosphorylation and RsbX-dependent dephosphorylation of RsbR in the RsbR-RsbS complex of *Bacillus subtilis*. *J. Bacteriol.* 186, 6830–6836. doi: 10.1128/JB.186.20.6830-6836.2004
- Cho, Y. H., Lee, E. J., Ahn, B. E., and Roe, J. H. (2001). SigB, an RNA polymerase sigma factor required for osmoprotection and proper differentiation of *Streptomyces coelicolor*. *Mol. Microbiol.* 42, 205–214. doi: 10.1046/j.1365-2958.2001.02622.x
- Chu, F., Kearns, D. B., Branda, S. S., Kolter, R., and Losick, R. (2006). Targets the master regulator of biofilm formation in *Bacillus subtilis*. *Mol. Microbiol.* 59, 1216–1228. doi: 10.1111/j.1365-2958.2005.05019.x

- Cogliati, S., Clementi, V., Francisco, M., Crespo, C., Argañaraz, F., and Grau, R. (2019). *Bacillus subtilis* delays neurodegeneration and behavioral impairment in the Alzheimer's disease model *Caenorhabditis elegans*. *J. Alzheimer's Dis.* 73, 1055–1072.
- Cramer, P., Bushnell, D. A., and Kornberg, R. D. (2001). Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. *Science* 292, 1863–1876. doi: 10.1126/science.1059493
- Dalton, T., Dowd, S. E., Wolcott, R., Sun, Y., Watters, C., Griswold, J., et al. (2011). An in vivo polymicrobial biofilm wound infection model to study interspecies interactions. *PLoS One* 6:e27317. doi: 10.1371/journal.pone.0027317
- de Been, M., Francke, C., Siezen, R. J., and Abee, T. (2011). Novel sigmaB regulation modules of Gram-positive bacteria involve the use of complex hybrid histidine kinases. *Microbiology* 157(Pt 1), 3–12. doi: 10.1099/mic.0.045740-0
- de Been, M., Tempelaars, M. H., van Schaik, W., Moezelaar, R., Siezen, R. J., and Abee, T. (2010). A novel hybrid kinase is essential for regulating the sigma(B)-mediated stress response of *Bacillus cereus*. *Environ. Microbiol.* 12, 730–745. doi: 10.1111/j.1462-2920.2009.02116.x
- Debarbouille, M., Martin-Verstraete, I., Klier, A., and Rapaport, G. (1991). The transcriptional regulator LevR of *Bacillus subtilis* has domains homologous to both s54 and phosphotransferase system-dependent regulators. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2212–2216. doi: 10.1073/pnas.88.6.2212
- Debois, D., Fernandez, O., Franzil, L., Jourdan, E., de Brogniez, A., Willems, L., et al. (2015). Plant polysaccharides initiate underground crosstalk with bacilli by inducing synthesis of the immunogenic lipopeptide surfactin: *Bacillus* lipopeptides induced by plant polymers. *Environ. Microbiol. Rep.* 7, 570–582. doi: 10.1111/1758-2229.12286
- Delumeau, O., Chen, C. C., Murray, J. W., Yudkin, M. D., and Lewis, R. J. (2006). High molecular weight complexes of RsbR and paralogues in the environmental signaling pathway of *Bacillus subtilis*. *J. Bacteriol.* 188, 7885–7892. doi: 10.1128/jb.00892-06
- DeMaio, J., Zhang, Y., Ko, C., and Bishai, W. R. (1997). *Mycobacterium tuberculosis* sigF is part of a gene cluster with similarities to the *Bacillus subtilis* sigF and sigB operons. *Tuber. Lung Dis.* 78, 3–12. doi: 10.1016/s0962-8479(97)90010-1
- DeMaio, J., Zhang, Y., Ko, C., Young, D. B., and Bishai, W. R. (1996). A stationary-phase stress-response sigma factor from *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U.S.A.* 93, 2790–2794. doi: 10.1073/pnas.93.7.2790
- Donato, V., Rodríguez Ayala, F., Cogliati, S., Bauman, C., Costa, J. G., Leñini, C., et al. (2017). *Bacillus subtilis* biofilm extends *Caenorhabditis elegans* longevity through downregulation of the insulin-like signaling pathway. *Nat. Commun.* 8:14332.
