*1st FEBS Advanced Lecture Course*

**Systems Biology: From Molecules &Modeling to Cells**

**Gosau, Austria, March 12-18, 2005**

# Hiding behind the Population Average - Cell Cycle Dynamics of Energy Metabolism during the Lifelines of Individual Yeast Cells


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***Dirk Müller, Luciano Aguilera-Vázquez, Ester Guerrero-Martín***

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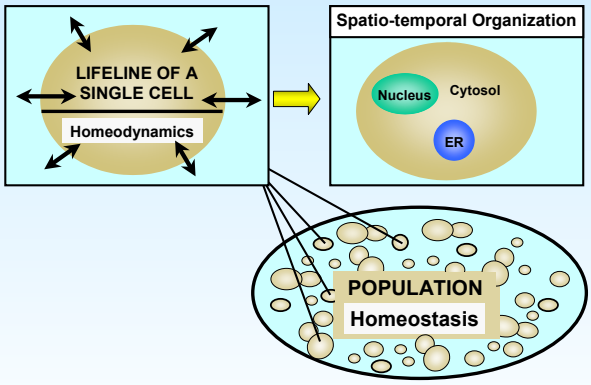
**University of Stuttgart/Germany**


- **MATHEMATICAL MODELING DOES NOT MAKE SENSE WITHOUT DEFINING, BEFORE MAKING THE MODEL, WHAT ITS USE IS AND WHAT PROBLEM IT IS INTENDED TO HELP TO SOLVE .**
- **FROM THE POINT OF VIEW OF MODELING WHOLENESS IS NEVER AN END IN ITSELF - THERE IS NO WHOLENESS IN ITS OWN RIGHT, IT MUST BE SUBMITTED TO THE PURPOSE OF THE MODEL.**

## Levels of Organization

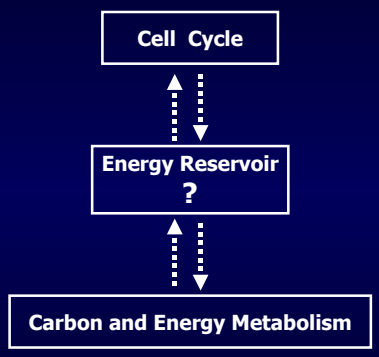



## Single cell models vs. Modeling at the level of population

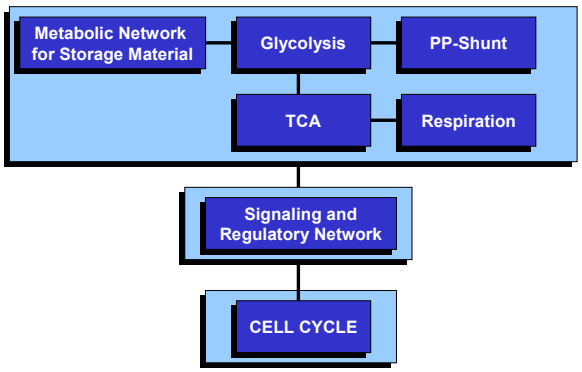
- Homeostasis
- Futile cycle
- Dynamics of signaltransduction between compartiments (spatial-temporal description)
- Synchronisation of autonomous oscillations at population level
- Circadian rythms

