A Modular Model of Cyclic AMP Signaling in Yeast: Linking Cell Cycle Progression and Energy Metabolism

D. Müller[1], L. Aguilera-Vázquez[1], H. Diaz-Cuervo[1], E. Guerrero-Martín[1], J. O. Marquetand[1], P.K. Murugan[1], M.A. Henson[2], and M. Reuss[1]

[1]: Institute of Biochemical Engineering, University of Stuttgart, Allmandring 31, D-70569 Stuttgart, Germany
[2]: Goessmann Laboratory, 686 North Pleasant St., University of Massachusetts Amherst, Amherst, MA 01003

Introduction

The pursuit of a systems-level understanding of biological processes constitutes one of the central goals of systems biology. In this work, a combination of experimental techniques and mathematical modeling has been applied to analyze the impact of signal transduction via the second messenger cyclic AMP (cAMP) on the coordination of energy metabolism and cell cycle progression in the yeast *Saccharomyces cerevisiae*. The aim of this work is to develop a single-cell model which provides a combined description of metabolic processes and signaling events in conjunction with a dynamic representation of the cell cycle.

In yeast, activation of protein kinase A (PKA) via a cAMP-dependent signaling cascade triggers a stimulation of glycolytic flux and stimulates breakdown of the storage carbohydrates glycogen and trehalose while downregulating their synthesis. The stimulation of glycolytic flux results from the joint action of increased storage carbohydrate breakdown and the upregulation of several glycolytic enzymes and inhibition of key gluconeogenetic enzymes, respectively, due to phosphorylation by PKA. This kinase also acts upon the cell cycle machinery by influencing the dynamics of G1 cyclin concentrations, which determine the critical cell size required for budding. PKA has also been shown to affect the timing of mitotic exit - and thus the cell size at division - by modifying the activity of the anaphase promoting complex (APC).

Experimental Analyses

Experiments have been performed in oscillating, partially-synchronized chemostat cultures as well as in synchronous cultures obtained by centrifugal elutriation. These allow to assay yeast cell cycle dynamics at the quasi-single cell level while providing sufficient biomass for biochemical assays, e.g., to determine intracellular concentrations of metabolites or second messengers. The population distribution of cell cycle stages in a cell population were quantified using different microscopic techniques and flow cytometry.

These investigations have demonstrated distinct dynamics of cAMP and downstream targets of PKA in energy metabolism during the cell cycle [1]. In particular, a peak in the cAMP concentration was observed at the transition from G1 to S phase at low growth rates, which was followed by increased storage carbohydrate breakdown, high rates of oxygen uptake and carbon dioxide excretion as well as temporary formation of the overflow metabolites ethanol and acetate. By contrast, cAMP was low during early mitosis, which agrees with the well-

known fact that a high APC activity is required for mitotic exit. This feature was found to be conserved at higher growth rates whereas the timing of the cAMP peak seemed to shift to later timepoints during the S/G2 phase in this case. The upstream signal responsible for the differential activation of this signaling pathway during the cell cycle has remained elusive so far. We have obtained evidence suggesting that intracellular nucleotide concentrations may play a role in linking cAMP signaling and hence cell cycle progression to the energetic state of the cell, at least under glucose-limited conditions.

Development of a Modular Mathematical Model

A mathematical model has been developed, which aims at capturing not only the dynamics of cAMP-PKA signal transduction, but also that of central carbon metabolism and the cell cycle machinery itself. The integrated single-cell model features a modular structure where each of the processes constitutes a module of its own. These submodels are interconnected via multiple feedback effects which occur in this network (see Figure 1).