- Dufour, A., and Haldenwang, W. G. (1994). Interactions between a *Bacillus subtilis* antisigma factor (RsbW) and its antagonist (RsbV). *J. Bacteriol.* 176, 1813–1820. doi: 10.1128/jb.176.7.1813-1820.1994
- Eymann, C., Schulz, S., Gronau, K., Becher, D., Hecker, M., and Price, C. W. (2011). In vivo phosphorylation patterns of key stressosome proteins define a second feedback loop that limits activation of *Bacillus subtilis* σ B. *Mol. Microbiol.* 80, 798–810. doi: 10.1111/j.1365-2958.2011.07609.x
- Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* 14, 563–575. doi: 10.1038/nrmicro.2016.94
- Fouet, A., Namy, O., and Lambert, G. (2000). Characterization of the operon encoding the alternative sigma B factor from *Bacillus anthracis* and its role in virulence. *J. Bacteriol.* 182, 5036–5045. doi: 10.1128/jb.182.18.5036-5045.2000
- Fredrick, K., and Helmann, J. D. (1996). FlgM is the primary regulator of sigD activity, and its absence restores motility to a *sinR* mutant. *J. Bacteriol.* 178, 7010–7013. doi: 10.1128/jb.178.23.7010-7013.1996
- Gaidenko, T. A., Bie, X., Baldwin, E. P., and Price, C. W. (2012). Two surfaces of a conserved interdomain linker differentially affect output from the RST sensing module of the *Bacillus subtilis* stressosome. *J. Bacteriol.* 194, 3913–3921. doi: 10.1128/jb.00583-12
- Gaidenko, T. A., Kim, T. J., Weigel, A. L., Brody, M. S., and Price, C. W. (2006). The blue-light receptor YtvA acts in the environmental stress signaling pathway of *Bacillus subtilis*. *J. Bacteriol.* 188, 6387–6395. doi: 10.1128/jb.00691-06
- Geiman, D. E., Kaushal, D., Ko, C., Tyagi, S., Manabe, Y. C., Schroeder, B. G., et al. (2004). Attenuation of late-stage disease in mice infected by the *Mycobacterium tuberculosis* mutant lacking the SigF alternate sigma factor and identification of SigF-dependent genes by microarray analysis. *Infect. Immun.* 72, 1733–1745. doi: 10.1128/iai.72.3.1733-1745.2004
- Giachino, P., Engelmann, S., and Bischoff, M. (2001). Sigma B activity depends on RsbU in *Staphylococcus aureus*. *J. Bacteriol.* 183, 1843–1852. doi: 10.1128/jb.183.6.1843-1852.2001
- Gitt, M. A., Wang, L. F., and Doi, R. H. (1985). A strong sequence homology exists between the major RNA polymerase sigma factors of *Bacillus subtilis* and *Escherichia coli*. *J. Biol. Chem.* 260, 7178–7185.
- Gottig, N., Pedrido, M. E., Méndez, M., Lombardia, E., Rovetto, A., Philippe, V., et al. (2005). The *Bacillus subtilis* SinR and RapA developmental regulators are responsible for the inhibition of spore development by alcohol. *J. Bacteriol.* 187, 2662–2672. doi: 10.1128/jb.187.8.2662-2672.2005
- Goya, F., Xue, C., Sampedro-Torres-Quevedo, S., Arnaouteli, L., Riquelme-Dominguez, A., Romanowski, J., et al. (2020). Probiotic *Bacillus subtilis* protects against α -Synuclein aggregation in *C. elegans*. *Cell Rep.* 14, 367–380. doi: 10.1016/j.celrep.2019.12.078
- Grau, R., de Oña, P., Kunert, M., Leñini, C., Gallegos-Monterrosa, R., Mhatre, E., et al. (2015). A duo of potassium-responsive histidine kinases governs the multicellular destiny of *Bacillus subtilis*. *mBio* 6:e00581-15. doi: 10.1128/mBio.00581-15
- Gruber, T. M., and Gross, C. A. (2003). Multiple sigma subunits and the partitioning of bacterial transcription space. *Annu Rev Microbiol.* 57, 441–466. doi: 10.1146/annurev.micro.57.030502.090913
- Guilhen, C., Forestier, C., and Balestrino, D. (2017). Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. *Mol. Microbiol.* 105, 188–210. doi: 10.1111/mmi.13698
- Haldenwang, W. G. (1995). The sigma factors of *Bacillus subtilis*. *Microbiol. Rev.* 59, 1–30. doi: 10.1128/mmb.59.1.1-30.1995
- Haldenwang, W. G., and Losick, R. (1979). A modified RNA polymerase transcribes a cloned gene under sporulation control in *Bacillus subtilis*. *Nature* 282, 256–260. doi: 10.1038/282256a0
- Hecker, M., Pané-Farré, J., and Völker, U. (2007). SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. *Annu. Rev. Microbiol.* 61, 215–236. doi: 10.1146/annurev.micro.61.080706.093445
- Hecker, M., Schumann, W., and Völker, U. (1996). Heat-shock and general stress response in *Bacillus subtilis*. *Mol. Microbiol.* 19, 417–428. doi: 10.1046/j.1365-2958.1996.396932.x
- Hecker, M., and Völker, U. (2001). General stress response of *Bacillus subtilis* and other bacteria. *Adv. Microb. Physiol.* 44, 35–91. doi: 10.1016/s0065-2911(01)44011-2
- Helmann, J. D. (1991). Alternate sigma factors and the regulation of flagellar gene expression. *Mol. Microbiol.* 5, 2875–2882. doi: 10.1111/j.1365-2958.1991.tb01847.x
- Helmann, J. D. (2002). The extracytoplasmic function (ECF) sigma factors. *Adv. Microb. Physiol.* 46, 47–110. doi: 10.1016/s0065-2911(02)46002-x
