

**BACTERIAL SERINE AND TYROSINE PROTEIN KINASES
CONTAINING A WALKER MOTIF**

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Several bacterial Ser, Thr and Tyr protein kinases have recently been described to contain a Walker motif A as nucleotide binding site and to exhibit a domain organization different from that in eukaryotic protein kinases. Two of these protein kinases have been studied in more detail. One is HPr kinase/phosphorylase (HprK/P), a Ser protein kinase acting as a sensor enzyme of carbon catabolite repression in Gram-positive bacteria. This bifunctional enzyme catalyses the phosphorylation and dephosphorylation at Ser-46 in HPr, a phosphocarrier protein of a sugar uptake and phosphorylation system (PTS). P-Ser-HPr functions as co-repressor by interacting with the catabolite repressor CcpA and allowing it to bind to operator sites preceding catabolite-repressed transcription units. The two antagonistic activities of HprK/P are regulated by metabolites, such as fructose-1,6 bisphosphate (stimulates the kinase activity) and inorganic phosphate (Pi) (inhibits the kinase activity). In addition, HprK/P can use two different phosphoryl donors: ATP and pyrophosphate (PPi). Only the ATP-dependent kinase activity is stimulated by fructose-1,6 bisphosphate.

The crystal structures of *Lactobacillus casei* and *Staphylococcus xylosus* HprK/P have been determined. In both enzymes, Pi was bound to the Walker motif A at the site occupied by the β -phosphate of ATP. This explained the inhibitory effect of Pi on the ATP-dependent kinase activity. However, Pi was also reported to stimulate the HprK/P-catalysed dephosphorylation of P-Ser-HPr, which was unusual, as Pi was expected to be the product of P-Ser-HPr dephosphorylation. In fact, P-Ser-HPr dephosphorylation turned out to follow an unusual mechanism. Pi bound to the Walker motif functions as substrate and carries out a nucleophilic attack on the phosphorous atom of P-Ser-HPr, thus leading to the formation of pyrophosphate and HPr. P-Ser-HPr dephosphorylation is therefore a phosphorolysis and not a hydrolysis reaction. In fact, it is the reversal of the PPi-dependent HPr phosphorylation. The structures of HprK/P in complex with HPr and P-Ser-HPr have also been determined and confirmed the proposed P-Ser-HPr dephosphorylation mechanism.

The second class of Walker motif-containing bacterial protein kinases are Tyr kinases controlling the synthesis of exopolysaccharides. These enzymes, which contain the Walker motifs A and B, exhibit low ATP hydrolysis and autophosphorylate at a C-terminal tyrosine cluster. They have been studied in *B. subtilis*, *E. coli* and several other bacteria. For *B. subtilis* YwqD we could demonstrate that this protein not only autophosphorylates, but that it can also phosphorylate the UDP-glucose dehydrogenases TuoD (implicated in

teichuronic acid synthesis) and YwqF (Ivan Mijakovic and Josef Deutscher, unpublished results). However, phosphorylation requires the presence of a transmembrane protein YwqC, which probably acts as phosphorylation modulator by bringing the protein kinase and its substrate into contact. Phosphorylation of TuaD and YwqF is necessary for their UDP-glucose dehydrogenase activity. When partly phosphorylated TuaD or YwqF, purified after overexpression in *E. Coli*, was dephosphorylated with YwqE, a P-Tyr-protein phosphatase exhibiting no similarity to eukaryotic P-Tyr-phosphatases, a complete loss of the activity of the two enzymes was observed. In contrast to HprK/P, YwqD is not bifunctional and cannot use PP_i as alternate phosphoryl donor.