PROBING STRUCTURE AND DYNAMICS OF CELL CYCLE CONTROL

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NUTRIENTS AND CELL CYCLE IN BUDDING YEAST

NUTRIENTS

Nutrient uptake

nutrient sensing

Metabolism

AMP/ATP

etc

Signal Transduction

Ras/Tor/Sch9/Snf1 /Ck2/Hog1

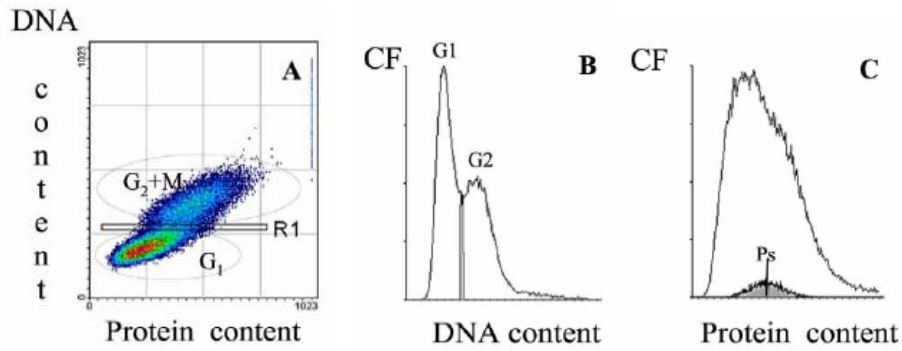
Transcriptional remodeling

Sfp1 etc

Cell cycle machinery

Cyclin/Cdk/Cki/SCF/ APC etc

all together
± 1000 gene products



asymmetrical cell division
in balanced exponential growth

Cell size distribution
Critical cell size (Ps)

Mass duplication time (T)

$$T = \ln 2 / \lambda$$

$$\lambda = K_2 \rho - 1/T_2$$

$$2^{-TP/T} + 2^{-TD/T} = 1$$

A ROADMAP FOR MODULAR SYSTEMS BIOLOGY

Global functional analysis of a process
Construction of a "coarse-grain" model, describing the more relevant modules of the process
Validation of its dynamics by simulation

↓

Iterative rounds of 4**M** strategy for each module
Mining of data bases
Manipulation of genetic and metabolic conditions
Measurements and localization of relevant molecular components
Modeling and simulation of a molecular network for the module

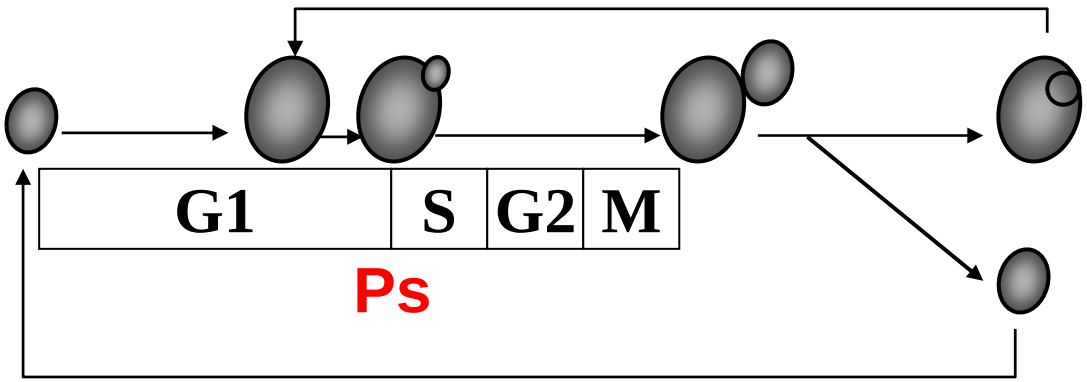
} Network identification

↓

Achievement of a satisfactory molecular model able to **quantitatively** account for the entire process