- Helmann, J. D. (2019). Where to begin? Sigma factors and the selectivity of transcription initiation in bacteria. *Mol. Microbiol.* 112, 335–347. doi: 10.1111/mmi.14309
- Helmann, J. D., Wu, M. F., Gaballa, A., Kobel, P. A., Morshedi, M. M., Fawcett, P., et al. (2003). The global transcriptional response of *Bacillus subtilis* to peroxide stress is coordinated by three transcription factors. *J. Bacteriol.* 185, 243–253. doi: 10.1128/jb.185.1.243-253.2003
- Hirata, A., Klein, B. J., and Murakami, K. S. (2008). The X-ray crystal structure of RNA polymerase from Archaea. *Nature* 451, 851–854. doi: 10.1038/nature06530
- Hölscher, T., Bartels, B., Lin, Y. C., Gallegos-Monterrosa, R., Price-Whelan, A., Kolter, R., et al. (2015). Motility, chemotaxis and aerotaxis contribute to competitiveness during bacterial pellicle biofilm development. *J. Mol. Biol.* 427, 3695–3708. doi: 10.1016/j.jmb.2015.06.014
- Hong, H., Khaneja, R., Tam, N., Cazzato, A., Tan, S., Urdaci, M., et al. (2009). *Bacillus subtilis* isolated from the human gastrointestinal tract. *Res. Microbiol.* 160, 134–143. doi: 10.1016/j.resmic.2008.11.002
- Huckauf, J., Nomura, C., Forchhammer, K., and Hagemann, M. (2000). Stress responses of *Synechocystis* sp. strain PCC 6803 mutants impaired in genes encoding putative alternative sigma factors. *Microbiology* 146(Pt 11), 2877–2889. doi: 10.1099/00221287-146-11-2877
- Impens, F., Rolhion, N., Radoshevich, L., Bécavin, C., Duval, M., Mellin, J., et al. (2017). N-terminomics identifies Prli42 as a membrane miniprotein conserved in Firmicutes and critical for stressosome activation in *Listeria monocytogenes*. *Nat. Microbiol.* 2:e17005.

- Jefferson, K. (2004). What drives bacteria to produce a biofilm? *FEMS Microbiol. Lett.* 236, 163–173. doi: 10.1111/j.1574-6968.2004.tb09643.x
- Johnson, W. C., Moran, C. P. Jr., and Losick, R. (1983). Two RNA polymerase sigma factors from *Bacillus subtilis* discriminate between overlapping promoters for a developmentally regulated gene. *Nature* 302, 800–804. doi: 10.1038/302800a0
- Kaneko, T., Tanaka, N., and Kumasaka, T. (2005). Crystal structures of RsbQ, a stress-response regulator in *Bacillus subtilis*. *Protein Sci.* 14, 558–565. doi: 10.1110/ps.041170005
- Kaplan, J. B. (2010). Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. *J. Dent. Res.* 89, 205–218. doi: 10.1177/0022034509359403
- Kazmierczak, M. J., Mithoe, S. C., Boor, K. J., and Wiedmann, M. (2003). *Listeria monocytogenes* sigma B regulates stress response and virulence functions. *J. Bacteriol.* 185, 5722–5734. doi: 10.1128/jb.185.19.5722-5734.2003
- Kearns, D. B., Chu, F., Branda, S. S., Kolter, R., and Losick, R. (2005). A master regulator for biofilm formation by *Bacillus subtilis*. *Mol. Microbiol.* 55, 739–749. doi: 10.1111/j.1365-2958.2004.04440.x
- Kearns, D. B., and Losick, R. (2003). Swarming motility in undomesticated *Bacillus subtilis*. *Mol. Microbiol.* 49, 581–590. doi: 10.1046/j.1365-2958.2003.03584.x
- Kim, K. H., Kabir, E., and Jahan, S. A. (2017). Exposure to pesticides and the associated human health effects. *Sci. Total Environ.* 575, 525–535. doi: 10.1016/j.scitotenv.2016.09.009
- Kim, T. J., Gaidenko, T. A., and Price, C. W. (2004a). A multicomponent protein complex mediates environmental stress signaling in *Bacillus subtilis*. *J. Mol. Biol.* 341, 135–150. doi: 10.1016/j.jmb.2004.05.043
- Kim, T. J., Gaidenko, T. A., and Price, C. W. (2004b). In vivo phosphorylation of partner switching regulators correlates with stress transmission in the environmental signaling pathway of *Bacillus subtilis*. *J. Bacteriol.* 186, 6124–6132. doi: 10.1128/jb.186.18.6124-6132.2004
- Kint, N., Alves Feliciano, C., Hamiot, A., Denic, M., Dupuy, B., and Martin-Verstraete, I. (2019). The σ_B signalling activation pathway in the enteropathogen *Clostridioides difficile*. *Environ. Microbiol.* 21, 2852–2870. doi: 10.1111/1462-2920.14642
- Kint, N., Janoir, C., Monot, M., Hoys, S., Soutourina, O., Dupuy, B., et al. (2017). The alternative sigma factor σ_B plays a crucial role in adaptive strategies of *Clostridium difficile* during gut infection. *Environ. Microbiol.* 19, 1933–1958. doi: 10.1111/1462-2920.13696
- Kovács, ÁT. (2016). Bacterial differentiation via gradual activation of global regulators. *Curr. Genet* 62, 125–128. doi: 10.1007/s00294-015-0524-8
- Kovács, ÁT., and Dragoš, A. (2019). Evolved biofilm: review on the experimental evolution studies of *Bacillus subtilis* Pellicles. *J. Mol. Biol.* 431, 4749–4759. doi: 10.1016/j.jmb.2019.02.005
- Kumar, A., Grimes, B., Fujita, N., Makino, K., Malloch, R. A., Hayward, R. S., et al. (1994). Role of the sigma 70 subunit of *Escherichia coli* RNA polymerase in transcription activation. *J. Mol. Biol.* 14, 405–413.
