Abstract

Reversible protein phosphorylation is considered the universal language for intracellular communication in all living organisms. This process, catalysed by protein kinases and phosphatases, enables the translation of extracellular signals into cellular responses and also allows for adaptation to a constantly changing environment. In recent years, a number of bacterial eukaryotic-type Ser/Thr protein kinases and phosphatases have been identified. However, their precise functions and substrates are not yet well defined.

The genome of opportunistic human pathogen *Pseudomonas aeruginosa* contains at least five genes encoding putative eukaryotic-type Ser/Thr protein kinases and phosphatases. In the first part of this study, we have attempted to establish the role of Ser/Thr protein kinase PpkA and phosphatase PppA, which belong to type VI secretion system H1-T6SS.

Double mutant strain Δ*pppA-ppkA* was prepared in *P. aeruginosa* PAO1 background. Phenotypic studies revealed that the mutant grew slower than the wild-type strain in minimal media and exhibited reduced secretion of pigment pyocyanin. In addition, the mutant had altered sensitivity to oxidative and hyperosmotic stress conditions. Consequently, mutant cells had an impaired ability to survive in murine macrophages and an attenuated virulence in the plant model of infection. Whole-genome transcriptome analysis revealed that *pppA-ppkA* deletion affects the expression of oxidative stress-responsive genes, stationary phase σ-factor RpoS-regulated genes as well as *quorum-sensing* regulons. The transcriptome of the *pppA-ppkA* mutant was also analysed under conditions of oxidative stress and showed an impaired response to the stress, manifested by a weaker induction of stress adaptation genes as well as the genes of the SOS regulon. In addition, expression of either quorum-sensing-dependent genes or RpoS-regulated genes and genes of Pho regulon was also affected. Our results suggest that in addition to its crucial role in controlling the activity of *P. aeruginosa* H1-T6SS at the post-translational level, the PppA-PpkA pair also affects the transcription of stress-responsive genes. Based on these data, it is likely that the reduced virulence of the mutant strain results from an impaired ability to survive in the host due to the limited response to stress conditions.

In the second part of this study, we focused on interactions of Ser/Thr protein kinase Stk1 with phosphatase Stp1 and their putative substrate protein Fha2. These proteins belong to the type VI secretion system H2-T6SS.

We prepared recombinant protein Fha2 and proved its phosphorylation by the Stk1 kinase *in vitro*. Immunodetection with anti-phospho-treonine antibody revealed further that Fha2 is phosphorylated by the Stk1 on threonine residue(s). However, our attempts to determine precise site of phosphorylation of Fha2 by the site directed mutagenesis of predicted target amino acid residues failed. We have also studied the effect of Stp1 phosphorylation on its activity. Site directed mutagenesis of previously identified phospho-threonine 90 did not affect phosphatase activity of Stp1. Furthermore, no phosphorylation of Stp1 by Stk1 in *in vitro* conditions was observed.