in: Alberghina and Westerhoff, eds., Systems Biology, Springer Verlag 2005

GLOBAL FUNCTIONAL ANALYSIS OF CELL CYCLE CONTROL

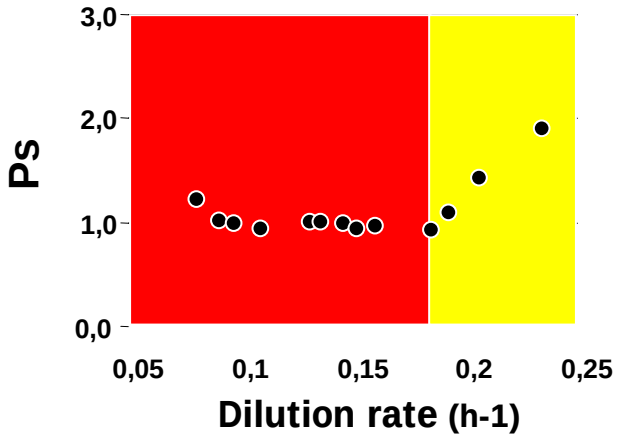


- The **coupling of cell growth to cell division** is a **universal** but poorly understood feature of the cell cycle

- The main regulatory event takes place at START, when cells must reach a **critical cell size (Ps)** to enter into S phase.

- This key regulatory function has been known for thirty years, but its molecular basis is still under investigation.

IN BUDDING YEAST Ps IS MODULATED BY GENETIC BACKGROUND AND BY METABOLIC CONDITIONS



Respiratory metabolism

Respiro-fermentative metabolism

Porro D. et al. *FEMS Microbiol. Letters,* 229 *, 165-171,* **2003**

The Cln3-Cdk1 complex is the most upstream activator of entrance into S phase

Changes in the availability of Cln3 modulate the length of the G1 phase:

Cln3 over-expressed → shorter G1, smaller cells

Cln3 deletion → longer G1, larger cells

Is there a link between Cln3 amount in the cell/nucleus and critical cell size?

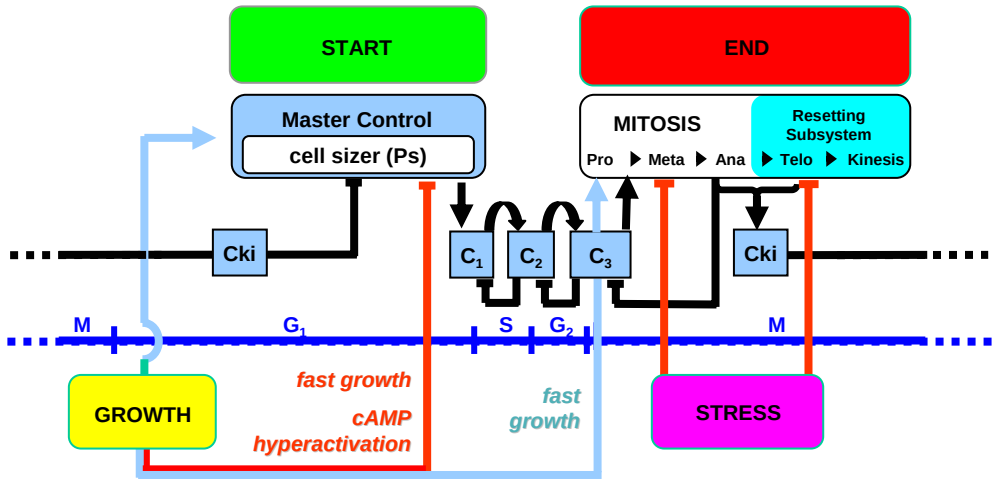
Growth on rich medium → increased Cln3 level → shorter G1, larger cells

Reconstructing the G_1 to S network:
THE BASIC EVENTS

upstream activator	transcription activation	Cyclin.Cdk complexes to enter S phase	
Cln3.Cdk1	SBF/MBF	Cln1,2.Cdk1	BUDDING
		Clb 5,6.Cdk1	DNA REPLICATION

Δsfp1 **cells, unable to stimulate the synthesis of ribosomes when growing on fermentable sugars (high growth rates), have a smaller critical cell size than wild type cells**

