# Phenotypic plasticity: the role of a phosphatase family in the genetic regulation of Bacilli

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**Abstract**

In the last two decades, an increasing number of bacterial species have been recognized that are able to generate a phenotypically diverse population that shares an identical genotype. This ability is dependent on a complex genetic regulatory network that includes cellular and environmental signals, as well as stochastic elements. Among *Bacilli*, a broadly distributed family of Rap (Response-regulator aspartyl phosphate) phosphatases is known to modulate the function of the main phenotypic heterogeneity regulators by controlling their phosphorylation. Even more, their related extracellular Phr (Phosphatase regulator) peptides function as quorum sensing signals, creating a cell-cell communication network that regulates the phenotypic development of the entire population. In this review, we examine the role that the Rap phosphatases and their Phr peptides play in the regulation of *B. subtilis* phenotypic differentiation, and in other members of the *Bacillus* genus. We also highlight the contribution of these regulatory elements to the fitness of bacterial cells and mobile genetic elements, e.g. prophages and conjugative vectors.

Phenotypic plasticity: the role of a phosphatase family in the genetic regulation of *Bacilli*

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Running title: Rap phosphatase regulatory network of *Bacilli*

**Abstract**

In the last two decades, an increasing number of bacterial species have been recognized that are able to generate a phenotypically diverse population that shares an identical genotype. This ability is dependent on a complex genetic regulatory network that includes cellular and environmental signals, as well as stochastic elements. Among*Bacilli* , a broadly distributed family of Rap (*R* esponse-regulator *a* spartyl *p* hosphate) phosphatases is known to modulate the function of the main phenotypic heterogeneity regulators by controlling their phosphorylation. Even more, their related extracellular Phr (*Ph* osphatase*r* egulator) peptides function as quorum sensing signals, creating a cell-cell communication network that regulates the phenotypic development of the entire population. In this review, we examine the role that the Rap phosphatases and their Phr peptides play in the regulation of *B. subtilis* phenotypic differentiation, and in other members of

the *Bacillus* genus. We also highlight the contribution of these regulatory elements to the fitness of bacterial cells and mobile genetic elements, e.g. prophages and conjugative vectors.

**Keywords**

Rap phosphatase, differentiation, phenotypic heterogeneity, Bacillus, biofilm

## Introduction

Bacteria in natural settings are constantly exposed to changing environmental conditions, and they must adapt to those changes in order to survive. Developing a phenotypically heterogeneous population is a strategy that bacteria utilize to increase their environmental fitness, and as a survival mechanism . This is due to the benefit for the population as a whole driven by cell-level phenotypic differences. Phenotypic heterogeneity can allow specific cells to survive sudden environmental changes that kill other members of the population. It can also lead to division of labour between individuals, which can increase the population's growth rate and facilitate the development of new biological functions . In the last decade, the study of phenotypic heterogeneity among microbial populations and communities has become a major research focus, and new techniques and models are being generated to explore this facet of microbiology .

*Bacillus subtilis* is a Gram-positive non-pathogenic bacterium that has been studied for over a century in a wide range of topics , and has become a model organism for the study of bacterial differentiation, including community movement on semi-solid agar surfaces, swarming and sliding , sporulation , and biofilm formation . An interesting characteristic of *B. subtilis,* both under planktonic and biofilm conditions, is that its cells divide into discrete subpopulations, each with a different phenotype although all still possessing the same genotype . This phenotypic differentiation leads to division of labor or bet hedging, providing an important ecological advantage to this bacterium . In the following sections we address the role that the family of Rap phosphatases and their Phr peptides play in the regulation of *B. subtilis* phenotypic differentiation among *Bacilli* , their mechanism of action and structural functionality, as well as the ecological and genetic reasons that may explain their wide distribution in this genus.

## Master regulators of phenotypic differentiation in *B. subtilis*

In *B. subtilis* , the response regulators DegU, Spo0A, and ComA are recognized as the master switches that control the development of population heterogeneity . The activity of these three heterogeneity modulators depends on the ratios of the respective proteins in their non-phosphorylated and phosphorylated states (phosphorylated regulators are henceforth indicated with P). In general, the phosphorylation state affects the regulator's affinity for the promotor regions of the genes that they regulate . Delicate modulation of these ratios allows *B. subtilis* to develop a heterogeneous population, where cells adapt to small environmental differences (micro-niches) within the population, especially in the spatial structure of biofilms .