## Relevance of Single Cell Behavior: Cancer and Apoptosis

adapted from
http://www.sigmaaldrich.com/cellsignaling

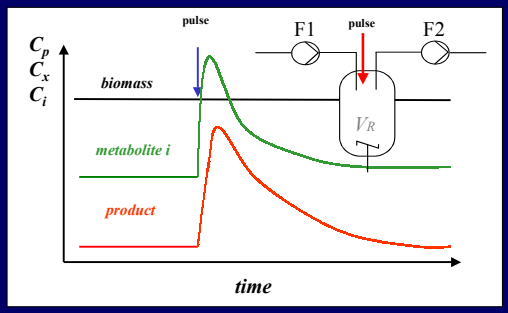


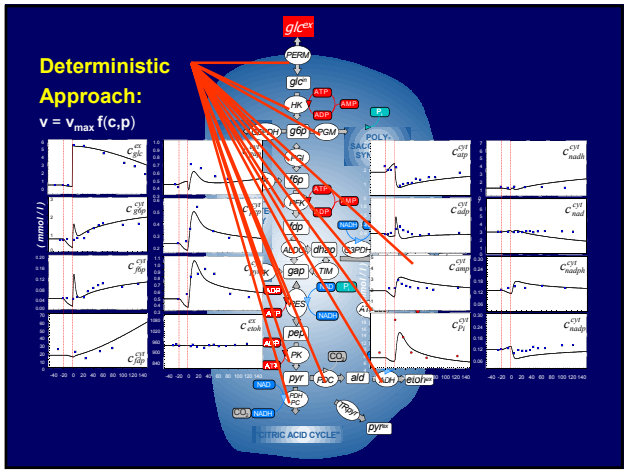
## MODULARISATION

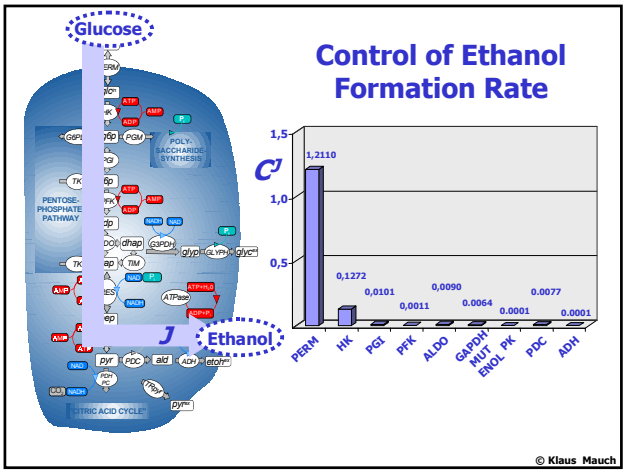


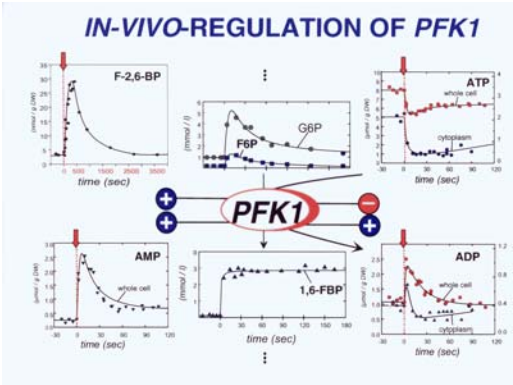

## Control of glycolytic flux /Strategy

Measurement of intracellular metabolites at continuous cultivation (steady state conditions) and after disturbance the steady state by a pulse of glucose (stimulus/response reaction)



