GUEST COMMENTARIES

A Unique Sensor Histidine Kinase[▽]

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Sporulation initiation in *Bacillus* species is controlled by an extended version of two-component signal transduction systems, termed phosphorelay (4). The cascade of His→Asp→His→Asp phosphotransfer is typically initiated by the ATP-dependent autophosphorylation of the histidine kinase domain. In this issue, Scaramozzino et al. (11) reported that a sporulation histidine kinase, BA2291, in *Bacillus anthracis* exclusively utilizes GTP for this purpose. BA2291, while complementing the sporulation deficiency of *Bacillus subtilis* kinase mutants, was shown to inhibit sporulation in *B. subtilis* when cloned on a multicopy plasmid (1), and the prediction that BA2291 can function as a phosphorylated Spo0F (Spo0F~P) phosphatase is confirmed in this study (11). The ability of BA2291 to specifically generate GTP through the reverse flow of a phosphoryl group from Spo0F~P is also demonstrated (11). Interestingly, a point mutation in the linker region that connects the sensor domain to the kinase domain converts BA2291 to a sporulation inhibitor. The identification of the reversible GTPase activities together with other unique regulatory features that modulate the enzymatic activity of BA2291 provides new insight into the sophisticated nature of bacterial signal transduction mechanisms and raises an intriguing question, whether the BA2291 kinase plays a role in both sporulation initiation and inhibition in *B. anthracis*.

In *B. subtilis*, upon sensing as-yet-unidentified signals, five histidine kinases phosphorylate the single-domain response regulator Spo0F, with the phosphoryl group being transferred to the ultimate master regulator, Spo0A. Spo0A then controls the expression of over 500 genes either directly or indirectly (2, 6). The dominant cytoplasmic kinase KinA is responsible for more than 90% of signal input under laboratory conditions. Negative regulatory inputs function via two aspartyl-phosphate phosphatase family proteins that target either Spo0F~P or Spo0A~P and are mediated by growth conditions antithetical to sporulation (8, 9). Similarly, five histidine kinases regulate sporulation, with BA2291 being the most actively transcribed one in *B. anthracis* (1). Although inactivating any of the kinases does not result in a significant sporulation deficiency, the deletion of BA2291 kinase caused the most noticeable sporulation delay in *B. anthracis* (1). Curiously, BA2291 is found in all *B. anthracis* strains but has no ortholog in *B. subtilis*. Due to its cytoplasmic localization, the direct activating ligand of BA2291

must reside intracellularly, and the activation results in the GTP-dependent autophosphorylation of BA2291. No other nucleotides, including the commonly used ATP, serve as substrates for the autophosphorylation. Attempts to understand the selectivity toward GTP have revealed a prominent role of a D-to-N substitution in the G1 box of the nucleotide binding domain in excluding ATP binding (11). As pointed out by those authors, ~5% of histidine kinases have an asparagine at this position, and whether this substitution can serve as an indicator for GTP selectivity remains to be explored.

Interestingly, BA2291 on a multicopy plasmid completely blocked sporulation in the wild-type *B. subtilis* strain, suggesting that it elicits a dominant negative role when artificially elevated in the cells. Thus, the ability of BA2291, when overexpressed, to efficiently deplete Spo0F~P and possibly sequester Spo0F from receiving phosphoryl groups from other sporulation kinases provides a new strategy for dampening sporulation initiation. Many histidine kinases exhibit phosphatase activity toward their cognate response regulators under certain physiological conditions, but this is the first example showing that merely overproducing the kinase protein converts it from a positive regulator to a negative regulator. Scaramozzino et al. also showed that an S147L mutation of BA2291, located in the linker region between the sensor domain and the kinase domain, yielded a slower autophosphorylation rate than that of the wild-type kinase and, as a result, changed the BA2291 kinase to a sporulation inhibitor even when expressed from a single-copy gene. A possible explanation is that the mutation altered the equilibrium state between the kinase and the phosphatase activities of BA2291 with respect to signal activation. It remains to be determined whether choosing GTP over ATP as the phosphoryl group donor provides mechanism feasibility for such functional switching and if the signal relay communications from the sensor domain to the catalytic domain differ between ATP- and GTP-dependent autokinases.

The propensity of the BA2291 kinase to switch to a negative sporulation regulator appears to be specifically targeted by virulence plasmid-encoded sporulation inhibitors. Two single-domain proteins with high degrees of sequence similarity to the sensor domain of BA2291 were demonstrated to inhibit sporulation initiation in *B. subtilis* in a BA2291-dependent manner (13). Both proteins are encoded on the virulence plasmids: one gene, the pXO1-118 gene, is encoded divergently from the *atxA* gene on pXO1 and is controlled by the toxin regulator AtxA, while the other, the pXO2-61 gene, is located on plasmid pXO2 and adjacent to the *cap* operon, coding for capsule expression. The acquisition of pXO1 and pXO2, which are

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responsible for the expression of toxin and capsule, respectively, allows *B. anthracis*, the etiological agent of anthrax, to infect mammals, including humans, through contact with, ingestion of, or inhalation of its spores. However, while the spore is required to initiate the infection, only encapsulated vegetative cells are able to evade the host immune response during infection, and sporulation does not occur in the host (5). Data presented in the study by Scaramozzino et al. (11) and in previous reports (1, 13) point to a likely regulatory mechanism where signal sequestration by these plasmid-encoded sensor-like proteins impedes the BA2291 kinase function and/or stimulates its phosphatase activity or other negative regulatory effectors, although confirmation of the precise control mechanism is yet to be elucidated.

Is there any underlying rationale in which the *B. anthracis* BA2291 kinase selectively utilizes GTP to initiate the phosphorelay leading to sporulation? Do GTP and its derivatives serve additional roles as indicators of environmental and/or nutritional changes through the unique GTP-dependent activity of BA2291 in pathogenic *B. anthracis*? The stringent response mediated by the hyperphosphorylated guanosine nucleotide ppGpp, which is derived from GTP and ATP, induces sporulation in *B. subtilis* and was shown recently to also affect sporulation in *B. anthracis*. However, this effect in *B. subtilis* was shown to be caused by a decrease in the intracellular GTP pool (7), a condition sensed by CodY. CodY, analogous to BA2291, specifically binds GTP (3), and the GTP-bound form functions as a repressor that controls adaptation to conditions of nutrient limitation during early stationary phase and represses sporulation initiation (10, 12). GTP binding, but not hydrolysis, is necessary for CodY-dependent regulation (3). As the expression of BA2291 is highly induced upon entry into stationary phase (1), could the GTPase activity of BA2291 affect the intracellular GTP level and thus synergistically derepresses CodY-controlled sporulation genes for a more prompt sporulation initiation? Alternatively, would the Spo0F~P phosphatase activity of BA2291 that potentially leads to GTP synthesis coordinate with CodY to inhibit sporulation under other physiological conditions? Interestingly, CodY was reported to be essential for

the synthesis of toxins and for virulence in a mouse model of anthrax (12), events that are antithetical to sporulation initiation. It appears that GTP-dependent BA2291 kinase behaves as a switch for sporulation initiation or inhibition in *B. anthracis*. Whether the unique features of the BA2291 kinase are evolved from the acquisition of its pathogenesis potential by *B. anthracis* awaits the identification and characterization of its activating signals.

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