# Thiamine-related regulation of metabolism and gene expression in the yeast *Saccharomyces cerevisiae*

**Dominik Mojzita**

**Department of Cellular and Molecular Biology, Göteborg University**
**Medicinaregatan 9C, Box 462, SE-405 30 Göteborg**

## *Abstract*

Thiamine (vitamin $B_1$) is an indispensable compound for all living organisms. In the form of thiamine diphosphate (ThDP), it works as an essential coenzyme of many enzymes involved in carbon metabolism. Its importance is especially apparent in the metabolism of pyruvate, which can not be further processed in the cell without ThDP-mediated reactions. Yeast and other organisms (such as bacteria or plants but not animals) can produce thiamine *de novo* by diverse biochemical pathways. Thiamine biosynthesis is a tightly regulated process primarily on the level of transcriptional control. In order to prevent energy wasting when thiamine is available in the environment, yeast has evolved efficient systems for thiamine uptake, sensing and regulation of gene expression program.

This thesis is dealing with thiamine related metabolism and its regulation in the yeast *Saccharomyces cerevisiae*. The first part of the thesis is focused on central metabolism, where thiamine plays a crucial role. Then a current understanding of thiamine utilization and biosynthesis is provided with particular emphasis on mechanistic descriptions of the processes involved. Next part of the thesis contains a review on regulatory mechanisms including also less characterized genes of the THI-regulon. In the last sections new concepts in gene expression regulation are presented by examples of metabolic enzymes playing a role in direct transcriptional control. A modeling approach for thiamine uptake system is also briefly introduced.

The thesis is based on four papers covering the topic of thiamine related metabolism. In *Paper I.*, a genome-wide as well as a gene-specific analysis is presented. The main conclusions of the paper are: further characterization of the THI-regulon, providing evidence of a key role of the transcription factor Pdc2 and description of four new genes of the regulon, *THI71*, *THI72*, *THI73* and *THI74*. In *Paper II.*, a detailed structural and functional analysis of the transcription factor Pdc2 is presented, including demonstration of its DNA-binding ability, transactivation and dimerization. In *Paper III.*, a systems biology approach is applied to study thiamine transport and phosphorylation. New aspects of the process are revealed such as quantitative properties of the system's components. New regulatory circuits in thiamine metabolism are also suggested. In *Paper IV.*, the mechanism of thiamine dependent transcriptional control is studied using protein-protein interaction analysis. Important findings are presented concerning the physical interaction between Pdc2 and the ThDP sensor Thi3 and between Pdc2 and a nuclear form of Pdc1. Functional properties of two isoforms of pyruvate decarboxylase, Pdc1 and Pdc5, are also analyzed.