A COARSE-GRAIN MODEL OF CELL CYCLE



Two major areas of control

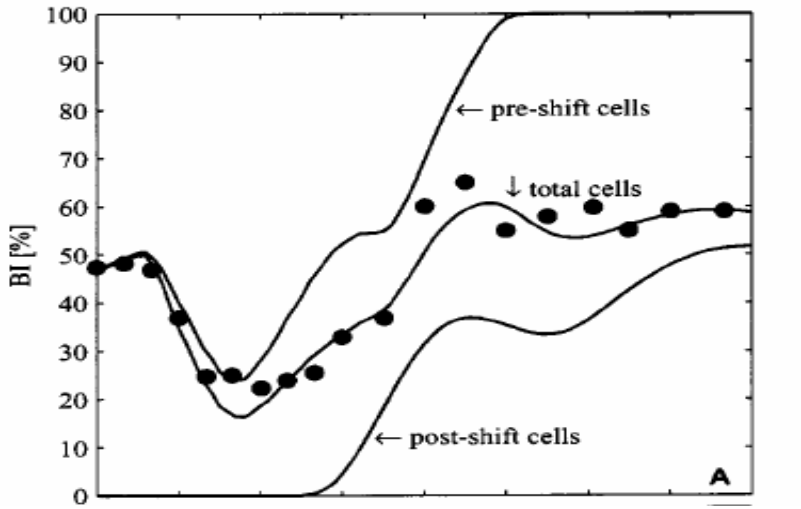
- *a cell sizer* control (involving Cki and modulated by growth conditions) at the G1 to S transition
- *delays of mitosis* execution, at metaphase/anaphase (End2) and at anaphase/telophase (End3), modulated by stress (DNA and spindle damages, conflicting metabolic signals, etc.)

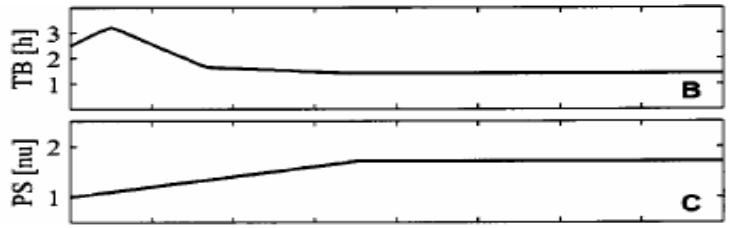
Alberghina et al - Oncogene 20, 1128-1134, 2001

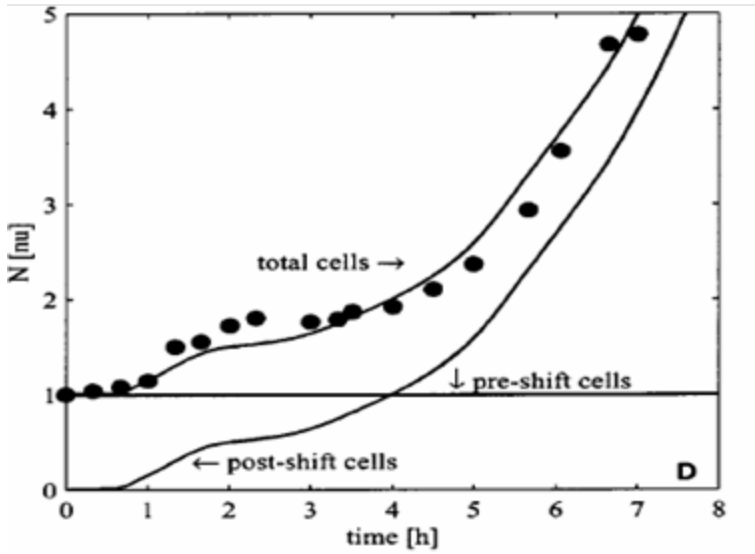
Alberghina et al - Current Genomics 5, 615-627, 2004

ANALYSIS OF A SHIFT UP BY SIMULATION

The model predicts, for cell population during transitory state, a continuous increase of Ps and an increase in duration of budded phase, followed by its decrease to the new steady state

