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REVIEW

Bacterial differentiation via gradual activation of global regulators

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Abstract Bacteria have evolved to adapt to various conditions and respond to certain stress conditions. The ability to sense and efficiently reply to these environmental effects involve versatile array of sensors and global or specific regulators. Interestingly, modulation of the levels of active global regulators enables bacteria to respond to diverse signals via a single central transcriptional regulator and to activate or repress certain differentiation pathways at a spatio-temporal manner. The Gram-positive *Bacillus subtilis* is an ideal bacterium to study how membrane bound and cytoplasmic sensor kinases affect the level of phosphorylated global regulator, Spo0A which in response activates genes related to sliding, biofilm formation, and sporulation. In addition, other global regulators, including the two-component system DegS-DegU, modulate overlapping and complementary genes in *B. subtilis* related to surface colonization and biofilm formation. The intertwinement of global regulatory systems also allows the accurate modulation of differentiation pathways. Studies in the last decade enable us to get a deeper insight into the role of global regulators on the smooth transition of developmental processes in *B. subtilis*.

Keywords Global regulator · Sensor kinase · Sliding · Biofilm formation · Sporulation · *Bacillus subtilis*

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Introduction

Bacteria appeared on earth billions of years ago and have well customized to diverse environmental conditions. Studying the microbial responses and the adjustments of bacterial life styles helps us to understand how certain signals are perceived by these tiny organisms and how certain regulatory proteins contribute to the adaptation processes. Deeper insights into the control of gene regulatory networks reveal how the complexity of signal perception and fine-tuning the transcription of hundreds of genes affect the bacterial differentiation processes.

Soil dwelling bacteria are well adapted to respond to the environmental changes and commit to a certain differentiation pathway depending on the ecological conditions. The Gram-positive bacterium, *Bacillus subtilis* shows various differentiation traits under laboratory conditions that are related to its survival in the environment. For example, extracellular matrix dependent biofilm formation of *B. subtilis* enables the bacterium to attach and colonize the root surface (Bais et al. 2004). Depending on the circumstances, *B. subtilis* can also colonize surfaces using flagellum-driven single cell based movement (swimming), motility-dependent multicellular spreading (swarming), or growth-powered passive surface translocation (sliding) (Kearns 2010). Swarming and sliding are generally observed in wild isolates of *B. subtilis* and lost in domesticated laboratory strains (Kinsinger et al. 2003; Patrick and Kearns 2009; Pollak et al. 2015). While the cultivating conditions determine which process is activated, it seems that the activation of cellular machineries required for translocation is modulated by precise level of certain sets of global regulators (Grau et al. 2015).