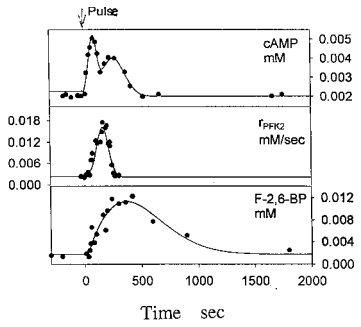



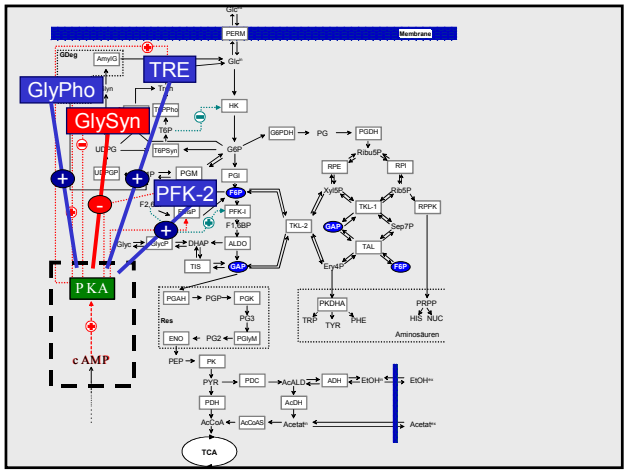




## cAMP Signaling and Metabolism

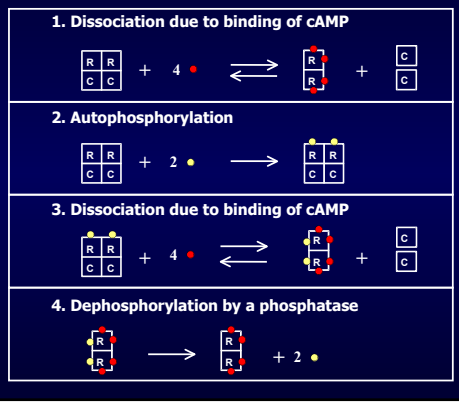
## cAMP Signaling and Metabolism

## Dynamics of the PFK2 System during a Glucose Pulse

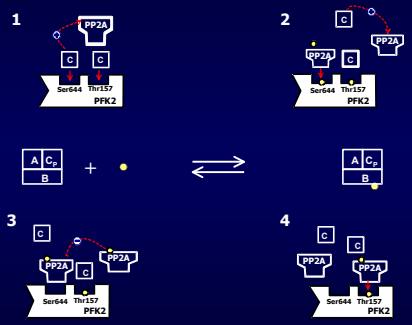


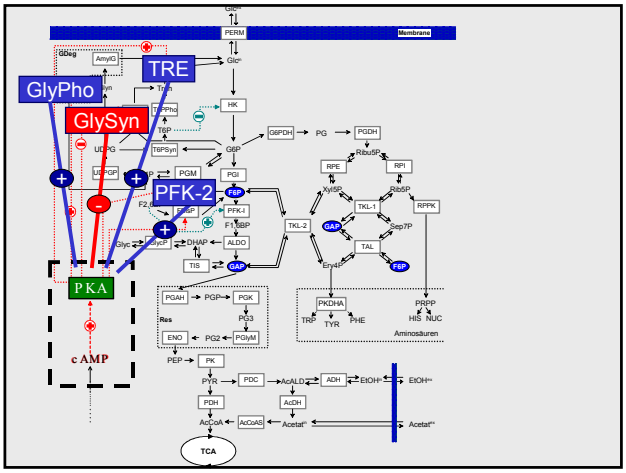



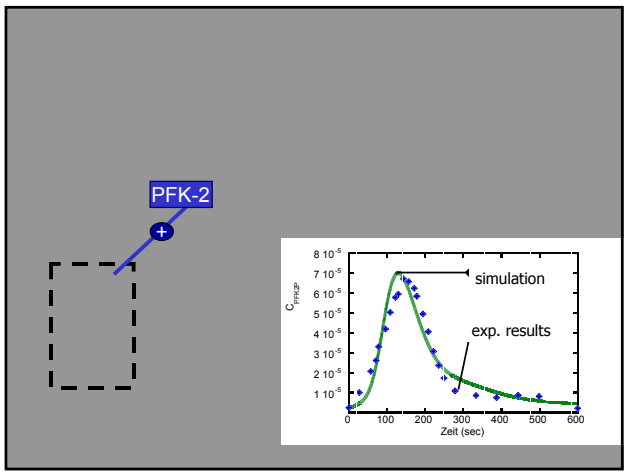

## Mechanism of PKA Activation

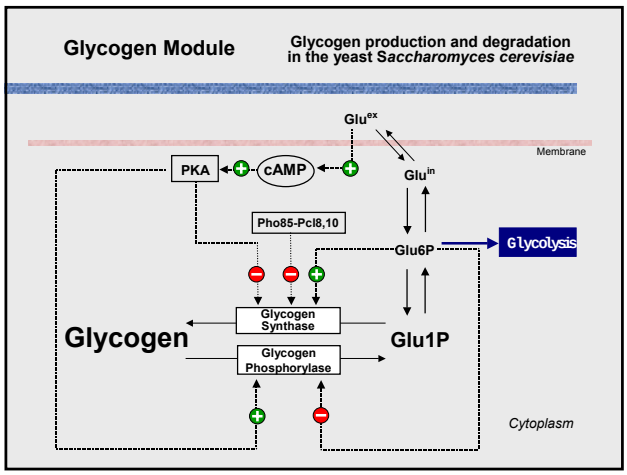


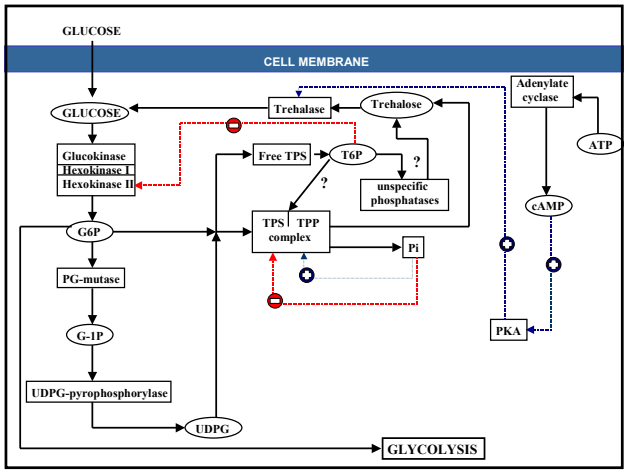

## Target Protein PFK 2: Phosphorylation and Dephosphorylation

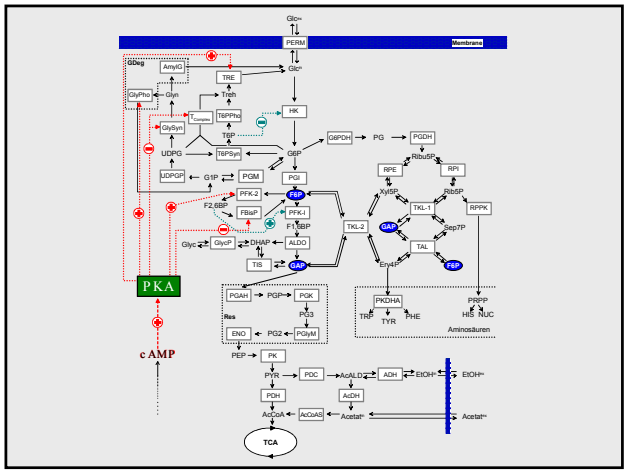


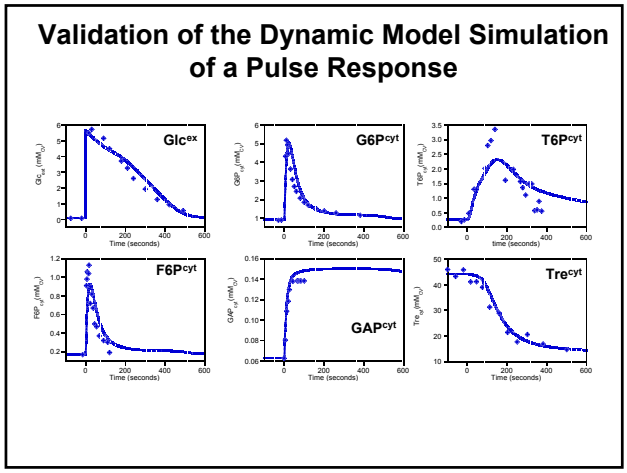



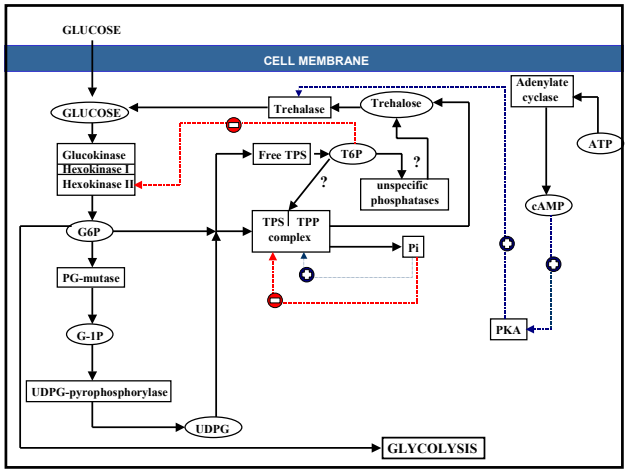

PFK-2
+
simulation
exp. results
Zeit (sec)


Glycogen Module
Glycogen production and degradation in the yeast Saccharomyces cerevisiae
Membrane
PKA
cAMP
Pho85-Pcl8,10