- Kunst, F., Ogasawara, N., Moszer, I., Albertini, A. M., Alloni, G., Azevedo, V., et al. (1997). The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* 390, 249–256.
- Kuo, S., Demeler, B., and Haldenwang, W. G. (2008). The growth-promoting and stress response activities of the *Bacillus subtilis* GTP binding protein Obg are separable by mutation. *J. Bacteriol.* 190, 6625–6635. doi: 10.1128/jb.00799-08
- Kussell, E., and Leibler, S. (2005). Phenotypic diversity, population growth, and information in fluctuating environments. *Science* 309, 2075–2078. doi: 10.1126/science.1114383
- Kwon, E., Pathak, D., Kim, H. U., Dahal, P., Ha, S. C., Lee, S. S., et al. (2019). Structural insights into stressosome assembly. *I.U.Cr.J.* 6(Pt 5), 938–947. doi: 10.1107/s205225251900945x
- Lee, E. J., Cho, Y. H., Kim, H. S., Ahn, B. E., and Roe, J. H. (2004). Regulation of sigmaB by an anti- and an anti-anti-sigma factor in *Streptomyces coelicolor* in response to osmotic stress. *J. Bacteriol.* 186, 8490–8498. doi: 10.1128/jb.186.24.8490-8498.2004
- Lewis, P. J., Doherty, G. P., and Clarke, J. (2008). Transcription factor dynamics. *Microbiology* 154(Pt 7), 1837–1844.
- Liebal, U. W., Millat, T., Marles-Wright, J., Lewis, R. J., and Wolkenhauer, O. (2013). Simulations of stressosome activation emphasize allosteric interactions between RsbR and RsbT. *BMC Syst Biol.* 7:3. doi: 10.1186/1752-0509-7-3
- Liu, J., Prindle, A., Humphries, J., Gabalda-Sagarra, M., Asally, M., Lee, D. Y., et al. (2015). Metabolic co-dependence gives rise to collective oscillations within biofilms. *Nature* 523, 550–554. doi: 10.1038/nature14660
- Locke, J. C., Young, J. W., Fontes, M., Hernández Jiménez, M. J., and Elowitz, M. B. (2011). Stochastic pulse regulation in bacterial stress response. *Science* 334, 366–369. doi: 10.1126/science.1208144
- Lombardia, E., Rovetto, A. J., Arabolaza, A. L., and Grau, R. (2006). A LuxS dependent cell-to-cell language regulates social behavior and development in *Bacillus subtilis*. *J. Bacteriol.* 188, 4442–4452. doi: 10.1128/jb.00165-06
- Losick, R., and Pero, J. (1981). Cascade of sigma factors. *Cell* 25, 582–584.
- Losick, R., and Pero, J. (2018). For want of a template. *Cell* 172, 1146–1152. doi: 10.1016/j.cell.2018.02.041
- Losick, R., and Stragier, P. (1992). Crisscross regulation of cell-type-specific gene expression during development in *Bacillus subtilis*. *Nature* 355, 601–604. doi: 10.1038/355601a0
- Luo, C., Zhou, H., Zou, J., Wang, X., Zhang, R., Xiang, Y., et al. (2015). Bacillomycin L and surfactin contribute synergistically to the phenotypic features of *Bacillus subtilis* 916 and the biocontrol of rice sheath blight induced by *Rhizoctonia solani*. *Appl. Microbiol. Biotechnol.* 99, 1897–1910. doi: 10.1007/s00253-014-6195-4
- Luo, Y., and Helmann, J. D. (2012). Analysis of the role of *Bacillus subtilis* σ_M in β -lactam resistance reveals an essential role for c-di-AMP in peptidoglycan homeostasis. *Mol. Microbiol.* 83, 623–639. doi: 10.1111/j.1365-2958.2011.07953.x
- Marles-Wright, J., Grant, T., Delumeau, O., van Duinen, G., Firbank, S. J., Lewis, P. J., et al. (2008). Molecular architecture of the "stressosome," a signal integration and transduction hub. *Science* 322, 92–96. doi: 10.1126/science.1159572
- Martinez, L., Reeves, A., and Haldenwang, W. (2010). Stressosomes formed in *Bacillus subtilis* from the RsbR protein of *Listeria monocytogenes* allow σ_B activation following exposure to either physical or nutritional stress. *J. Bacteriol.* 192, 6279–6286. doi: 10.1128/jb.00467-10