DegU is the response regulator of the DegS/U two component system. DegS is a cytoplasmic sensor histidine kinase that directly phosphorylates DegU. The DegU regulon is extensive and includes genes associated to motility (e.g. flagellum production) and biofilm formation (e.g. hydrophobin protein and exoenzymes needed for substrate degradation) . Non-phosphorylated DegU activates competence development, while depending on the relative amount of DegU P different sets of genes of its regulon are transcribed that provide a smooth transition from surface spreading to settlement during biofilm development .

Spo0A is a transcriptional regulator that controls the expression of hundreds of genes and operons in *B. subtilis* important among others for biofilm matrix production and the generation of spores . Spo0A is phosphorylated via a phosphorelay that can be initiated by any of five known independent histidine kinases (KinA, KinB, KinC, KinD, and KinE) . Once activated by their cognate signals, the Kin kinases phosphorylate the response regulator Spo0F, which in turn transfers the phosphoryl group to a secondary response regulator, Spo0B, which finally phosphorylates Spo0A. Like DegU, the amount of Spo0A P in the cells determines which of its target genes are expressed .

The third major master switch of population heterogeneity in *B. subtilis* , ComA, is directly modulated by

a quorum sensing (QS) system . QS is a common cell-communication strategy that relies on the production and detection of extracellular autoinducer signaling molecules by cells of the same species . ComA-related QS system uses the ComX peptide as its autoinducer and the membrane localized ComP histidine kinase as sensor. The extracellular ComX signal activates ComP, leading to autophosphorylation and transfer of phosphate to ComA . Once phosphorylated, ComA controls the production of surfactin (an important surfactant lipopeptide), and the development of competence in *B. subtilis* cells .

**A family of regulatory phosphatases**

The genetic network of *B. subtilis* contains multiple cross-talk points between the activities of the three main regulators described above that guarantees that population differentiates accordingly to its environmental conditions . Furthermore, *B. subtilis* possesses a family of QS-regulated Rap phosphatases that fine-tunes this intertwinned genetic network .

The Rap phosphatases are conserved proteins (>25% of sequence identity) of ca. 380 amino acids that are able to hinder the phosphorylation of Spo0A, DegU, or ComA, thus preventing or delaying the expression of genes that depends of high levels of the phosphorylated versions of these regulators . Early studies showed that the primary function of these proteins is to directly dephosphorylate their target regulators. The exception is the regulation of Spo0A, where the cognate Rap phosphatases act on upstream members of the phosphorelay, such as Spo0F P . Some Rap phosphatases can also bind to their target transcriptional regulators, forming a complex that can no longer adhere to DNA . Shortly after the discovery of the first Rap phosphatases (RapA and RapB), it was recognized that a small gene directly following*rapA* was involved in RapA regulation: its expression results in the production of a five-amino acid peptide that binds and inhibits the activity of RapA, and thus was called PhrA, *ph* osphatase*r* egulator A .

Subsequent studies have later revealed a wide variety of *rap*genes in the genome of *B. subtilis* , most of which are followed (and slightly overlapped) by *phr* genes that code for small proteins of ca. 40 amino acids known as Phr pro-peptides. The*rap-phr* gene pairs are often recognized as cassettes, and the production of their respective proteins is translationally coupled . Once produced, the Rap phosphatases can immediately exert their regulatory function, either by dephosphorylating or preventing the DNA-binding of their target transcriptional regulator (Fig. 1). The pro-peptides encoded by the *phr* genes follow a more complicated path to become active. Phr pro-peptides contain export signal sequences in their N-terminal portion, followed by cleavage peptidase signal domains and hydrophilic C-terminal domains. The Phr pro-peptides are mobilized to the cell membrane, where they are processed by peptidases that produce 5-6 amino acid Phr peptides in the extracellular space. The mature Phr peptides, upon reaching threshold concentrations, are imported back into the cell by the Opp oligopeptide permease. Once inside the cell, Phr peptides can finally directly bind to their respective cognate Rap phosphatase and induce a conformational change that blocks Rap activity .