Glycolysis
Glu6P
Glycogen Synthase
Glycogen
Glu1P
Glycogen Phosphorylase
Cytoplasm


GLUCOSE
CELL MEMBRANE
Adenylate cyclase
Trehalase
Trehalose
ATP
Glucokinase
Hexokinase I
Hexokinase II
Free TPS
T6P
unspecific phosphatases
cAMP
G6P
TPS
TPP
complex
Pi
PG-mutase
PKA
G-1P
UDPG-pyrophosphorylase
UDPG
GLYCOLYSIS


PERM
Membrane
PKA
cAMP
TCA


Validation of the Dynamic Model Simulation of a Pulse Response
Glcex
G6Pcyt
T6Pcyt
F6Pcyt
GAPcyt
Trecyt
Time (seconds)


GLUCOSE
CELL MEMBRANE
Adenylate cyclase
Trehalase
Trehalose
ATP
Glucokinase
Hexokinase I
Hexokinase II
Free TPS
T6P
unspecific phosphatases
cAMP
G6P
TPS
TPP
complex
Pi
PG-mutase
PKA
G-1P
UDPG-pyrophosphorylase
UDPG
GLYCOLYSIS

## Model of the cAMP-PKA Cascade

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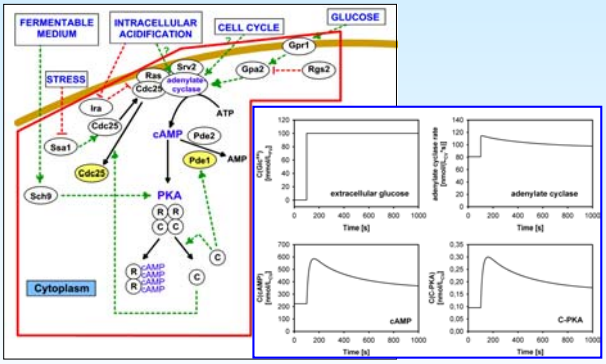
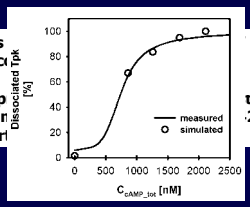



