Histidine Kinases and Phosphatases from yeast to humans

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Schizosaccharomyces pombe is a fission yeast as it divides by medial division and is one of the most extensively used model organisms in molecular biology. It is a unicellular, rod shaped eukaryotic organism with three chromosomes as well as 4970 open reading frames. It can be used to express various proteins. In one project a human phosphohistidine phosphatase (PHPT1) has been studied. There are two splice variants of PHPT1 in humans one of which is truncated by an error in the annotation by one nucleotide. PHPT1 is 14 kD protein that removes phosphate group from histidine residues in proteins. The aim of this thesis is to express these two splice variants in *S. pombe* and compare their enzymatic activity with wild type variant by using an effective and improved PHPT1 assay.

For expression of PHPT1, the splice variants were first expressed in *E. coli* cells. Then these splice variants were inserted into two yeast vectors and transformed into *S. pombe* for the expression of recombinant protein. Protein was collected from the yeast cells and the activity of PHPT1 as well as its splice variants will be measured.

A second project is also going on to study a histidine kinase, CHK1, in *Candida albicans,* which is homologous to a histidine kinase in *S. pombe*. *C. albicans* is a commensal organism which means that it is not harmful in healthy individual but becomes pathogenic in immunocompromised patients with HIV or cancer. It has 3 histidine kinases, among which *CHK1* is more important for the synthesis of cell wall and virulency. To become infectious *C. albicans* switches its morphology from yeast growth to hyphal growth. The *CHK1* gene is the main responsible factor for this transition. The aim of this project is to set up a drug screen with substances inhibiting the Chk1 activity.

For this investigation, *CHK1* gene was first amplified and then sequenced. After that the gene will be expressed in a *S. pombe* vector. The three histidine kinase genes in *S. pombe* are important to prevent mating. When the yeast lacks these genes, it can mate and sporulate. By crossing, a *S. pombe* strain will be constructed which lacks the three endogenous histidine kinase genes and then the *S. pombe* expression vector with *CHK1* gene will be used to transform this strain. Then the transformed strain will be cultured in nutrient rich media and checked for the doubling time whether it can complement the deleted endogenous histidine kinase genes. By checking the growth rate, it is possible to screen for drugs that will inhibit Chk1 which in turn inhibit *C. albicans* growth rate.

Master project, 30 hp, and autumn 2012
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