On the intracellular pH of baker's yeast

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This thesis describes our current knowledge of intracellular pH ($pH_i$) regulation in the yeast *Saccharomyces cerevisiae*. The way we perceive this regulation has changed considerably from when we set out to study this subject. The $pH_i$ proved to be more than a permissive condition for all biochemical reactions in the cell to occur, but was shown to be an actual part of signal transduction as a response to changing environmental conditions.

The cytosolic pH ($pH_c$) in yeast cells growing on glucose has been determined by us and others to be maintained around neutrality. The amount of free protons in the cytosol under these conditions cannot be more than 3000. In contrast, the amount of buffer molecules in the cytosol, e.g. ionic metabolites and amino acid side chains, that can accept or donate a proton are at least hundreds of millions. This makes the ionization states of these molecules very important as they largely contribute to $pH_c$. This observation changed our view of pH in a cell from the classical view in which free protons in a bulk of water determine the pH to a more complex view in which the relevant property is the combined protonation state of all charged molecules in the cell. This property is affected both by the influx or efflux of free protons, but also by changes in concentrations of weakly acidic or basic compounds in the cell. The relevance of this way of regarding $pH_c$ becomes immediately apparent in the research into the molecular mechanisms of growth inhibition by anti-microbial compounds, such as weak organic acids, where the anion can accumulate to high levels (hundreds of millimolars) in the cell.

Another observation we made is that $pH_i$ is not a static value, but a highly dynamic property of cells that is sensitive to changes in environmental conditions. Upon glucose depletion, $pH_c$ was shown to rapidly decline. If this decline is an active mechanism or a result of the inability of the cell to keep a proton gradient over the plasma membrane due to declining ATP reserves remains to be properly elucidated. Pma1p, the plasma membrane P-type ATPase and the main regulator of $pH_c$, pumps protons out of the cell at the cost of ATP. Our experiments show that $pH_c$ declines to medium pH (our unpublished results). Similarly, Dechant *et al.* showed that at external pH of 7.4
glucose depletion does not lead to a decline in pHc, suggesting a passive means of acidification as a result of energy depletion [1]. In accordance with this, the same paper described that excess of 2-deoxy-glucose, a glucose analog that cannot be metabolized beyond the hexokinase reaction and leads to ATP depletion, added to yeast growing on glucose, lead to a decline in pHc. Although pHc regulation seems to be linked to ATP levels in the cell, additional studies are needed to uncover if these observations are the result of a direct lack of ATP to activate Pma1p, or if some other signaling pathway is involved.

Even if the regulation of pHi by glucose is a passive mechanism, yeast cells have evolved to use this mechanism to regulate processes important for growth. The necessity of cells to halt the metabolic processes that regulate growth in case of carbon source depletion is self-explanatory. One of the processes that needs to be stopped is lipid metabolism, as lipids are the building blocks of biological membranes. In chapter 5 we showed that the main repressor of lipid metabolism, Opi1p, is regulated by glucose availability through pHc [2]. This raises the question if other transcriptional regulators are governed by a similar mechanism as the effect of glucose depletion results in a complete change in cellular physiology involving a plethora of transcriptional effectors. Figure 1 depicts a model of a proposed mechanism such regulation. Currently we have only identified Opi1 as a pH-dependent transcriptional regulator, but we hypothesize that there are other processes required for growth, such as cell cycle processes or cell division that could be regulated in a similar fashion.

One of the mechanisms that we revealed to be under the control pHc is cellular growth rate (chapter 3). It seems unlikely that the pathway controlled by Opi1p is in control of this regulation, since the experiments in chapter 3 were performed in excess inositol. In these conditions Opi1p cannot regulate growth as inositol is no longer limiting. We identified 19 mutants in our screen that had a growth rate that was too high for their corresponding growth rate. These mutants were enriched for genes involved in pyrophosphate biosynthesis. The apparent uncoupling of these mutants from the pHc-growth rate relationship suggest a role for these genes in the transmitting a signal from pHc to the mechanism that control growth. However, the precise mechanism that constitutes this signaling function remains to be elucidated.
Figure 1: Regulation of and by pH$_6$ - Mechanistic model of nutrient coupling to cell proliferation. Nutrients like glucose have been shown to be essential for pH maintenance. Glycolysis produces ATP that can be used by the ATPases to transport protons over membranes to raise pH$_6$. Low pH$_6$ protonates phosphatidic acid and prevents Opi1p to repress lipid synthesis of inositol, an essential component of membranes (chapter 5). We suggest more signal transduction pathways are regulated similarly by pH$_6$.

The nature of regulation of cellular processes by pH$_6$ is so simple and seems so universal it is hard to imagine that it would not be a conserved biological phenomenon across all cellular organisms. The utilization in all cellular systems of inorganic phosphate as a cellular buffer and phosphorylation as a way to
activate proteins, that are both sensitive to pH changes in a physiologically relevant pH range, are strong indications of this hypothesis. We believe that we need to be more aware of these basic mechanisms when designing experimental procedures. The uncovering of additional mechanisms under control of intracellular pH promises to be an important and exciting topic for future research.

1. Dechant, R., et al., Cytosolic pH is a second messenger for glucose and regulates the PKA pathway through V-ATPase. EMBO J, 2010.