## Model of cAMP Signal Transduction

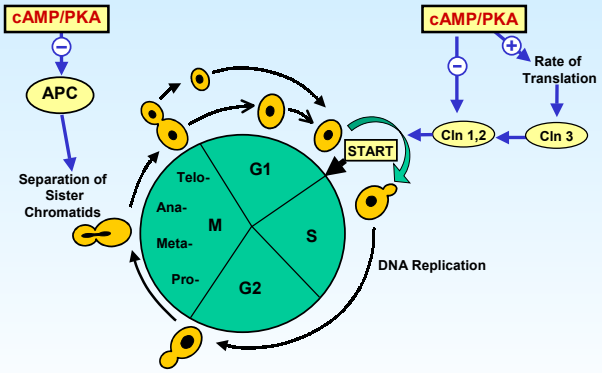
- quantitative protein levels from large-scale study (Ghaemmaghami et al. (2003) Nature 425:737-41) as starting point for simulation

- model consists
(combinatorial c
- 'quantitative' p
(Ghaemm
as a star

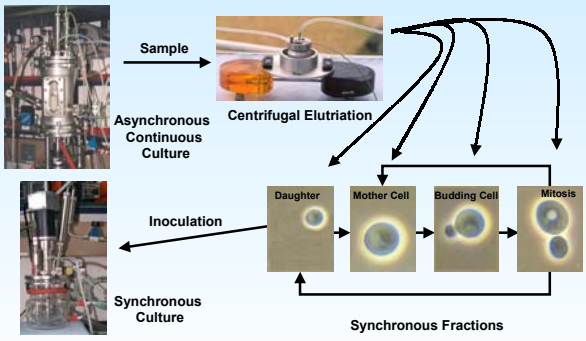



Target Protein: Kemptide
tudy
25:737-41)

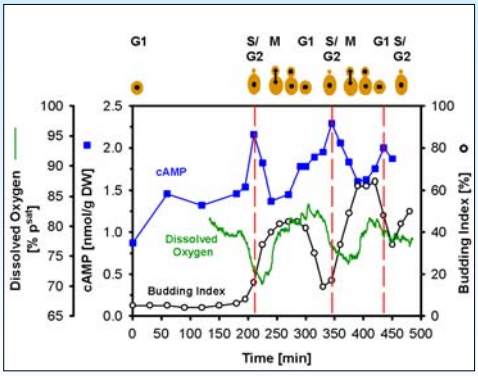
## cAMP Signalling Cascade - Cell Cycle Effects




## Preparation and Cultivation of Synchronous Cultures

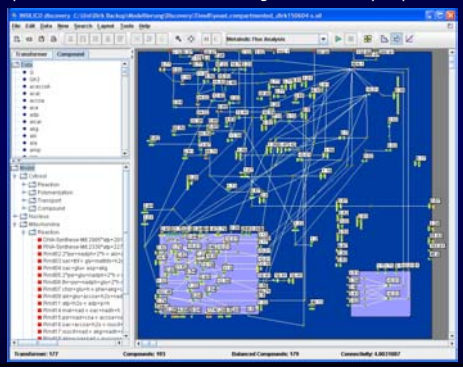



## cAMP Dynamics in Synchronous Culture



## Metabolic Flux Analysis

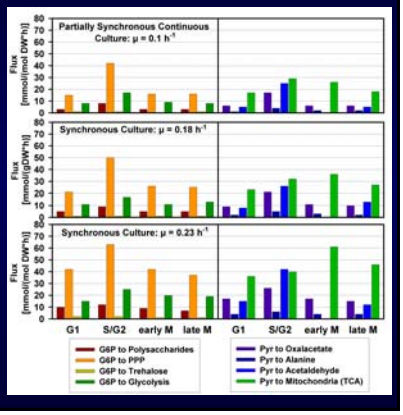
- compartmented stoichiometric model (193 compounds, 177 reactions)
- captures variation in metabolism during different cell cycle phases



## Metabolic Flux Analysis

Preliminary Results:

- breakdown of storage carbohydrates induces flux increase and production of overflow metabolites at G1/S transition
- increased flux to TCA cycle due to uptake of previously excreted ethanol and acetate during early mitosis




## The Missing Link - Hypotheses

*CDB*: Cyclin Destruction Box

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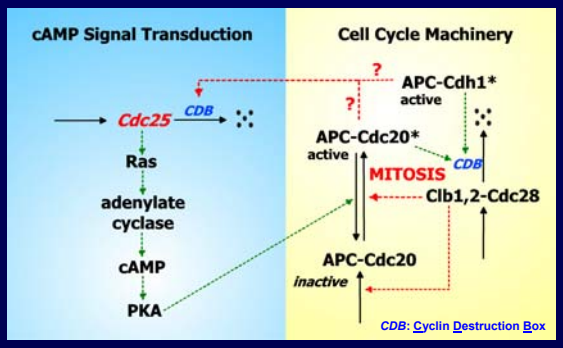
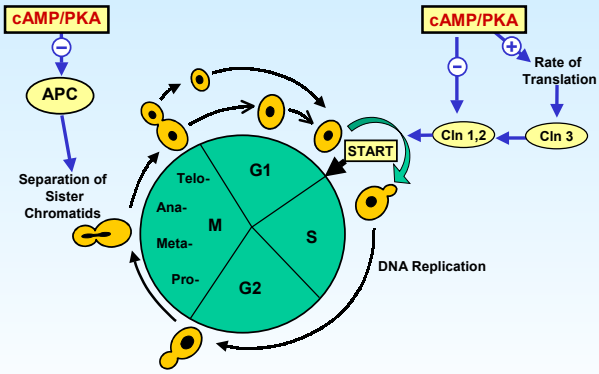