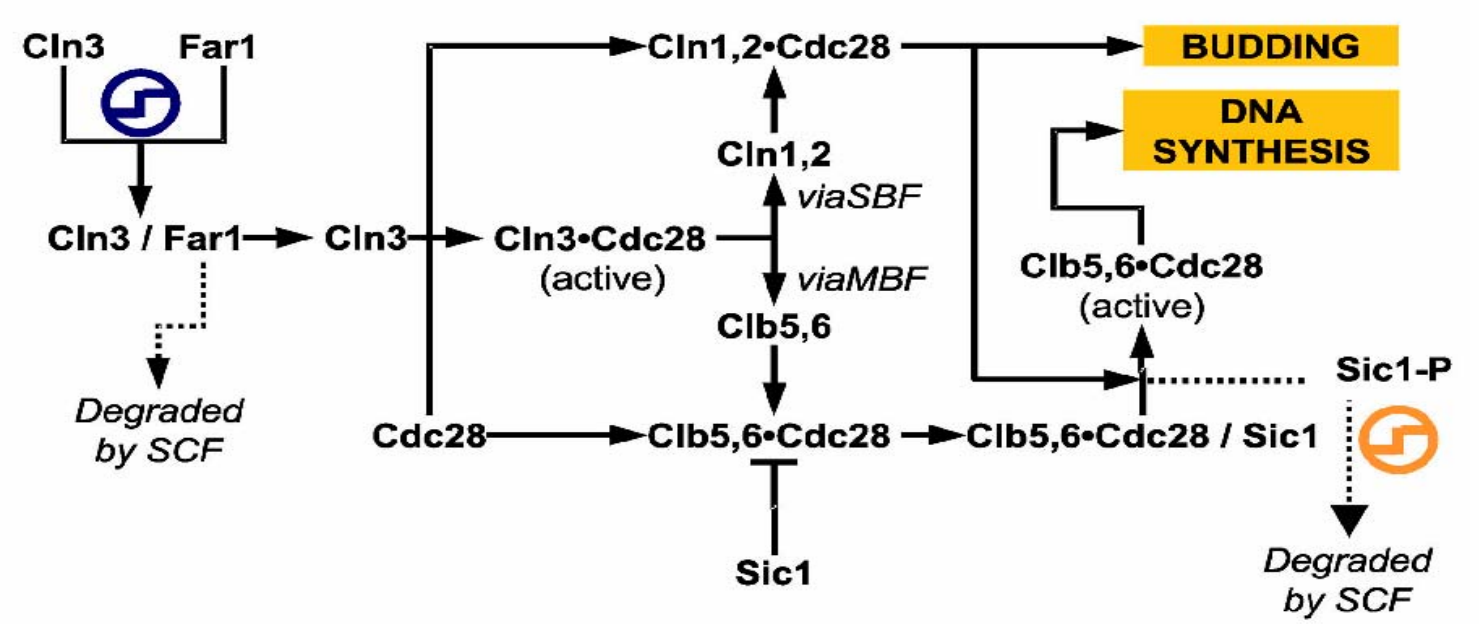






Alberghina et al., Oncogene 20, 1128-1134, 2001

A NEW ROLE IN MITOTIC CYCLE FOR FAR1 PREVIOUSLY KNOWN TO BE INVOLVED IN MATING DEPENDENT ARREST AT G1/S



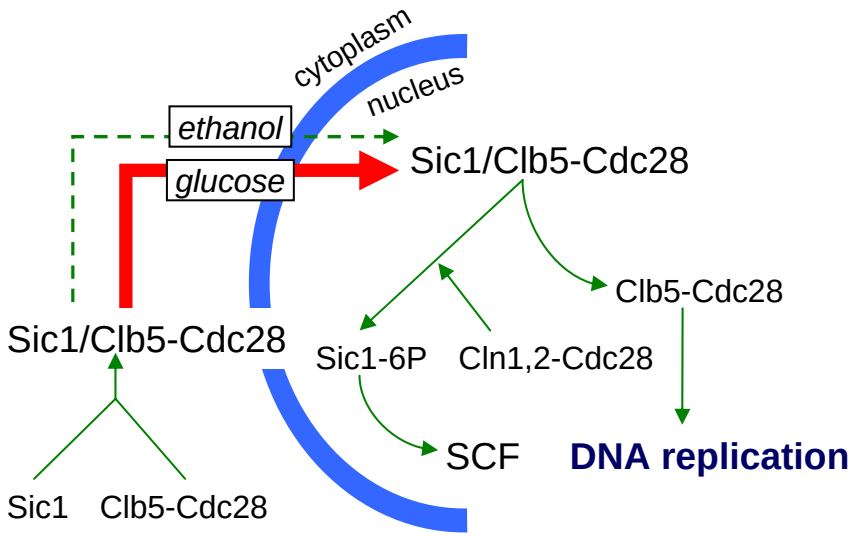
- The Cln3 level in G1 cells is constant and therefore the amount of accumulated Cln3 is proportional to cell mass

