Genetic and biochemical analyses of sensor kinase A in *Bacillus subtilis* sporulation

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**ABSTRACT.** Temporal and spatial regulation of gene expression during endospore formation in *Bacillus subtilis* prompted us to investigate the molecular mechanisms that coordinate the phosphorelay. We targeted KinA for random mutagenesis. In addition, we constructed KinA-GFP transcriptional fusions for verification, via fluorescence. Four distinct types of sporulation-defective mutants were identified as inactive (no sporulation), hypoactive (low sporulation efficiency), isoactive (same efficiency as wild-type), and hyperactive (high efficiency) mutants. Surprisingly, the β-galactosidase activity of hyperactive mutants was barely greater than that of the wild-type strain; the only noticeable difference was early synthesis of KinA, which could allow them to activate Spo0A precociously, undergo sporulation earlier, and yield more spores. There was no fluorescence emission by the spore-defective mutant, which confirmed the presence of a truncated KinA (nonsense mutation) in inactive strains; other mutants harbored missense or silent mutations. We determined the nucleotide sequences of KinA mutants and found a conserved C-terminus region; more variability was observed in the N-terminus region, involving the PAS-A and PAS-C domains. We speculate that PAS-A, notwithstanding its ATPase activity, has only a minor role in KinA activity, whereas PAS-B was found to be indispensable. Our results emphasize the importance of
temporal coordination of gene expression during the sporulation process and corroborate the necessity of Spo0A phosphorylation by KinA, which stimulates SpoIIG expression. We further propose a novel hypothetical model that purposely dubbed the “C-shaped intertwined model”, which requires both homodimerization and spatial proximity between PAS-A and histidine H$_{405}$ of two different KinA molecules.

**Key words**: KinA; Sporulation; C-shaped intertwined model; *Bacillus subtilis*