*CDB*: Cyclin Destruction Box

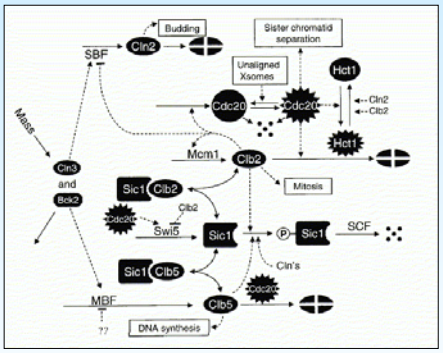
## The Missing Link - Hypotheses

*CDB*: Cyclin Destruction Box

## cAMP Signalling Cascade - Cell Cycle Effects



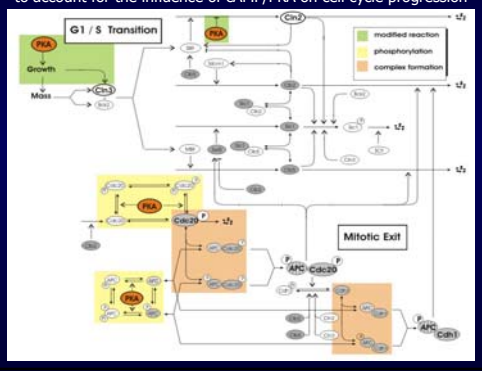
## Cell Cycle Model (Chen et al., 2000)



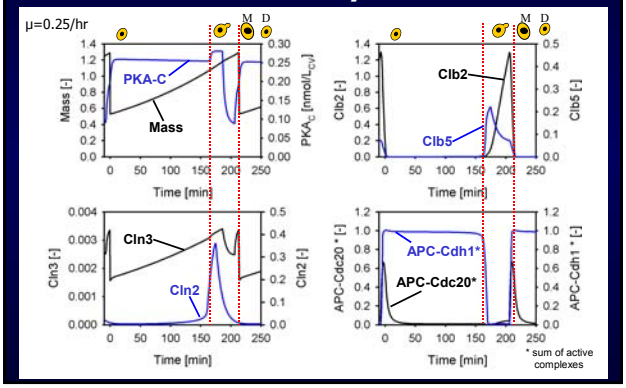

Chen KC, Csikasz-Nagy A, Gyorffy B, Val J, Novak B, Tyson JJ (2000) Mol Biol Cell **11**:369-391

## Extended Cell Cycle Model

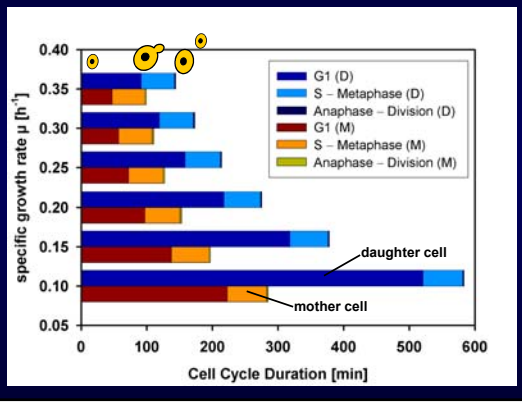
Extension of the cell cycle model developed by Chen *et al.* (2000) and Cross (2003) to account for the influence of cAMP/PKA on cell cycle progression