- While the average Cln3 level is much higher in fast-growing cells as compared to slow-growing ones, the average Cln3/Far1 ratio is almost constant

Alberghina L. et al., J. Cell. Biol., 167, 433-443, 2004

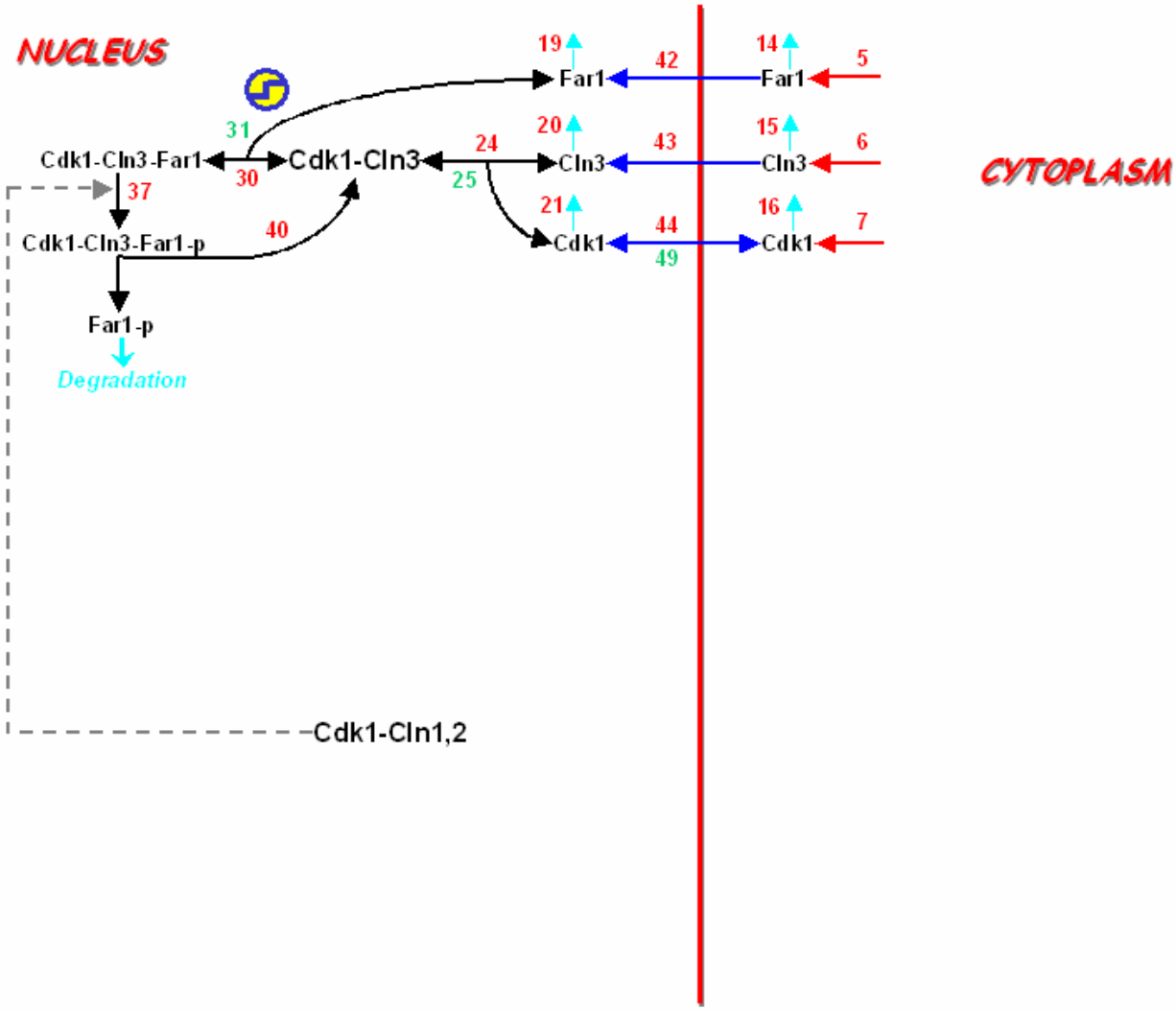
CARBON SOURCE MODULATES THE NUCLEO/CYTOPLASMIC LOCALIZATION OF Sic1

- **In unbudded G1 cells grown**
  - **in glucose** Sic1 is localized in the nucleus
  - **in ethanol** Sic1 is localized both in the nucleus and in cytoplasm
- Sic1 requires a NLS to be imported in the nucleus
- Sic1 facilitates nuclear accumulation of Clb5



