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Cytosolic pH and Environmental Signalling in Yeast

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Summary

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Cytosolic pH (pH_c) is a crucial parameter in the cell because it is highly dynamic and its changes affect the function of all biomolecules containing weakly acidic or basic groups. Although it can influence most biological processes, our understanding of how pH_c is controlled and what are the consequences of changes in pH_c is rather limited. The objective of our research was to investigate these two aspects of cytosolic pH signalling within the context of environmental signalling in the budding yeast *Saccharomyces cerevisiae*.

In **Chapter 1** we review the current understanding of intracellular pH regulation and its effects focusing on the yeast model. We first discuss the importance of pH_c changes and how those can affect processes either as a second messenger or a parameter affecting the efficiency of biological process or a combination of both. Because of the relevance of pH_c changes, mechanisms evolved that keep pH_c tightly controlled. We recapitulate known mechanisms for the regulation of the main players involved in pH homeostasis, the plasma membrane H^+-pump Pma1 and the vacuolar H^+-ATPase, V-ATPase. Glucose starvation and re-addition trigger inactivation and re-activation of the pumps, respectively. Several nutrient signalling pathways are involved in such process. Environmental signalling pathways are not only involved in pH_c control but are in turn downstream of pH_c regulation. In the last part of Chapter 1, we discuss the implications of pH_c changes on general cell function and address the effects of pH_c on specific environmental signalling pathways.

Similar to pH_c, the nutrient signalling pathway Protein Kinase A (PKA) promotes growth in response to the presence of fermentable carbon sources. PKA seems to regulate both Pma1 and V-ATPase, but the implications for pH_c control are not known. In **Chapter 2**, we study the effects of PKA on pH_c regulation. By monitoring pH_c dynamics in a set of PKA pathway mutants for we identify PKA as an important regulator of pH_c specifically upon glucose depletion. Thus, pH_c is tightly controlled not only during growth, but also in the absence of a carbon source, when ATP levels are low. Careful assessment of the time of PKA activation by cAMP shows that PKA activity prior glucose depletion promotes the pH_c decrease occurring after glucose depletion. PKA is activated by glucose and, accordingly, decreasing the initial concentration of glucose supplied to the cultures, reduces starvation-associated acidification via PKA. The active control of starvation pH_c also implies a role of pH_c on the adaptation and survival in stationary phase, when cultures

are starved for all carbon sources. Specific manipulation of starvation pH_c shows that cytosolic acidification reduces survival, all in all supporting a model in which glucose availability reduces stationary phase survival via modulation of pH_c downstream of PKA.

Nutrient limitation is a key input regulating growth and survival. How nutrient availability signals are integrated with other growth and survival regulators such as pH_c is not known. In **Chapter 3**, we study the effects of nitrogen on pH_c. Analysis of pH_c dynamics on two nitrogen sources, glutamic acid and ammonium sulphate, shows that nitrogen concentration does not affect pH_c during growth but promotes the pH_c decrease upon glucose starvation. This indicates that nitrogen limits growth via mechanisms other than pH_c and establishes a role for nitrogen on the control of pH_c changes upon carbon starvation. Upon acute glucose starvation, only glutamic acid availability but not ammonium sulphate promotes acidification. In rapid controlled starvations, the effect of glutamic acid on pH_c is also reduced and transient, altogether suggesting that nitrogen is mainly sensed during growth to promote acidification after glucose depletion (similar to PKA and glucose in Chapter 2). Next we show that the nitrogen sensing pathway Target of Rapamycin Complex1 1 (TORC1) controls starvation pH_c in response to nitrogen abundance. The effects on pH_c of glutamic acid abundance do not affect survival because lifespan is already very long in media containing only a single nitrogen source. We therefore propose that pH_c may have an effect at later time-points or influence the commitment towards starvation-induced differentiation programs.

Cytosolic acidification is a signal limiting growth, effect that has been proposed to be signalled by a reduction of PKA activity. Low pH_c, however, has been also suggested to activate PKA. In **Chapter 4** we set up an *in vivo* PKA assay, TriPP-TRAP, to investigate the effects on PKA of changes in pH_c under several growth conditions. TriPP-TRAP responds rapidly to both PKA activation and inhibition and thus can be used to monitor PKA dynamics. Analysis of the effects of changes on pH_c shows that pH_c regulates PKA in a condition-dependent manner. Low pH_c can inhibit PKA in the presence of glucose, but the effects are only transient and therefore cannot explain the growth rate reduction caused by low pH_c. In glucose de-repressed cultures, however, acidification activates PKA. Overall our results highlight the importance of metabolic factors in the regulation of signalling pathways.

In **Chapter 5** we study the mode of action of low extracellular pH (pH_{ex}) stress, a treatment with relevance in bioethanol production. Mutants in the cell wall integrity (CWI) pathway, a stress signalling pathway that responds to cell wall damage, are hypersensitive to low pH_{ex} stress.

Indeed, CWI mutants loss viability upon low pH_{ex} treatment, indicating that cell damage occurs extracellularly at the level of the cell wall/envelope. The viability loss under these conditions is not caused by intracellular acidification. In contrast, intracellular acidification improves viability of CWI mutants in an acidic environment. We therefore conclude that the stress-induced decrease in pH_c protects the cells from stress via growth reduction.

Chapter 6 summarizes the main findings of this thesis and discusses its implications in a broader perspective. We conclude that, although PKA regulates pH_c and pH_c regulates PKA, these effects take place on different conditions or upon distinct nutrient transitions and therefore PKA and pH_c are not part of a feedback regulatory loop. In contrast, TORC1 activity and pH_c seem to regulate each other in a feedback regulatory loop, at least under some conditions. Further research, however is necessary to dissect the interaction between them. We suggest that pH_c fulfils different roles in the cell, again in a condition-dependent manner. Thus, pH_c emerges as a signal integrated within the signalling network and future work will to define its targets and functions to understand cell decision making.