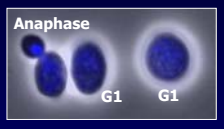

## Concentration Trajectories of the Extended Cell Cycle Model



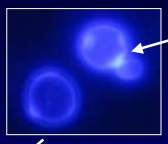

## Cell Cycle Model - Phase Durations




## Cell Cycle Model - Parameter Estimation




Nuclear Morphology (DAPI) - discriminate different cell cycle phases

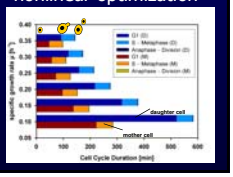


Birth Scars (Calcofluor) - differentiate between mother and daughter cells

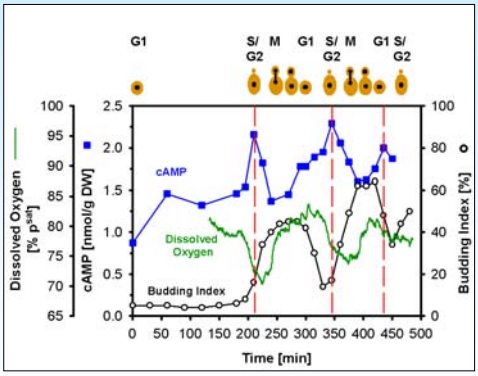
Population Balance Model

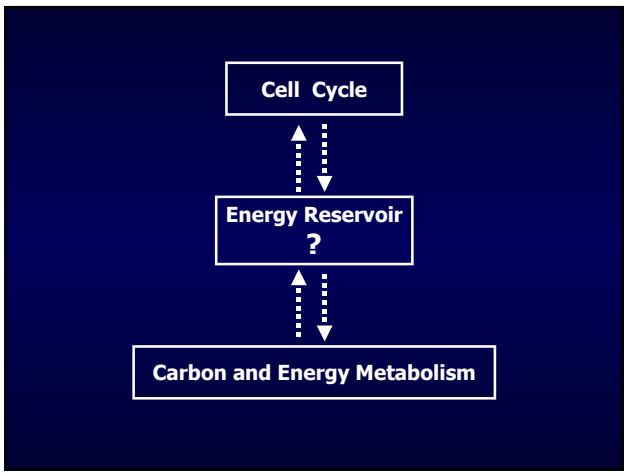
Phase Durations of Mother and Daughter Cell Cycles

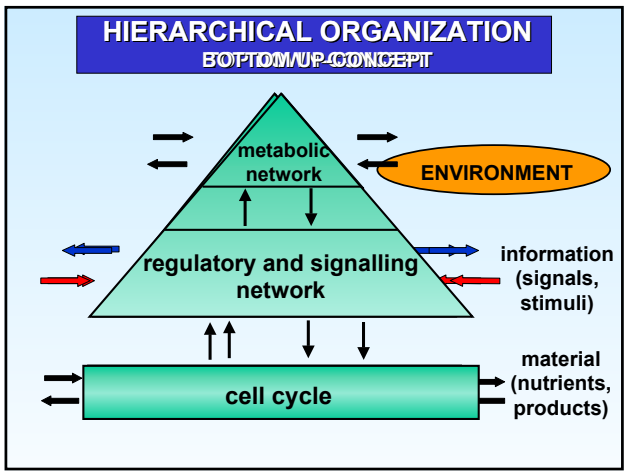
Parameter estimation by nonlinear optimization



