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Crystal Structure of Histidine Phosphotransfer Protein ShpA, an Essential Regulator of Stalk Biogenesis in *Caulobacter crescentus*

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Cell-cycle-regulated stalk biogenesis in *Caulobacter crescentus* is controlled by a multistep phosphorelay system consisting of the hybrid histidine kinase ShkA, the histidine phosphotransfer (HPt) protein ShpA, and the response regulator TacA. ShpA shuttles phosphoryl groups between ShkA and TacA. When phosphorylated, TacA triggers a downstream transcription cascade for stalk synthesis in an RpoN-dependent manner. The crystal structure of ShpA was determined to 1.52 Å resolution. ShpA belongs to a family of monomeric HPt proteins that feature a highly conserved four-helix bundle. The phosphorylatable histidine His56 is located on the surface of the helix bundle and is fully solvent exposed. One end of the four-helix bundle in ShpA is shorter compared with other characterized HPt proteins, whereas the face that potentially interacts with the response regulators is structurally conserved. Similarities of the interaction surface around the phosphorylation site suggest that ShpA is likely to share a common mechanism for molecular recognition and phosphotransfer with yeast phosphotransfer protein YPD1 despite their low overall sequence similarity.

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Abbreviations used: HPt, histidine phosphotransfer; HK, histidine kinase; RR, response regulator; P-His, phosphorylatable histidine; ASU, asymmetric unit; TEV, tobacco etch virus; MAD, multiwavelength anomalous diffraction.

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Introduction

Two-component signaling systems, the predominant signal transduction pathways in bacteria, are essential for the survival, growth, and development of bacteria by enabling them to adapt to the environment.[1,2] In a canonical two-component system, the translation of extracellular environmental signals into cellular responses is achieved by two proteins. A cytoplasmic histidine kinase (HK), often fused to an extracellular sensor domain (sensor HK) through a transmembrane helix, autophoshorylates at a histidine residue using ATP upon ligand binding to the sensor domain. The phosphoryl group is then transferred to an aspartate residue of a response regulator (RR), which becomes activated upon phosphorylation. The activated RR triggers appropriate downstream responses (e.g., gene expression). Bacteria, lower eukaryotes, and plants have also evolved a more intricate phosphorelay system in which an additional histidine phosphotransfer (HPt) protein accepts the phosphoryl group from the first RR domain (R1) and transfers it to the second RR domain (R2). This arrangement coordinates integration of multiple signals from different sources, propagation of one signal to multiple targets, and more complex regulation scenarios.[3]

Two-component systems are critical for controlling cell division and development in *Caulobacter crescentus*, which has become an important model system for studying the regulation of the cell cycle and cellular differentiation.[4–6] *C. crescentus* divides asymmetrically, producing two cells with different structures and functions: a stalk daughter cell and a motile swarmer daughter cell with a single polar flagellum and pili. The swarmer cell cannot initiate DNA replication but can differentiate into a stalk cell by shedding its polar flagellum, retracting its pili, and synthesizing a stalk at the former flagellum site after 30–45 min of swimming. The adhesive polysaccharide holdfast is then synthesized, concomitantly with initiation of a single round of DNA replication. The stalk of the pre-divisional cell is then extended, while a new polar flagellum and pili are built at the opposite pole of the stalk, followed by a new round of cell division (Fig. 1a). The master cell cycle regulator CtrA (an RR) controls several key cell cycle events.[7] Phosphorylated CtrA directly activates or represses the transcription of 95 genes that are essential for cell cycle processes, such as DNA methylation, morphogenesis, and cell division. CtrA is responsible for activation of the genes involved in the biosynthesis of pili, holdfast, and flagellum.[6,8] Phosphorylated CtrA also inhibits the initiation of DNA replication in the swarmer cell by binding to the replication origin.

Bacterial stalks are likely nutrient-scavenging antennae that allow stalked bacteria to survive in nutrient-limited environments.[9,10] Stalk synthesis is regulated by the cell cycle, and the stalk length is controlled by both cell cycle and environmental cues. The cell-cycle-regulated stalk biogenesis is controlled by an ShkA–ShpA–TacA multistep phosphorelay system (Fig. 1b) in a sigma factor σ54 (RpoN)-dependent manner.[11] ShkA (stalk biogenesis HK A) is a cytoplasmic hybrid HK that contains an HK domain and a C-terminal RR domain. ShpA (stalk biogenesis histidine phosphotransferase A) is a small monomeric HPt protein (112 residues) that transfers the phosphoryl group from ShkA to TacA. TacA is an RpoN-dependent, NtrC-like transcription activator with an RR domain at its N-terminus.[12,13]

DNA microarray analysis has identified 30 genes involved in stalk biogenesis controlled by this phosphorelay system.[11] This pathway was shown to be essential for stalk biogenesis as Δ*shkA*, Δ*shpA*, and Δ*tacA* mutants are stalkless and ΔShkA and ΔShpA deletions can be rescued by the TacA D54E mutant, which functionally mimics phosphorylated TacA. Phosphorylated TacA collaborates with RpoN to activate the expression of genes involved in the cell-cycle-regulated stalk biogenesis. Details about the upstream signal(s) for ShkA and how the target genes of TacA function together have yet to be fully explored. Several proteins involved in stalk synthesis, such as penicillin-binding protein 2, RodA, and MreB, are also required for cell elongation, suggesting that stalk synthesis is a special type of cell elongation.[14]

At least two types of HPt proteins are found in two-component systems,[1] Spo0B-like proteins assemble