Global regulators influence the differentiation of *B. subtilis*

Intriguingly, the transition among the differentiation pathways to slide, to develop highly wrinkled colony biofilms, or to enter sporulation depends on the level of the phosphorylated Spo0A (Spo0A~P) protein in *B. subtilis* (Grau et al. 2015). The global regulator, Spo0A has been originally identified due to its requirement for sporulation (Trowsdale et al. 1978). Analysis of the Spo0A~P modulated genes revealed that the list of genes up- or downregulated depends on the level of Spo0A~P in the cells. While the transcription of *abrB* gene that codes for a transition state regulator in *B. subtilis* is repressed already at low levels of Spo0A~P, activation of sporulation related gene expression requires high levels of Spo0A~P (Fujita et al. 2005). Such a differential control of genes is achieved by variance in the Spo0A~P binding sites located in the promoter and regulatory regions of directly affected genes that defines the binding properties of Spo0A~P to these sites and therefore transcriptional activation or repression. The gradual increase in the phosphorylation level of Spo0A is mediated by various histidine kinases in *B. subtilis* (Fujita and Losick 2005). *B. subtilis* harbours five histidine kinases, the cytoplasmic KinA and the membrane-bound kinases, KinB to KinE (Jiang et al. 2000). Increased levels of KinA, KinB, or KinC trigger the entry into the sporulation pathway (Fujita and Losick 2005). Upon activation of these kinases, phosphorylation of Spo0A is achieved via a phosphorelay including Spo0F and Spo0B proteins (Hoch 1993). Numerous signals have been described that are perceived by these kinases (Mhatre et al. 2014), including anaerobic conditions [KinA and KinB (Kolodkin-Gal et al. 2013)], plant-derived signals [KinC and KinD (Beauregard et al. 2013; Chen et al. 2013)], polyketides [KinC (López et al. 2009)], combination of glycerol and manganese [KinD (Shemesh and Chai 2013)], osmotic conditions [KinD (Rubinstein et al. 2012)], and potassium level [KinB (Grau et al. 2015)]. In response to these or other so far unknown signals affecting the kinase activity of Kin proteins, Spo0A~P level increases in the cells and modulates the transcription of certain sets of genes. Importantly, the increase in the Spo0A~P level is heterogeneous within a clonal population (Veening et al. 2005). Positive feedback loops contribute to the hetero-chronic timing of Spo0A~P dependent activation (Chastanet et al. 2010; de Jong et al. 2010) that greatly determine the level of phenotypic heterogeneity between individual cells. Heterogeneity is proposed to originate from the variable gene activities of phosphorelay components that result in high cell–cell variability (de Jong et al. 2010). The versatility of single master regulator modulated pathways is not restricted to Spo0A. The level of phosphorylated DegU (DegU~P) determines whether *B. subtilis* commits to competence for DNA uptake (DegU in the non-phosphorylated form), swims or swarms (low DegU~P level), activate genes related to biofilm formation (medium DegU~P level) or increase protease production (high DegU~P level) (Kobayashi 2007; Verhamme et al. 2007). Importantly, several genes and phenotypes are affected by both Spo0A~P and DegU~P, including biofilm formation (Marlow et al. 2014) and protease secretion (Veening et al. 2008a) presenting a naturally occurring logic-AND gates for the expression of certain genes (Veening et al. 2008b).

The level of phosphorylated Spo0A defines the activation of sliding, biofilm formation or sporulation

The seemingly continuous transition between the different differentiation pathways was recently reported in *B. subtilis* using identical environmental conditions, but synthetically altering the level of Spo0A~P (Grau et al. 2015). Depending on the induction level of the *sad*67 gene that codes for a phosphorylation-independent active Spo0A protein (Spo0A*), distinct phenotypic traits could be stimulated (Fig. 1). Using semi-solid nutrient rich medium (lysogeny broth medium with 0.7 % agar), low, medium, or high levels of Spo0A* activate sliding, architecturally complex colony biofilm development or high level of spore production, respectively. Both sliding and biofilm formation require the production of exopolysaccharide (EPS) and an amphiphilic protein (BslA). Expansion of colony biofilm is proposed to be driven through the secretion of EPS (Seminara et al. 2012; van Gestel et al. 2014) by generating osmotic pressure gradient in the extracellular space. Similar mechanism may act during sliding, where the EPS driven colony expansion additionally requires hydrophobins, BslA and surfactin (Grau et al. 2015). It is appealing to hypothesize that precise levels of these components determine whether cells colonize a surface via sliding or through slight expansion of the colony biofilm. Alternatively, sliding perceives spatial fixing of cells in the biofilm, i.e., surface attachment and fruiting body formation. However, it is clear that gradual activation of a global activator controls the transition between surface spreading and biofilm formation, previously believed to be antagonistic and independent prokaryotic social behaviours (Grau et al. 2015). The importance of single cell based motility has recently been reported during *B. subtilis* biofilm development on the air-medium interface verifying the ecological connection of distinctive pathways (Hölscher et al. 2015).