Rossi R.L. et al., Cell Cycle 4, 1798-1807, 2005

MATHEMATICAL MODEL OF THE G_1 TO S TRANSITION STARTING FROM SMALL UNBUDDED CELLS



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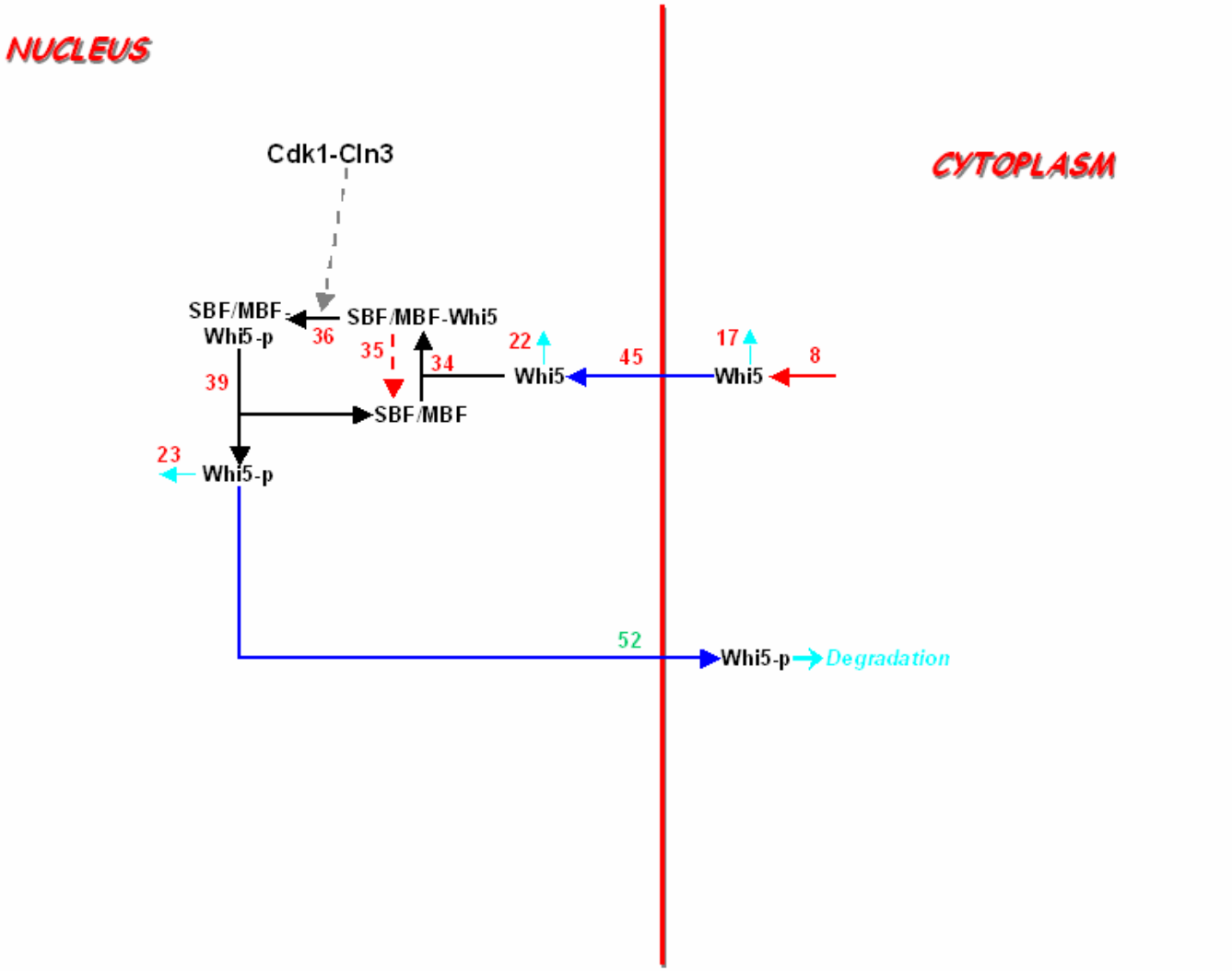