- Mascher, T., Margulis, N. G., Wang, T., Ye, R. W., and Helmann, J. D. (2003). Cell wall stress responses in *Bacillus subtilis*: the regulatory network of the bacitracin stimulator. *Mol. Microbiol.* 50, 1591–1604. doi: 10.1046/j.1365-2958.2003.03786.x
- McDougald, D., Rice, S. A., Barraud, N., Steinberg, P., and Kjelleberg, S. (2011). Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. *Nat. Rev. Microbiol.* 10, 39–50. doi: 10.1038/nrmicro2695
- Méndez, M., Orsaria, L., Philippe, V., Pedrido, M. E., and Grau, R. (2004). Novel Roles of the master transcription factors Spo0A and σ_B for survival and sporulation of *Bacillus subtilis* at low growth temperature. *J. Bacteriol.* 186, 989–1000. doi: 10.1128/jb.186.4.989-1000.2004
- Mendez, R., Gutierrez, A., Reyes, J., and Márquez-Magaña, L. (2012). The extracytoplasmic function sigma factor SigY is important for efficient maintenance of the Sp β prophage that encodes sublincan in *Bacillus subtilis*. *DNA Cell Biol.* 31, 946–955. doi: 10.1089/dna.2011.1513
- Michele, T. M., Ko, C., and Bishai, W. R. (1999). Exposure to antibiotics induces expression of the *Mycobacterium tuberculosis* sigF gene: implications for chemotherapy against mycobacterial persisters. *Antimicrob. Agents Chemother.* 43, 218–225. doi: 10.1128/aac.43.2.218
- Minakhin, L., Bhagat, S., Brunning, A., Campbell, E. A., Darst, S. A., Ebright, R. H., et al. (2001). Bacterial RNA polymerase subunit omega and eukaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly. *Proc. Natl. Acad. Sci. U.S.A.* 98, 892–897. doi: 10.1073/pnas.98.3.892
- Moore, C. M., Nakano, M. M., Wang, T., Ye, R. W., and Helmann, J. D. (2004). Response of *Bacillus subtilis* to nitric oxide and the nitrosating agent sodium nitroprusside. *J. Bacteriol.* 186, 4655–4664. doi: 10.1128/jb.186.14.4655-4664.2004
- Moran, C. P. Jr., Lang, N., LeGrice, S. F. J., Lee, G., Stevens, M., Sonenshein, A. L., et al. (1982). Nucleotide sequences that signal the initiation of transcription and translation in *Bacillus subtilis*. *Mol. Gen. Genet.* 186, 339–346. doi: 10.1007/bf00729452
- Mukherjee, K., Nagai, H., Shimamoto, N., and Chatterji, D. (1999). GroEL is involved in activation of *Escherichia coli* RNA polymerase devoid of the omega

- subunit *in vivo*. *Eur. J. Biochem.* 266, 228–235. doi: 10.1046/j.1432-1327.1999.00848.x
- Murakami, K. (2015). Structural biology of bacterial RNA polymerase. *Biomolecules* 5, 848–864. doi: 10.3390/biom5020848
- Murray, E. J., Strauch, M. A., and Stanley-Wall, N. R. (2009). Sigma X is involved in controlling *Bacillus subtilis* biofilm architecture through the AbrB homologue Abh. *J. Bacteriol.* 191, 6822–6832. doi: 10.1128/jb.00618-09
- Nadezhdin, E., Murphy, N., Dalchau, N., Phillips, A., and Locke, J. C. W. (2020). Stochastic pulsing of gene expression enables the generation of spatial patterns in *Bacillus subtilis* biofilms. *Nat Commun.* 19:950.
- Nicholson, W. L., Sun, D. X., Setlow, B., and Setlow, P. (1989). Promoter specificity of sigma G-containing RNA polymerase from sporulating cells of *Bacillus subtilis*: identification of a group of forespore-specific promoters. *J. Bacteriol.* 171, 2708–2718. doi: 10.1128/jb.171.5.2708-2718.1989
- Nicolas, P., Mader, U., Dervyn, E., Rochat, T., Leduc, A., Pigeonneau, N., et al. (2012). Condition-dependent transcriptome reveals high-level regulatory architecture in *Bacillus subtilis*. *Science* 335, 1103–1106.