**Regulatory function and mechanisms of the Rap-Phr cassettes**

The regulatory function and mechanism of action of the Rap phosphatases and Phr peptides has been intensively studied. The known Rap phosphatases have been studied in diverse strains, finding an apparent redundancy in their function: most Rap phosphatases act upon Spo0F P, ComA P, or both . Furthermore, these investigations have revealed that certain Rap-Phr cassettes are encoded on plasmids, and that these regulatory modulators are a common feature of other members of the*Bacillus* genus . Table 1 presents the known function of those Rap phosphatases that have been studied or reported independently, along with their possible action mechanism.

All known Rap phosphatases share a high sequence homology, however they regulate structurally distinct targets . Initial structural predictions of Rap phosphatases based on amino acid sequence suggested a two-domain architecture consisting of an N-terminal 3-helix bundle domain connected to a tetratricopeptide repeat (TPR) domain. This structure is strikingly different from other known bacterial phosphatases . TPR domains consist of 3 to 16 repeats of a degenerate 34 amino acid sequence motif, these repeats create a right-handed superhelix structure with an internal ligand-binding concave surface. TPR domains are known

to function as protein-protein interaction domains . Rap proteins appear to possess 6 canonical TPR motifs distributed along most of their length, with a further non-canonical TPR motif separating TPR5 and TPR6 . Parashar*et al.* found that Rap proteins undergo a major conformational change in their N-terminal domain when complexed with their target proteins: the N-terminal 3-helix bundle is flipped and merged with the existing C-terminal TPR domains . Further comparison of the crystal structures of RapI, RapH and RapF (these last two in complex with their target proteins) revealed that these conformational changes can generate different interacting surfaces that block their target's active site (in the case of Spo0F), or DNA-binding domain (in the case of ComA) .

The regulatory mechanism of the Phr peptides has also been structurally studied. Binding of Rap proteins to their cognate Phr peptides is mediated by their C-terminal TPR domains, and causes a pronounced rotation of the N-terminal 3-helix bundle; this creates two helix-turn-helix structures that pack against the existing C-teminal TPR domain. This rearrangement generates a compression along the whole TPR superhelical axis, which causes the loss of the ligand-binding concave surface normally present in Rap proteins. Furthermore, the Phr peptides can interact with the residues of multiple TPR repeats (up to six, in the case of RapF-PhrF complexes), leading to intramolecular interactions that stabilize the "closed" conformation of the Rap protein . These multi-TPR motif interactions confer a high specificity to Rap-Phr binding, with some Phr residues determining protein anchoring and orientation, and others mediating the interaction with the residues of the Rap protein. Gallego del Sol and Marina (2013) demonstrated that specific residues of RapF are required to bind its PhrF inhibitor, and that these residues are independent from the ability of RapF to bind to its target regulator ComA. The conservation of similar residues among Rap proteins, and additional experimental evidence from previous studies , suggest that this is a common Phr-binding mechanism for all Rap proteins.

Interestingly, a few known *rap* genes lack the concomitant gene for a specialized Phr peptide, but can be regulated by Phr peptides produced by other Rap-Phr cassettes (see SubtiWiki http://subtiwiki.uni-goettingen.de) . This is the case of RapB, which lacks a specialized Phr but is regulated by PhrC *in vitro* . Another example is *rapJ* , which is not followed by a *phr*gene. RapJ plays its regulatory role by dephosphorylating Spo0F P, and it binds to PhrC, forming a complex that is no longer able to interact with Spo0F P . Moreover, at least one Rap protein is known to be insensitive to regulation by its cognate Phr peptide. The*rapP-phrP* cassette is encoded in the pBS32 plasmid present in the undomesticated strain of *B. subtilis* NCIB 3610. RapP regulates biofilm formation, sporulation, and competence development by directly dephosphorylating Spo0F P , and by a ComA-dependent mechanism . However, RapP is not inhibited by PhrP, either when PhrP is overexpressed*in vivo,* or tested *in vitro* with exogenously added peptides derived from the C-terminal sequence of *phrP* . Conspicuously, RapP of *B. subtilis* NCIB 3610 shows an asparagine-to-threonine mutation at position 236 that is not present in the corresponding *rapP* alleles of other *Bacillus* strains. Omer Bendori *et al* . (2015) showed that this single amino acid substitution is responsible for the observed resistance of RapP to inhibition by PhrP, and that this inhibition could be restored by repairing the N236T mutation. Similarly, the plasmid encoded Rap63-Phr63 and Rap8-Phr8 modules synergistically moderate sporulation and biofilm formation of *B. thuringiensis* .