Sensor kinases influence which differentiation pathways are activated by Spo0A~P

The gradual activation of Spo0A~P is believed to be mediated by the differential activation of histidine kinases of *B.*

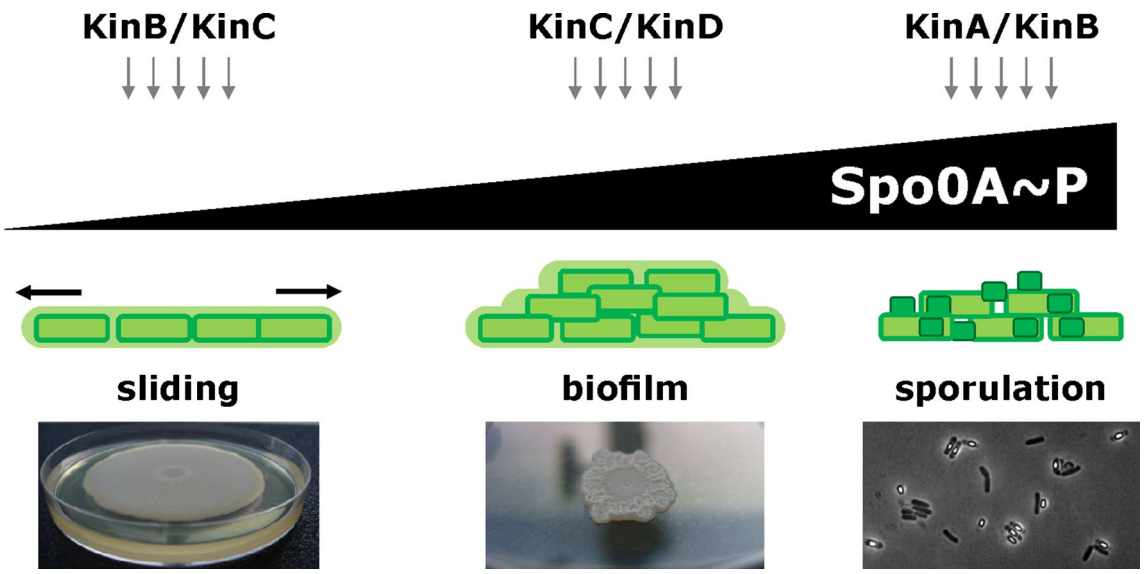

Fig. 1 Gradual increase in the level of Spo0A~P defines which developmental pathways are activated in *B. subtilis*. Sliding, biofilm development or production of resistant spores are induced at certain levels of Spo0A~P protein in the bacterial cells (Grau et al. 2015)

subtilis. While effective sliding was shown to be dependent on both KinB and KinC (Grau et al. 2015), the KinC and KinD proteins seems to mainly trigger biofilm development under aerobic conditions (Mhatre et al. 2014), and KinA and KinB present the major input during entry into sporulation. KinB-initiated sliding of *B. subtilis* was proposed be triggered by the level of potassium (Grau et al. 2015). Sensing of potassium in the medium requires a protein domain of KinB that shows an intriguing homology to the conserved K^+-filter sequence of eukaryotic and prokaryotic potassium channels. It is hypothesized that while both KinB and KinC are required for the activation of sliding, these kinases might respond to differential levels of potassium (Grau et al. 2015). Finally, it is reasonable to assume that these kinases control the spatial activation of these pathways to guarantee that these traits are sequentially activated during surface colonization. Indeed, colony biofilms were reported to enclose spatially and temporally separated populations with activation of genes related to flagellum-dependent motility, matrix production or spore development (Vlamakis et al. 2008). Thus, it will be fascinating to examine how the activation of these differentiation pathways is mediated during sliding at the single cell levels and determine how heterogeneity contribute to the smooth transition between these phenotypes.

Protein phosphorylation is not only restricted to regulatory proteins in bacteria, but also found in diverse processes, including proteins with enzymatic function or those involved in cell division (Derouiche et al. 2015). By adjusting protein phosphorylation, bacteria have implemented a general way to modulate the function of global regulators depending on the environmental cues. Sensory kinases take an important role in differentiating the signals and adequately modifying activities of regulators that initiate certain bacterial differentiation processes.

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