- O'Byrne, C. P., and Karatzas, K. A. (2008). The role of sigma B (sigma B) in the stress adaptations of *Listeria monocytogenes*: overlaps between stress adaptation and virulence. *Adv. Appl. Microbiol.* 65, 115–140. doi: 10.1016/s0065-2164(08)00605-9
- Ondrusch, N., and Kretsch, J. (2011). Blue and red light modulates SigB-dependent gene transcription, swimming motility and invasiveness in *Listeria monocytogenes*. *PLoS One* 6:e16151. doi: 10.1371/journal.pone.0016151
- Ongena, M., and Jacques, P. (2008). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol.* 16, 115–125. doi: 10.1016/j.tim.2007.12.009
- Ongena, M., Jacques, P., Toure, Y., Destain, J., Jabrane, A., and Thonart, P. (2005). Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.* 69, 29–38. doi: 10.1007/s00253-005-1940-3
- Pané-Farré, J., Jonas, B., Hardwick, S., Gronau, K., Lewis, R., Hecker, M., et al. (2009). Role of RsbU in controlling SigB activity in *Staphylococcus aureus* following alkaline stress. *J. Bacteriol.* 191, 2561–2575. doi: 10.1128/jb.01514-08
- Pané-Farré, J., Lewis, R. J., and Stülke, J. (2005). The RsbRST stress module in bacteria: a signalling system that may interact with different output modules. *J. Mol. Microbiol. Biotechnol.* 9, 65–76. doi: 10.1159/000088837
- Pedrido, M. E., de Oña, P., Ramirez, W., Leñini, C., Goñi, A., and Grau, R. (2013). Spo0A links de novo fatty acid synthesis to sporulation and biofilm development in *Bacillus subtilis*. *Mol. Microbiol.* 87, 348–367. doi: 10.1111/mmi.12102
- Petersohn, A., Bernhardt, J., Gerth, U., Höper, D., Koburger, T., Völker, U., et al. (1999). Identification of sigma(B)-dependent genes in *Bacillus subtilis* using a promoter consensus-directed search and oligonucleotide hybridization. *J. Bacteriol.* 181, 5718–5724. doi: 10.1128/jb.181.18.5718-5724.1999
- Pietiainen, M., Gardemeister, M., Mecklin, M., Leskelä, S., Sarvas, M., and Kontinen, V. P. (2005). Cationic antimicrobial peptides elicit a complex stress response in *Bacillus subtilis* that involves ECF-type sigma factors and two-component signal transduction systems. *Microbiology* 151, 1577–1592. doi: 10.1099/mic.0.27761-0
- Piewngam, P., Yue, Z. P., Nguyen, T. H., Dickey, S. W., Joo, H. S., Villaruz, A. E., et al. (2018). Pathogen elimination by probiotic *Bacillus* via signaling interference. *Nature* 562, 532–537. doi: 10.1038/s41586-018-0616-y
- Piggot, P. J., and Hilbert, D. W. (2004). Sporulation of *Bacillus subtilis*. *Curr. Opin. Microbiol.* 7, 579–586.
- Pingali, P. L. (2012). Green revolution: impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12302–12308. doi: 10.1073/pnas.0912953109
- Predich, M., Nair, G., and Smith, I. (1992). *Bacillus subtilis* early sporulation genes *kinA*, *spo0F*, and *spo0A* are transcribed by the RNA polymerase containing sigma H. *J. Bacteriol.* 174, 2771–2778. doi: 10.1128/jb.174.9.2771-2778.1992
- Price, C. W. (2000). "Protective function and regulation of the general stress response in *Bacillus subtilis* and related Gram-positive bacteria," in *Bacterial Stress Responses*, eds G. Storz and R. Hengge-Aronis (Washington, DC: ASM Press), 179–197.
- Price, C. W. (2002). "General stress response," in *Bacillus subtilis and its Closest Relatives. From Genes to Cells*, eds A. L. Sonenshein, J. A. Hoch, and R. Losick (Washington, DC: ASM Press), 369–384.
- Raaijmakers, J. M., De Bruijn, I., Nybroe, O., and Ongena, M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiol. Rev.* 34, 1037–1062. doi: 10.1111/j.1574-6976.2010.00221.x
- Raja, N. (2013). Biopesticides and biofertilizers: ecofriendly sources for sustainable agriculture. *J. Biofertil. Biopestic.* 4, 1–2.
- Reder, A., Albrecht, D., Gerth, U., and Hecker, M. (2012a). Cross-talk between the general stress response and sporulation initiation in *Bacillus subtilis* - the sigma(B) promoter of spoE represents an AND-gate. *Environ. Microbiol.* 14, 2741–2756. doi: 10.1111/j.1462-2920.2012.02755.x
- Reder, A., Gerth, U., and Hecker, M. (2012b). Integration of sigma B activity into the decision-making process of sporulation initiation in *Bacillus subtilis*. *J. Bacteriol.* 194, 1065–1074. doi: 10.1128/jb.06490-11
- Reeves, A., and Haldenwang, W. G. (2006). Isolation and characterization of dominant mutations in the *Bacillus subtilis* stressosome components RsbR and RsbS. *J. Bacteriol.* 189, 1531–1541. doi: 10.1128/jb.01649-06
- Roels, S., Driks, A., and Losick, R. (1992). Characterization of spoIVA, a sporulation gene involved in coat morphogenesis in *Bacillus subtilis*. *J. Bacteriol.* 174, 575–585. doi: 10.1128/jb.174.2.575-585.1992
- Rothstein, D. M., Lazinski, D., Osburne, M. S., and Sonenshein, A. L. (2017). A Mutation in the *Bacillus subtilis* rsbU gene that limits RNA synthesis during sporulation. *J. Bacteriol.* 199, e212–e217.
