On the intracellular pH of baker’s yeast

Orij, P.J.

Citation for published version (APA):
SUMMARY

The work in this thesis focuses on unraveling the mechanisms that regulate intracellular pH (pH$_i$) in baker’s yeast. The pH$_i$ is an important physiological parameter that is able to influence all ions in the cell and therefore has a profound influence on nearly all biochemical reactions. Because pH$_i$ has a big influence on the cell's biochemistry, tight regulation of this parameter seems essential. Regulation of pH$_i$ implies the cell has to have means of sensing the proton level at all times in order to correct deviating values. In this thesis we study what defines pH$_i$, what mechanisms sense and regulate it, as well as the implications of this regulation in terms of signaling.

Chapter 1 of this thesis summarizes the current knowledge obtained from research on pH$_i$ up to now, with a heavy focus on yeast. We discuss how pH$_i$ is defined and what methods are commonly used for its determination. The method we have used for all pH$_i$ determinations in this thesis makes use of a pH-sensitive green fluorescent protein (GFP). Wild-type GFP has 2 excitation peaks, and the ratio between the emission intensity of light that is emitted from the GFP as a result of excitation is basically the same at all pH values. The technique we utilize makes use of a particular mutant of GFP named pHluorin [1], which shows pH-dependent variation in the emission intensity of the protein, effectively turning it into an in vivo pH meter. The advantages of this method are that it is non-invasive, it can be used for organelle-specific measurements and it is fast. Chapter 1 continues by describing the effects pH$_i$ has on various cellular components like metabolites, proteins and phospholipids. Next, pH$_i$ regulation is discussed. The main contributors to the pH regulation of various organelles are the ATPases that reside in the membranes of yeast. Pma1p in the plasma membrane is considered the master regulator of pH$_i$ [2], but V-APases have also been shown to be important for pH$_i$ maintenance [3] as well as various cationic transporters and exchangers. The last part of Chapter 1 focuses on how yeast responds to pH$_i$ perturbations as a result of environmental changes. These perturbations appear to have important signaling functions.

Chapter 2 describes our efforts to prepare the pHluorin tool for measurements in yeast [4]. The GFP variant was expressed in the cytosol and mitochondrial
matrix to measure pH\textsubscript{i} in response to nutrient availability, respiratory chain activity, shifts in environmental pH and stress induced by addition of sorbic acid. To establish proof of principle, we reproduced the known pH\textsubscript{i} response of rapid acidification followed by alkalization upon addition of glucose to starved cells. Subsequent experiments showed cytosolic pH (pH\textsubscript{c}) of 7.2 and a mitochondrial (pH\textsubscript{m}) of 7.5 in growing cells that were unaffected by shifts in external pH (pH\textsubscript{ex}) between pH\textsubscript{ex} 3.0 and 7.5. We further show that pH\textsubscript{c} and pH\textsubscript{m} are differentially regulated by carbon source and respiratory chain inhibitors. Experiments with the weak-acid preservative sorbic acid showed a remarkable correlation between pH\textsubscript{i} recovery and the resumption of growth that was identically observed as a response to glucose availability. These results were a first indication of a possible relationship between pH\textsubscript{i} and growth rate.

In chapter 3 the relation between pH\textsubscript{i} and growth rate was further investigated. We set out to identify genes involved in pH\textsubscript{c} regulation by expressing pHluorin in the complete haploid gene deletion collection, and measurement of intracellular pH in each mutant during exponential growth on glucose. This revealed that genetically, pH\textsubscript{c} is a very tightly controlled mechanism as very few mutants showed a deviation in pH\textsubscript{c} and that this deviation was never more than 0.3 pH units. Furthermore, our global analysis revealed a striking relation between pH\textsubscript{c} and growth rate, which we confirmed and analyzed in wild-type strains. To establish causality, we used a specific mutant with a hypomorphic allele of PMA1 (pma1-007). In this mutant we were able to titrate pH\textsubscript{c}, which resulted in a decrease in growth rate quantitatively matching the one predicted. This effectively proved that pH\textsubscript{c} controls growth rate. The mechanism by which pH\textsubscript{c} controls growth rate remains unclear. However, it seems likely pH\textsubscript{c} has a role as a second messenger. Evidence for this was provided by the fact that not all mutants that showed deviating pH\textsubscript{c} adhered to the pH\textsubscript{c}-growth rate relationship. Several mutants in the inositol pyrophosphate biosynthesis pathway showed growth rates too high for their corresponding pH\textsubscript{c} and were uncoupled from the pH\textsubscript{c}-growth rate relationship, suggesting a role in pH signaling to growth for these metabolites.

In chapter 4 we studied the mechanisms that regulate pH\textsubscript{m}, and pH difference over the mitochondrial inner membrane (ΔpH), that drives ATP production...
during growth on non-fermentable carbon sources. This first genome-wide study of pH$_i$ revealed that the pH of different compartments is not independently maintained, but rather controlled by general mechanisms involved in regulating the proton availability in the entire cellular system. Additionally, we determined that under fermentation and respiration conditions ∆pH remained virtually the same, suggesting that the absolute ∆pH is not regulated to maximize energy generating capacity.

**Chapter 5** shows an important example of how pH$_i$ regulation as a result of changes in glucose availability can influence a specific signaling pathway. The pathway focused on is involved in lipid signaling. In yeast, phospholipid metabolism is regulated by the transcriptional repressor Opi1p, part of a lipid-sensor complex in the endoplasmic reticulum (ER). In the absence of inositol, Opi1p resides in the ER where it is bound to both phosphatidic acid (PA) and the tail-anchored ER protein Scs2 [5]. Addition of inositol to the medium results in a rapid depletion of PA, as it is converted into phosphatidylinositol, and a relocation of Opi1p to the nucleus where it subsequently represses INO1, which encodes the rate-limiting enzyme in inositol biosynthesis. A genome-wide screen for inositol auxotrophy showed significant enrichment for genes involved in pH regulation. The inositol sensitivity could be rescued by a deletion of OPI1, which indicated a role for these genes upstream of the ER lipid-sensor. Detailed combination of microscopy, pH$_i$ measurements and biochemical analyses revealed that the interaction of Opi1p with PA was indeed controlled by pH$_i$. The physiological relevance for this pH dependence was demonstrated by glucose starvation experiments which lead to a decrease in pH$_i$ and the subsequent translocation of Opi1p to the nucleus to repress lipid metabolism.

In conclusion, we uncovered new roles for the central physiological parameter pH$_i$, that confirms its role as a second messenger and emphasizes its central and general regulatory role. We believe these findings will change the way we look at the performance of the cellular metabolic and signaling network, and the way we have to do experiments to take these findings into account. The experiments in chapter 3 and 5 suggest that pH$_i$ acts as a *bona fide* second messenger that transmits environmental signals to a number of pathways.
Summary