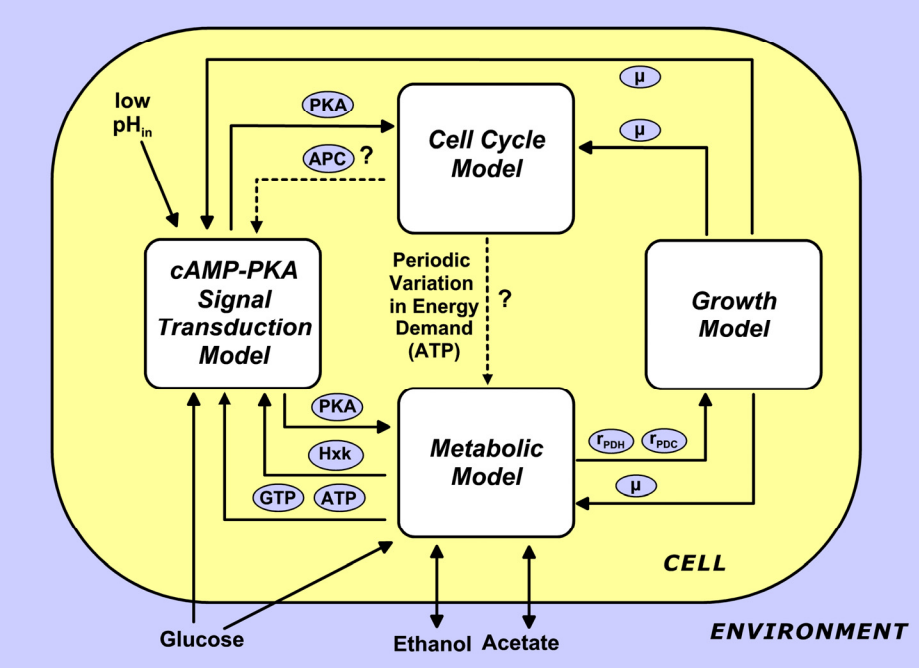

Figure 1 Sketch of the modular model describing the impact of cAMP and PKA on energy metabolism and cell cycle progression. The different modules are interconnected via feedback effects. Question marks and dotted lines indicate hypothetical interactions (*Hxk*: hexokinase, μ: specific cellular growth rate, pH_{in}: intracellular pH, r_{PDC}: rate of pyruvate decarboxylase, r_{PDH}: rate of pyruvate dehydrogenase).

The signaling module comprises the dynamics of cAMP synthesis and degradation as well as the resulting PKA activation. Simulation studies demonstrate that the model is able to reproduce, e.g., the adaptive response of the signaling pathway to a persistent extracellular

stimulus of extracellular glucose observed in experiments. This behavior results from the negative feedback of PKA activity on cAMP production.

The metabolic module is based on a previously established model of glycolysis and the pentose phosphate pathway [2, 3], which has been extended to include the dynamics of the storage carbohydrates trehalose and glycogen along with their associated regulation by PKA. Cell growth is modeled as a simple stoichiometric relation where the specific growth rate is directly dependent on the output of the metabolic module. Estimation of model parameters for this module was performed based on measurement data from stimulus-response experiments in chemostats.

A kinetic model of the yeast cell cycle machinery developed by Chen et al. [4] and Cross [5] constitutes the basis of the cell cycle module. The original model was amended to account for PKA influence on cell cycle progression (cf. Figure 2b). In particular, the delay in expression of G1 cyclins and the inhibition of APC activity during mitosis by high PKA levels have been accounted for. Population distributions of cell age and nuclear morphology were determined in chemostat cultures at steady state (μ=0.1 h^{-1}).

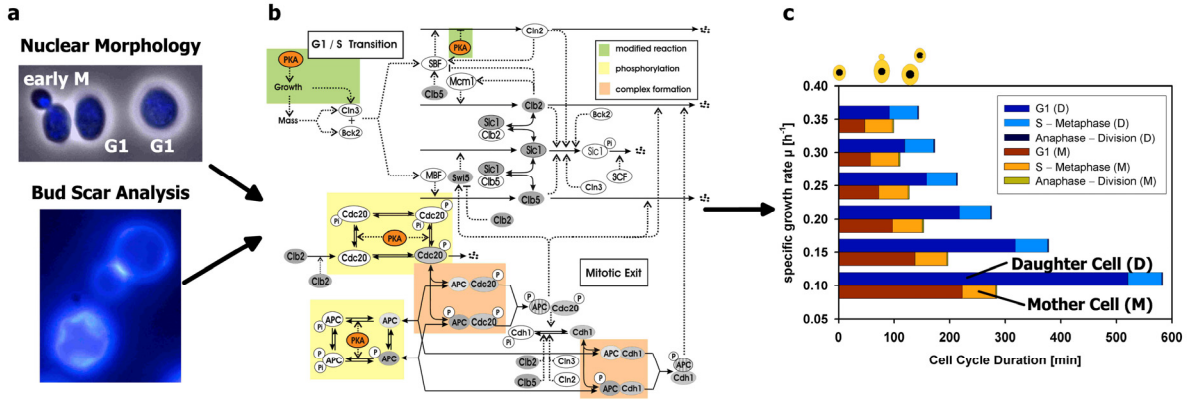

Figure 2 Development of the cell cycle module. Population distributions of nuclear morphology and bud scars (a) were used to estimate parameters of the extended cell cycle model (b). Highlighted areas in the scheme indicate reactions that have been added to or modified in the original model [4, 5]. (c) Simulated cell cycle duration of mother and daughter cell cycles at different specific cellular growth rates according to the dynamic model shown in (b).

