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Two-component systems in bacteria: how is the signal unidirectionally transmitted?

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In order to survive, living organisms need to adapt to environmental changes, relying on their signaling and regulatory capacities. Two-component systems (TCS) are one of the main signaling pathways in prokaryotes, also present in fungi and plants. In general, they are constituted of a sensor histidine-kinase (HK) that is able to autophosphorylate on a conserved histidine (His) according to presence/absence of the signal. HKs can then phosphorylate or dephosphorylate a conserved aspartate (Asp) of their cognate response regulator (RR) according to the pathways' signaling 'on'/'off' status. These pathways have shown to be highly specific and efficient, and canonical TCSs work with a "His-to-Asp" unidirectional, irreversible phosphoryl-transfer pathway that ensures a physiological output. However, there are more complex systems termed phosphorelays, which rely on additional intermediary phosphotransferase and RR-like shuttle domains that allow phosphate flow to occur bidirectionally (i.e., also "Asp-to-His"). This raises the question as to how a large number of TCSs only allow for unidirectional "His-to-Asp" phosphoryl transmission, avoiding futile phosphorylations or dephosphorylations, and minimizing "Asp-to-His" backtransfer, which would otherwise cause signaling shutdown. Set up to address these questions, and combining X-ray crystallography and biochemical in vitro assays, we have now determined the crystal structures of the binary complex between a bona fide HK and RR, the DesK-DesR complex from Bacillus subtilis. This TCS has minimal backtransfer phosphoryl flow [1], hence a model for a canonical TCS signaling pathway. The structures solved represent snapshots of the dephosphorylation- and phosphotransfer-competent states, distinctly crystallized likely due to the use of site-directed DesK mutants that we have previously shown to stabilize specific functional states [2,3]. Our findings uncover the means by which a concerted conformational switch linking the two partners in complex, has evolved to efficiently control disparate HK activities. We found that it is the HK that dictates the direction of catalysis (whether phosphatase or phosphotransferase), recognizing a unique, complex-specific conformation of the RR. Using PhosTag-SDS-PAGE assays, we demonstrated that the catalytic core residue Phe82 of DesR stabilizes the phosphorylated state of the RR. Combining the data from a phospho-DesR crystal structure previously solved in our lab, we now observed that this stabilization is accomplished by the bulky sidechain of Phe82 sterically shielding the phospho-Asp from the solvent, thereby avoiding immediate RR dephosphorylation. A comparative analysis of the catalytic sites of unidirectional TCSs (including the phosphotransfer-competent complex here presented) vs bidirectional phosphorelay complexes, suggests that the distance between His and Asp, and hence the relative position of the catalytic magnesium, are determinants for signal directionality: larger distances imply asymmetric location of the metal, and appear to lead to a dissociative phosphoryl-transfer mechanism, ultimately driving phosphoryl flow towards a unique "His-to-Asp" direction. In sum, the data we are now presenting contribute to understand how signaling occurs unidirectionally in these systems, ensuring effective connection between sensed stimulus to downstream adaptive output.

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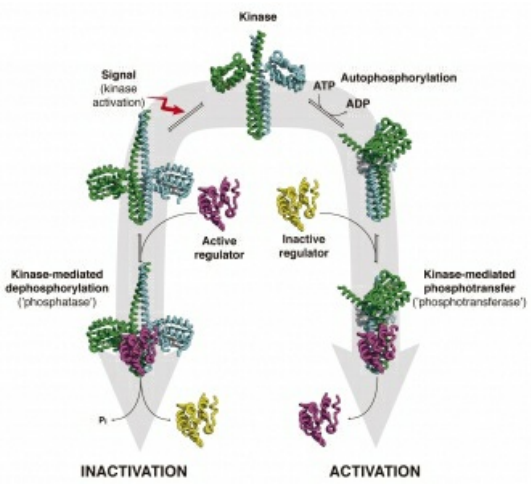

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