The structural insights from these studies suggest that there is a delicate balance between peptide-recognition specificity and regulatory plasticity in the Rap-Phr family. Studies that use various synthetic Phr peptides to investigate Rap-Phr interactions have found that, although usually one peptide shows strong affinity for a given Rap protein (normally the one coded in the same *rap-phr* cassette), other Phr peptides also show a partial ability to regulate the same Rap protein*in vitro* and *in vivo* . These alternative Phr are usually synthetized comprising the last 5 or 6 residues of the C-terminal end of Phr pro-peptides . However, it is important to consider that these results have been observed using artificial laboratory conditions and, in particular, *in vitro* experiments include the testing of a very limited number of peptides at a time. In natural settings, a complex network of Phr peptide cross-talk and co-regulation might exist among the populations of *Bacilli* to modulate the function of Rap phosphatases.

**Distribution and diversity of the Rap-Phr family in the*Bacillus* genus**

The Rap-Phr family of regulatory proteins is highly diversified and widespread in the *Bacillus* genus. Rap homologs have been found in the genomes of *B. subtilis* , *Bacillus amyloliquefaciens,Bacillus pumilus, Bacillus anthracis, Bacillus cereus, Bacillus halodurans* , *Bacillus stearothermophilus,* and *Bacillus clausii* . Also, one of these Rap-Phr cassettes has been studied heterologously in another *Bacillus* species (RapQ-PhrQ), and was found to be both functional and comparable to the native Rap phosphatases of the host . These reports support the idea that the Rap proteins and their Phr peptides play a common regulatory role in the entire *Bacillus* genus.

Interestingly, *Bacillus* species that have at least one Rap-Phr cassette normally possess multiple of these regulatory elements encoded in their genomes. The first study to identify RapA as a Spo0F phosphatase already established RapA and RapB as members of a protein-aspartate phosphatase family with multiple members within the same organism: *B. subtilis* strain JH642 . Subsequent studies have identified further members of this phosphatase family in diverse*B. subtilis* strains (see Table 1). In 2016, Even-Tov *et al* . compared over 400 *Bacillus* genomes, searching for Rap homologs based on their conserved N-terminal 3-helix bundle and C-terminal TPR domain structure. They found over 2500 *rap*homologs among the studied genomes, and that *B. subtilis* strains have, on average, 11 *rap* genes per strain, while *B. cereus*strains usually possess around 6 of these phosphatases .

How has the Rap-Phr family achieved such a widespread presence and diversity among *Bacilli* ? There are two main factors that can be considered when answering this question. First, bacteria commonly pass on genes among sibling cells or cells from closely related species. This ability, known as horizontal gene transfer (HGT), is an efficient mechanism for individual organisms to acquire genes, regardless of functionality . HGT of Rap-Phr cassettes is heightened due to the fact that many *rap-phr* genes are encoded within mobile genetic elements . In addition, the genes related to natural competence for the uptake of DNA from the environment are widely conserved in*Bacilli* . Even-Tov *et al* . recently estimated that up to 75% of Rap-Phr cassettes may be mobile, based on a GC-content comparison with their host strain . Furthermore, some Rap-Phr cassettes are able to regulate the mobility of the genetic element that contains them, be them plasmids , or transposons . These features could then favor a rapid expansion of Rap-Phr cassettes through HGT among*Bacilli* . Similarly, experimental selection for spores of *B. subtilis* increases the copy number of a cryptic prophage, phi3T, that carries Rap and Phr proteins . Interestingly, certain prophages, like SPβ, that are similar to phi3T do not carry such *rap* gene, but encode a biosynthetic gene cluster for a bacteriocin that presumably benefit the fitness of the host bacterium . The Rap protein coded within the phi3T prophage has been also hypothesized to contribute to phage fitness . Further, genome analysis combined with targeted experimental validation revealed that diversification of the autoinducer Phr peptides might be driven by promiscuous duplication events followed by adjustment of the Phr peptide in accordance with the respective evolutionary change of its cognate Rap phosphatase .

A second factor that can help explain the diversity of the Rap-Phr family is functional diversification through social selection. Experimental analyses and modeling suggest that acquisition of additional Rap-Phr system is facilitated by a facultative social cheating mechanism in *B. subtilis* . At low frequency, a strain harbouring an extra Rap-Phr system acts a cheater (i.e. exploiting the public good produced by the wild type), while at high frequency it returns to cooperation without fitness loss. Such social selection processes in combination with HGT ensure the diversification and maintainance of multiple copies of Rap-Phr systems in *Bacilli* .