Cell age was determined by scoring the number of bud scars on Calcofluor-stained cells under the fluorescence microscope. Similarly, cells stained with DAPI (4',6-diamidino-2-phenylindole), which yields nuclear DNA blue-fluorescent, were used to classify cells according to nuclear morphology into four categories: (i) cells with single nucleus and without bud, (ii) cells with single nucleus carrying a bud, (iii) budded cells with elongated spindle, and (iv) binucleate cells. These procedures were carried out for several samples and at least 800 cells were scored per sample in both cases. From this data, the fractions of the respective cell classes in the population were determined. Assuming balanced growth conditions [6, 7], it is possible to calculate the duration of the different cell cycle phases in both

mother and daughter cells, respectively, from these fractions. A modified version of the set of the population balance equations suggested by Bellgardt [8] and Alberghina et al. [9] was employed for this purpose. In this way, it is possible to determine the length of the cell cycle phases under well-controlled conditions from asynchronous chemostat cultures at steady state without the need to synchronize cells. This approach has the additional benefit that it relies on real single cell data while at the same time capturing the variation present in the real cell culture. The calculated duration of the phases were subsequently used to estimate newly-introduced parameters of the dynamic model (cf. Figure 2b) by non-linear optimization. The resulting model describes the impact of cAMP and PKA on the dynamics of cell cycle progression at different growth rates. Especially, the growing asymmetry in cell cycle duration between mother and daughter cells with decreasing growth rates observed in experiments is correctly reproduced by the model (cf. Figure 2c).

Conclusion

Upon integration of the developed modules, the resulting single-cell model will yield a dynamic description of the cAMP-dependent regulation of metabolism and cell cycle progression during the different cell cycle phases. Information from global datasets and published experimental data as well as own measurements in synchronous cultures and stimulus-response experiments in chemostats have been employed for estimation of model parameters. This guarantees the intimate connection of experiments and model development characteristic of systems biology. The chosen modular approach is potentially applicable to systems of medical importance where the link between signal transduction, energy metabolism, and the cell cycle is crucial, e.g. when modeling tumor cell behavior. Moreover, the model can also serve as a basis for a segregated description of heterogeneous cell populations [10, 11], an issue of major importance in the operation of large-scale bioprocesses.

References

[1] Müller, D., et al., *Cyclic AMP mediates the cell cycle dynamics of energy metabolism in Saccharomyces cerevisiae.* Yeast, 2003. **20**(4): p. 351-367.

[2] Rizzi, M., et al., *In vivo analysis of metabolic dynamics in Saccharomyces cerevisiae .2. Mathematical model.* Biotechnology and Bioengineering, 1997. **55**(4): p. 592-608.

[3] Vaseghi, S., et al., *In vivo dynamics of the pentose phosphate pathway in Saccharomyces cerevisiae.* Metabolic Engineering, 1999. **1**(2): p. 128-40.

[4] Chen, K.C., et al., *Kinetic analysis of a molecular model of the budding yeast cell cycle.* Molecular Biology of the Cell, 2000. **11**(1): p. 369-91.

[5] Cross, F.R., *Two redundant oscillatory mechanisms in the yeast cell cycle.* Developmental Cell, 2003. **4**(5): p. 741-52.

[6] Hartwell, L.H. and M.W. Unger, *Unequal Division in Saccharomyces cerevisiae and Its Implications for Control of Cell Division.* Journal of Cell Biology, 1977. **75**(2): p. 422-435.

[7] Lord, P.G. and A.E. Wheals, *Asymmetrical Division of Saccharomyces cerevisiae.* Journal of Bacteriology, 1980. **142**(3): p. 808-818.

[8] Bellgardt, K.H., *Baker's Yeast Production*, in *Bioreaction Engineering - Modeling and Control*, K. Schügerl and K.H. Bellgardt (Eds.). 2000, Springer: Berlin. p. 279-320.

[9] Alberghina, L., et al., *Analysis of Protein Distribution in Budding Yeast.* Biotechnology and Bioengineering, 1983. **25**(5): p. 1295-1310.
[10] Henson, M.A., D. Müller, and M. Reuss, *Cell population modelling of yeast glycolytic oscillations.* Biochemical Journal, 2002. **368**(Pt. 2): p. 433-446.
[11] Lapin, A., D. Müller, and M. Reuss, *Dynamic behavior of microbial populations in stirred bioreactors simulated with Euler-Lagrange methods: Traveling along the lifelines of single cells.* Industrial & Engineering Chemistry Research, 2004. **43**(16): p. 4647-4656.