Abstract

One of the key prerequisites for yeast cell growth is the uptake of essential compounds, such as potassium. Potassium is a vital monovalent cation and its sufficient intracellular concentration is crucial for various processes, for instance: regulation of membrane potential and cell turgor, enzymatic activity, and protein synthesis. A sufficient internal concentration of potassium is also one of the pivotal signals for cell division. However, as also excess of potassium might lead to unfavourable physiological consequences in yeast, such as deacidification of vacuoles and depolarization of plasma membrane, it is imperative for the yeast cells that the whole process of potassium acquisition is a tightly regulated affair, in order to maintain proper potassium homeostasis. In yeast *Saccharomyces cerevisiae*, uniporter Trk1 is considered a key player in potassium uptake. The presented thesis aimed to provide novel knowledge regarding Trk1, more specifically to study its ability to modify its capacity for potassium uptake, putative regulation by phosphorylation, and involvement in the survival of glucose-induced cell death (GICD). Additionally, potassium-uptake systems in selected non-conventional species were characterized as well.

The most distinctive feature of Trk1 is its alleged ability to switch between two affinity modes: low- and high-affinity mode, as a response to changes in external potassium concentration. We performed a detailed characterization of the process of 'affinity-switch' and found that rather than switching between two modes, Trk1 was able to adjust both its affinity and maximum velocity precisely in accordance with the availability of potassium. Additionally, we identified novel signals presumably driving the aforementioned changes in the capacity of Trk1-mediated uptake: internal potassium content and membrane potential. Our results also suggested the involvement of specific subdomains of Trk1, P-helices, in processes of adjustments of affinity.

Despite the indispensable role of Trk1 in yeast physiology, there is a substantial scarcity of detailed knowledge regarding its regulation. Transporter Trk1 has been previously shown to be expressed on a low level regardless of external conditions and its regulation is therefore thought to occur on a posttranslational level, mainly in form of phosphorylation. We identified two novel putative phosphorylation sites: Ser882 and Thr900 within the highly conserved intracellular subdomain of Trk1. Moreover, the involvement of small, regulatory 14-3-3 proteins in the orchestration of the activity of Trk1 was established, as well as their specific binding sites within Trk1.

In addition to Trk1, the genome of *S. cerevisiae* also encodes the second Trk-transporter and paralog of Trk1, Trk2. Despite the high degree of homology between the two, the role of Trk2 in

potassium uptake is considered marginal and its precise physiological role remains obscure. We studied the role of Trk-transporters in connection to GICD and found both Trk1 and Trk2 to be important for the survival of GICD with Trk2 fulfilling a major role. Additionally, a functional connection between Trk2 and H^+ -ATPase Pma1 was established unravelling putative, novel physiological roles of transporter Trk2.

Sufficient uptake of potassium and improved function of Trk1 has been shown multiple times to enhance the survival and performance of yeast during biotechnological production. It is therefore highly desirable to study processes of potassium acquisition and homeostasis also in biotechnologically relevant, often non-conventional species. We performed the initial characterization of two potassium uptake systems: Trk1 and Hak1 in the species *Kluyveromyces marxianus*. We found Hak1 to be a major player under low external potassium concentrations and Trk1 to presumably function under potassium non-limiting conditions. The individual contribution of both transporters to the survival of various stresses was studied as well.

Taken all together, the presented thesis provides novel results regarding Trk1 and its ability to react to changes in the availability of substrate by changing the overall capacity for uptake and its regulation by phosphorylation and association with 14-3-3 proteins. Presented results also point to the novel, previously uncharacterised physiological roles of transporter Trk2. Additionally, potassium uptake systems were also studied in non-conventional, biotechnologically relevant species *K. marxianus*.

Keywords: *Saccharomyces cerevisiae*, potassium homeostasis, Trk1, Trk2, regulation, affinity, phosphorylation, 14-3-3 proteins, *Kluyveromyces marxianus*, Hak1