**Social regulation of *B. subtilis* phenotypic adaptability through Rap phosphatases**

Rap phosphatases fine-tune the sociomicrobiology of *B. subtilis*by modulating the activity of the master regulators Spo0A, ComA and DegU and therefore their regulons (Fig. 2). This fine-tuning occurs at different levels: first, the Rap phosphatases themselves that may appear at first sight to have directly redundant regulatory roles, are expressed under different conditions. This leads to regulatory differences among Rap proteins with the same target. As an example, both RapA and RapB dephosphorylate Spo0F P, however, *rapA* expression is promoted by QS-dependent ComA, while *rapB* seems to be promoted only by the house-keeping sigma factor $\sigma^{A}$ . This difference means that RapB will be produced earlier and more consistently than RapA, leading to differences in the Spo0A/Spo0A P ratio in the cell population. A second level of

fine-tuning is given by the Phr peptides, which may comprise the most diverse family of QS autoinducers known to date . QS systems are used to coordinate social behavior that is most effective if a large number of cells participate, such as expression of virulence, or production of a biofilm matrix. The different Rap-Phr pairs have distinct influence on biofilm development of *B. subtilis* and colonization of the roots of the model plant*Arabidopsis thaliana* . In biofilm settings, the process-export-import regulatory pathway of Phr peptides provides*B. subtilis* cells with the opportunity to detect and integrate further environmental signals into their complex gene regulatory network. Cells in a biofilm live in micro-niches that lead to population heterogeneity , thus, the biofilm subpopulations will secrete different types and amounts of mature Phr peptides to the extracellular milieu. Furthermore, the flexibility of Phr peptides to serve as cell-cell communication signals has been demonstrated by the ability of non-producing cells to detect the Phr signals produced by other cell . Therefore, Phr peptides can form a biofilm-spanning communication network, where each biofilm subpopulation can participate in the developmental process of their neighbors.

*B. subtilis* is an environmentally ubiquitous bacterium, with numerous strains obtained from soil, animals, plants and aquatic habitats . Interestingly, although *B. subtilis* strains commonly show conservation among their main population heterogeneity regulators (Spo0A, ComA, DegU) , they show high variation among their Rap-Phr cassette content . Rap phosphatases determine the phenotypic memory of*B. subtilis* spores, the timing of spore formation and germination speed: the earlier the spores are formed, the faster these spores germinate driven by higher level of alanine dehydrogenase (i.e. high-quality spores), while delayed establishment of spores lead to higher number of spores in the population with reduced revival ability (i.e. high quantity spores) . Overexpression of *kinA* gene decelerates sporulation and therefore increase the spores yield, however, this gives rise to a lower fraction of spores growing out . The differences in the number and the diversity of the Rap-Phr family play an important role for the environmental adaptability of specific strains of *B. subtilis* by allowing them to fine-tune their metabolism to different ecological niches. For example, strains isolated from the digestive tract of animals can show differences in the timing of sporulation initiation influenced by Rap-Phr cassette variation. This variation effectively serves as an adaptation that allows them to sporulate at optimum rates according to the ecological niche in which they live . Ultimately, these differences in the amount of Rap phosphatases and the timing of sporulation eventually influence the quality-yield spore tradeoff in natural isolates .

Motivated by these observations related to the divergence in*rap -phr* modules and their influence on timing of sporulation, Gallegos-Monterrosa and colleagues constructed all possible combinations of single and double *rap -phr* deletions in*B. subtilis* NCIB 3610 and tested the fitness of these strains in a selection experiment . A mixture of the single and double mutants along with the wild-type strain was cultivated either as biofilms or as plaktonic cultures for 2- or 5-days, and spores were selected for re-inoculation again. After 9 cycles of spore selection, the abundance of strains in each culture condition was examined, which revealed that a shorter incubation time selects for higher diversity of strains, while longer cultivation selects for specific combination of *rap-phr*deletions . Additionally, fitness assays using the mutants that were selected under these conditions, e.g. derivative carrying a *rapB*or *rapH* deletion in addition to other *rap -phr*mutation, demonstrated the competitiveness of these strains compared to the wild-type strain. However, as these evolution experiments included spore selection, the cryptic phi3T prophage and its encoded Rap protein could have potentially influenced the selection dynamics . Nevertheless, this experimental approach highlighted the complexity of how the different Rap-Phr systems contribute to fitness of *B. subtilis* .