- Scott, J. M., and Haldenwang, W. G. (1999). Obg, an essential GTP binding protein of *Bacillus subtilis*, is necessary for stress activation of transcription factor sigma(B). *J. Bacteriol.* 181, 4653–4660. doi: 10.1128/jb.181.15.4653-4660.1999
- Shaw, L., Aish, J., Davenport, J., Brown, M., Lithgow, J., Simmonite, K., et al. (2006). Investigations into sigma B-modulated regulatory pathways governing extracellular virulence determinant production in *Staphylococcus aureus*. *J. Bacteriol.* 188, 6070–6080. doi: 10.1128/jb.00551-06
- Singh, P. K., Bartalomej, S., Hartmann, R., Jeckel, H., Vidakovic, L., Nadell, C., et al. (2017). *Vibrio cholerae* combines individual and collective sensing to trigger biofilm dispersal. *Curr. Biol.* 27, 3359–3366. doi: 10.1016/j.cub.2017.09.041
- Smolentseva, O., Gusarov, I., Gautier, I., Shamovsky, I., De Francesco, A., Losick, R., et al. (2017). Mechanism of biofilm-mediated stress resistance and lifespan extension in *Caenorhabditis elegans*. *Sci. Rep.* 7:7137.
- Sorokin, A., Bolotin, A., Purnelle, B., Hilbert, H., Lauber, J., Dusterhöft, A., et al. (1997). Sequence of the *Bacillus subtilis* genome region in the vicinity of the lev operon reveals two new extracytoplasmic function RNA polymerase sigma factors SigV and SigZ. *Microbiology* 143(Pt 9), 2939–2943. doi: 10.1099/00221287-143-9-2939
- Stacy, A., Everett, J., Jorth, P., Trivedi, U., Rumbaugh, K. P., and Whiteley, M. (2014). Bacterial fight-and-flight responses enhance virulence in a polymicrobial infection. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7819–7824. doi: 10.1073/pnas.1400586111
- Sun, D., Fajardo-Cavazos, P., Sussman, M. D., Tovar-Rojo, F., Cabrera-Martinez, R.-M., and Setlow, P. (1991). Effect of chromosome location of *Bacillus subtilis* forespore genes on their spo gene dependence and transcription by E-sigma F: identification of features of good E-sigma F-dependent promoters. *J. Bacteriol.* 173, 7867–7874. doi: 10.1128/jb.173.24.7867-7874.1991
- Tago, D., Andersson, H., and Treich, N. (2014). "Pesticides and health: a review of evidence on health effects, valuation of risks, and benefit-cost analysis," in *Advances in Health Economics and Health Services Research*, eds G. C. Blomquist and K. Bolin (Bingley: Emerald Group Publishing Ltd), 203–295. doi: 10.1108/s0731-2199_2014_0000024006
- Thackray, P. D., and Moir, A. (2003). SigM, an extracytoplasmic function sigma factor of *Bacillus subtilis*, is activated in response to cell wall antibiotics, ethanol, heat, acid, and superoxide stress. *J. Bacteriol.* 185, 3491–3498. doi: 10.1128/jb.185.12.3491-3498.2003
- Toledo-Arana, A., Dussurget, O., Nikitas, G., Sesto, N., Guet-Revillet, H., Balestrino, D., et al. (2009). The *Listeria* transcriptional landscape from saprophytism to virulence. *Nature* 459, 950–956. doi: 10.1038/nature08080
- Trach, K., and Hoch, J. A. (1989). The *Bacillus subtilis* spoB stage 0 sporulation operon encodes an essential GTP-binding protein. *J. Bacteriol.* 171, 1362–1371. doi: 10.1128/jb.171.3.1362-1371.1989
- Tran, V., Geraci, K., Midili, G., Satterwhite, W., Wright, R., and Bonilla, C. Y. (2019). Resilience to oxidative and nitrosative stress is mediated by the stressosome, RsbP and SigB in *Bacillus subtilis*. *J. Basic Microbiol.* 59, 834–845. doi: 10.1002/jobm.201900076

- van der Veen, S., and Abee, T. (2010). Importance of SigB for *Listeria monocytogenes* static and continuous-flow biofilm formation and disinfectant resistance. *Appl. Environ. Microbiol.* 76, 7854–7860. doi: 10.1128/aem.01519-10
- van Schaik, W., and Abee, T. (2005). The role of sigmaB in the stress response of Gram-positive bacteria – targets for food preservation and safety. *Curr. Opin. Biotechnol.* 16, 218–224. doi: 10.1016/j.copbio.2005.01.008
- van Schaik, W., Tempelaars, M. H., Wouters, J. A., de Vos, W. M., and Abee, T. (2004). The alternative sigma factor sigmaB of *Bacillus cereus*: response to stress and role in heat adaptation. *J. Bacteriol.* 186, 316–325. doi: 10.1128/jb.186.2.316-325.2004
- Vijay, K., Brody, M. S., Fredlund, E., and Price, C. W. (2000). A PP2C phosphatase containing a PAS domain is required to convey signals of energy stress to the σ B transcription factor of *Bacillus subtilis*. *Mol. Microbiol.* 35, 180–188. doi: 10.1046/j.1365-2958.2000.01697.x