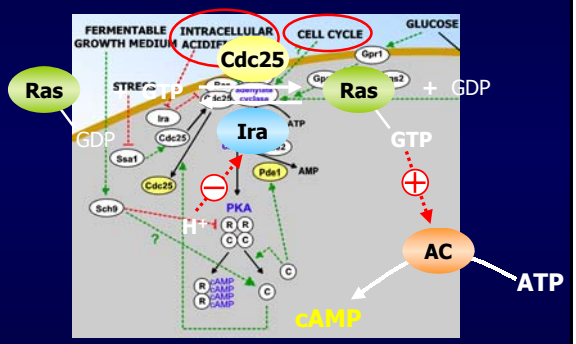
## cAMP Dynamics in Synchronous Culture



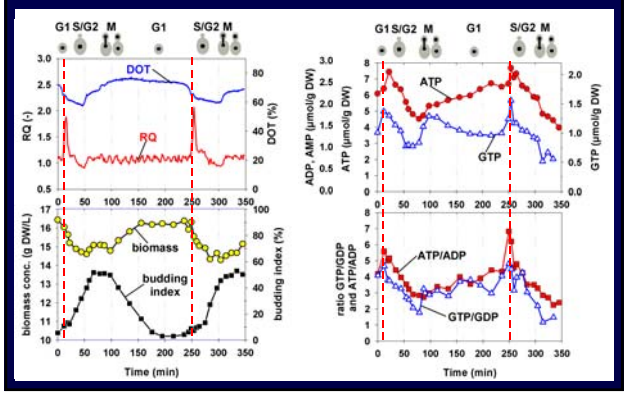



## The Missing Link - Hypotheses




## Nucleotide Dynamics during the Synchronization of Autonomous Cell Cycle Oscillations




## Integrated Model - Single Cell

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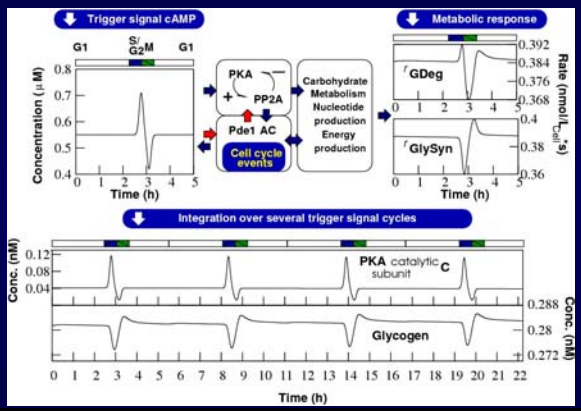
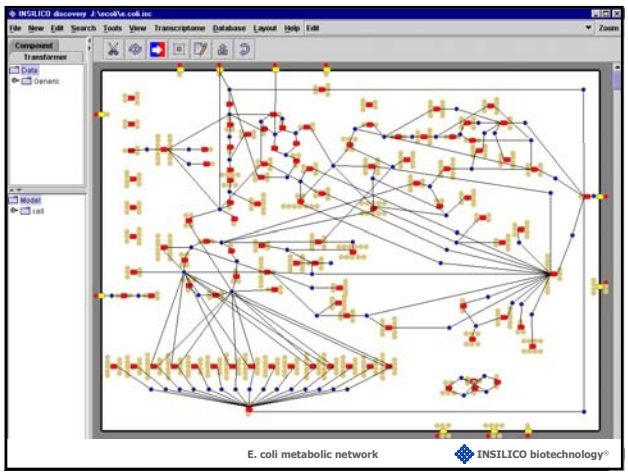
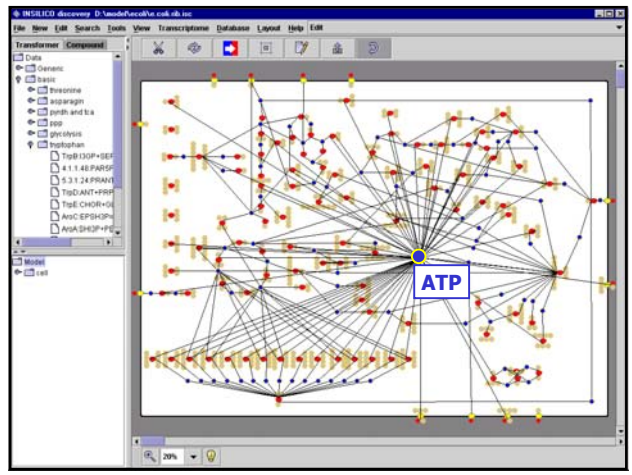
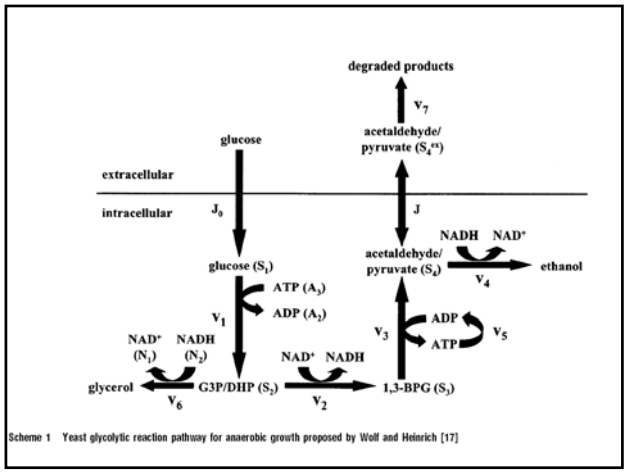




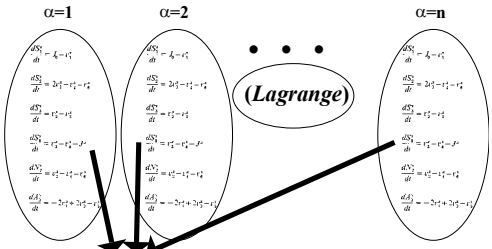




- **Decomposition of populations into single cells**
- **Aggregation of lifelines of individual cells to population behaviour**




Scheme 1 Yeast glycolytic reaction pathway for anaerobic growth proposed by Wolf and Heinrich [17]

Set of intra- and extracellular balance equations




**Transport:** $J^{\alpha} = \kappa(S_4^{\alpha} - S_4^{ex})$

**Acetaldehyde:** $\frac{dC_4^{ex}}{dt} = \frac{\varphi}{n}\sum_{\alpha=1}^{n} J^{\alpha} - v_7$ (*Euler*)

## Conclusions

- For sytem-oriented modeling we frequently need to replace homeostasis with a richer concept, that of homeodynamics
- Because of 'missing links' connection of functional units (modules) is a difficult task if the scientific community have investigated the functionality of the units in isolation

## People

Luciano Aguilera-Vázquez

Klaus Mauch

Ester Guerrero-Martín

Dirk Müller

Jens Marquetand

Anja Niebel