Regulatory Mechanism of Sensing by FixL-FixJ

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FixL/FixJ is a prokaryotic two-component regulatory system, where the FixL protein is a kinase and sensor, and the FixJ protein is a response regulator. FixL from *Rhizobium meliloti* was the first characterized hemeprotein functioning as a direct oxygen sensor. Under O_2 deprivation the FixL heme-binding domain activates its C-terminal kinase domain to promote autophosphorylation, and a further catalytic transfer of the phosphoryl group to FixJ. As a result, the phospho-FixJ regulates gene expression by directly binding to DNA.

A mechanistic model of sequential reaction (ping-pong mechanism) had been used to describe this system, but it turned out to be unlikely to be relevant *in vivo*. If FixL is first incubated with FixJ, there are profound kinetic and thermodynamic changes in this sensing system. The formation of the FixL-FixJ,complex, as an earlier step, improved FixJ turnover nearly 30 fold if compared to the ping-pong mechanism. Additionally, heme status was for the first time directly regulating phospho-FixJ turnover. This unprecedent mechanism for two-component system leads us to investigate its mechanistic regulation.

There are five steps at which binding of O_2 and other regulatory ligands to the heme could control the level of phospho-FixJ generated by the FixL enzyme:

a) Binding of ATP
b) Complexation of FixL with FixJ
c) Phospho transfer to histidine in FixL
d) Phospho transfer from FixL to FixJ
e) Dissociation of phospho-FixJ from Complex

Traditional fluorescence and polarization fluorescence have been employed along with enzymatic activity assay and gel filtration to address some of the points of regulation above mentioned. Fluorophore nucleotide probes have been used as well as selected fluorophore probes attached to FixL and FixJ to better define and measure the relevant mechanistic step involved in the oxygen regulation.

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