**Concluding remarks**

The efficiency of bacterial adaptation depends on the regulatory pathways that enable the cell to sense and respond to the external environment, that encompasses both abiotic and biotic factors. Biotic factors include the bacterial population itself and its density.*Bacilli* evolved to integrate population density using the Rap-Phr quorum sensing pathways that eventually diverged to modulate distinct, but partly overlapping, regulatory systems in the bacteria. Systemic dissection of the Rap-Phr systems (i.e. single- and multiple-deletions of the casettes) in different *Bacillus* species under diverse conditions, including their natural environments, will reveal their impact on the ecology of this group of microorganisms.

## AUTHOR CONTRIBUTIONS

Ramses Gallegos-Monterrosa: Writing – original draft. Ákos T. Kovács: Writing – original draft; Funding acquisition.

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## Tables and Figures

**TABLE 1** Known Rap-Phr proteins and their regulatory role in*B. subtilis* .

| **Rap protein** | **Phr peptide** | **Gene cluster localization** | **Target regulator and action mechanism** |
|---|---|---|---|
| RapA | PhrA | Chromosome | Dephosphorylation of Spo0F P |
| RapB | PhrC | Chromosome | Dephosphorylation of Spo0F P |
| RapC | PhrC | Chromosome | Blocks binding of ComA to DNA |
| RapD | Unknown | Chromosome | Inhibition of ComA activity |
| RapE | PhrE | Chromosome | Dephosphorylation of Spo0F P |
| RapF | PhrF | Chromosome | Blocks binding of ComA to DNA |
| RapG | PhrG | Chromosome | Blocks binding of DegU to DNA. Possible inhibition of ComA activity |
| RapH | PhrH | Chromosome | Dephosphorylation of Spo0F P. Blocks binding of ComA to DNA. |
| RapI | PhrI | Chromosome | Dephosphorylation of Spo0F P. Involved in the regulation of mobile genetic elements. |
| RapJ | PhrC | Chromosome | Possible dephosphorylation of Spo0F P |
| RapK | PhrK | Chromosome | Inhibition of ComA activity |

| Rap protein | Phr peptide | Gene cluster localization | Target regulator and action mechanism |
|---|---|---|---|
| RapLS20 | Unknown | Plasmid | Possible dephosphorylation of Spo0F P. Regulatory effect on sporulation. Involved in the regulation of mobile genetic elements. |
| RapP | PhrP (RapP is resistant to PhrP) | Plasmid | Dephosphorylation of Spo0F P. Inhibition of ComA activity |
| RapQ | PhrQ | Plasmid | Inhibition of ComA activity. Possible dephosphorylation of Spo0F P. Originally identified in *Bacillus amyloliquefaciens.* |
| Rap40 | Phr40 | Plasmid | Unknown |
| Rap50 | Phr50 | Plasmid | Unknown |
| Rap60 | Phr60 | Plasmid | Dephosphorylation of Spo0F P. Inhibition of ComA activity |

## References

## Figure legends

**FIGURE 1** General regulatory mechanism of Rap-Phr pairs. (i) the Rap protein is produced and, (ii) carries its regulatory role intracellularly; meanwhile, (iii) pre-Phr proteins are produced, (iv) processed and exported out of the cell as Phr peptides; (v) upon reaching threshold concentrations, (vi) mature Phr peptides can be imported into the cell via the Opp permease and (vii) inhibit its cognate Rap protein. Rap proteins are visualized using the RapF crystal structure (PDB doi: 10.2210/pdb4I9E/pdb), while prePhr is depicted using the structure based on AlphaFold as displayed in SubtiWiki . The figure was prepared on BioRender.com.

**FIGURE 2** Known cellular functions regulated by Rap phosphatases in *B. subtilis* . The centeral protein structure shows the conserved architecture of Rap phosphatases (RapF crystal structure, PDB doi: 10.2210/pdb4I9E/pdb). The biofilm colony images of wild type (WT) and Δ*rapP* mutant are taken from Nordgaard *et al.* (2021), scale bar represents 5 mm. The figure was prepared on BioRender.com.