- Vlamakis, H., Chai, Y., Beaugregard, P., Losick, R., and Kolter, R. (2013). Sticking together: building a biofilm the *Bacillus subtilis* way. *Nat. Rev. Microbiol.* 11, 157–168. doi: 10.1038/nrmicro2960
- Völker, U., Luo, T., Smirnova, N., and Haldenwang, W. (1997). Stress activation of *Bacillus subtilis* sigma B can occur in the absence of the sigma B negative regulator RsbX. *J. Bacteriol.* 179, 1980–1984. doi: 10.1128/jb.179.6.1980-1984.1997
- Völker, U., Völker, A., Maul, B., Hecker, M., Dufour, A., and Haldenwang, W. G. (1995). Separate mechanisms activate σ B of *Bacillus subtilis* in response to environmental and metabolic stresses. *J. Bacteriol.* 177, 3771–3780. doi: 10.1128/jb.177.13.3771-3780.1995
- Vrentas, C. E., Gaal, T., Ross, W., Ebright, R. H., and Gourse, R. L. (2005). Response of RNA polymerase to ppGpp: requirement for the omega subunit and relief of this requirement by DksA. *Genes Dev.* 19, 2378–2387. doi: 10.1101/gad.1340305
- Wiedmann, M., Arvik, T. J., Hurley, R. J., and Boor, K. J. (1998). General stress transcription factor sigmaB and its role in acid tolerance and virulence of *Listeria monocytogenes*. *J. Bacteriol.* 180, 3650–3656. doi: 10.1128/jb.180.14.3650-3656.1998
- Wiegert, T., Homuth, G., Versteeg, S., and Schumann, W. (2001). Alkaline shock induces the *Bacillus subtilis* sigma W regulon. *Mol. Microbiol.* 41, 59–71. doi: 10.1046/j.1365-2958.2001.02489.x
- Williams, E. P., Lee, J. H., Bishai, W. R., Colantuoni, C., and Karakousis, P. C. (2007). Mycobacterium tuberculosis SigF regulates genes encoding cell wall-associated proteins and directly regulates the transcriptional regulatory gene phoY1. *J. Bacteriol.* 189, 4234–4242. doi: 10.1128/jb.00201-07
- Wise, A. A., and Price, C. W. (1995). Four additional genes in the sigB operon of *Bacillus subtilis* that control activity of the general stress factor sigma B in response to environmental signals. *J. Bacteriol.* 177, 123–133. doi: 10.1128/jb.177.1.123-133.1995
- Wu, S., de Lencastre, H., and Tomasz, A. (1996). Sigma-B, a putative operon encoding alternate sigma factor of *Staphylococcus aureus* RNA polymerase: molecular cloning and DNA sequencing. *J. Bacteriol.* 178, 6036–6042. doi: 10.1128/jb.178.20.6036-6042.1996
- Yahav, S., Berkovich, Z., Ostrov, I., Reifen, R., and Shemesh, M. (2018). Encapsulation of beneficial probiotic bacteria in extracellular matrix from biofilm-forming *Bacillus subtilis*. *Artif. Cells Nanomed. Biotechnol.* 46, 974–982. doi: 10.1080/21691401.2018.1476373
- Yang, X., Kang, C. M., Brody, M. S., and Price, C. W. (1996). Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. *Genes Dev.* 10, 2265–2275. doi: 10.1101/gad.10.18.2265
- Zellmeier, S., Hofmann, C., Thomas, S., Wiegert, T., and Schumann, W. (2005). Identification of sigma V-dependent genes of *Bacillus subtilis*. *FEMS Microbiol. Lett.* 253, 221–229. doi: 10.1111/j.1365-2958.2006.05131.x
- Zerriouh, H., de Vicente, A., Perez-Garcia, A., and Romero, D. (2014). Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. *Environ. Microbiol.* 16, 2196–2211. doi: 10.1111/1462-2920.12271
- Zhang, G., Campbell, E. A., Minakhin, L., Richter, C., Severinov, K., and Darst, S. A. (1999). Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 Å resolution. *Cell* 98, 811–824. doi: 10.1016/s0092-8674(00)81515-9
- Zhang, S., and Haldenwang, W. G. (2005). Contributions of ATP, GTP, and redox state to nutritional stress activation of the *Bacillus subtilis* sigmaB transcription factor. *J. Bacteriol.* 187, 7554–7560. doi: 10.1128/JB.187.22.7554-7560.2005
- Zhang, S., Scott, J. M., and Haldenwang, W. G. (2001). Loss of ribosomal protein L11 blocks stress activation of the *Bacillus subtilis* transcription factor sigma(B). *J. Bacteriol.* 183, 2316–2321. doi: 10.1128/JB.183.7.2316-2321.2001
- Zheng, L., Halberg, R., Roels, S., Ichikawa, H., Kroos, L., and Losick, R. (1992). Sporulation regulatory protein GerE from *Bacillus subtilis* binds to and can activate or repress transcription from promoters for mother-cell-specific genes. *J. Mol. Biol.* 226, 1037–1050. doi: 10.1016/0022-2836(92)91051-p

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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