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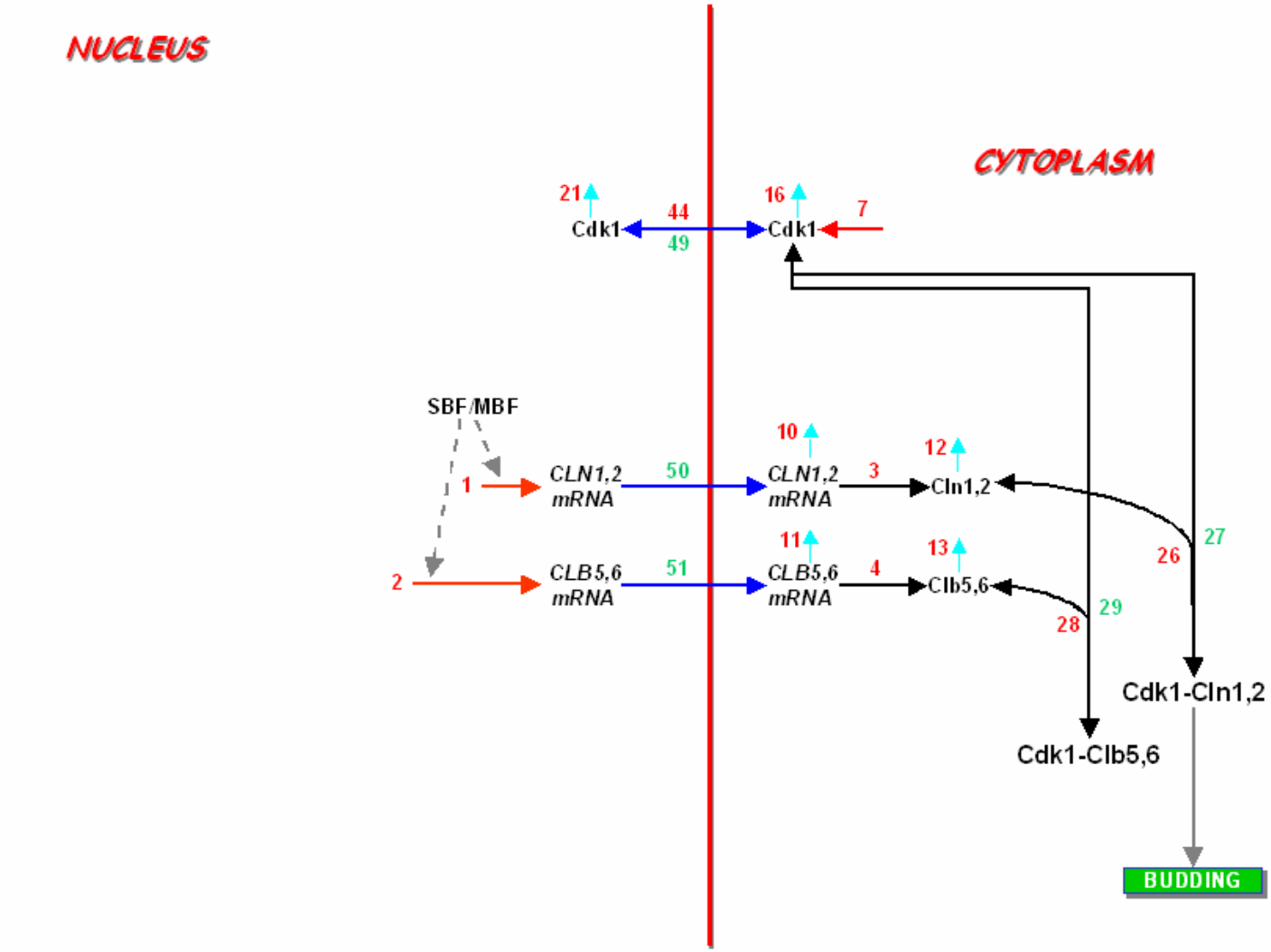

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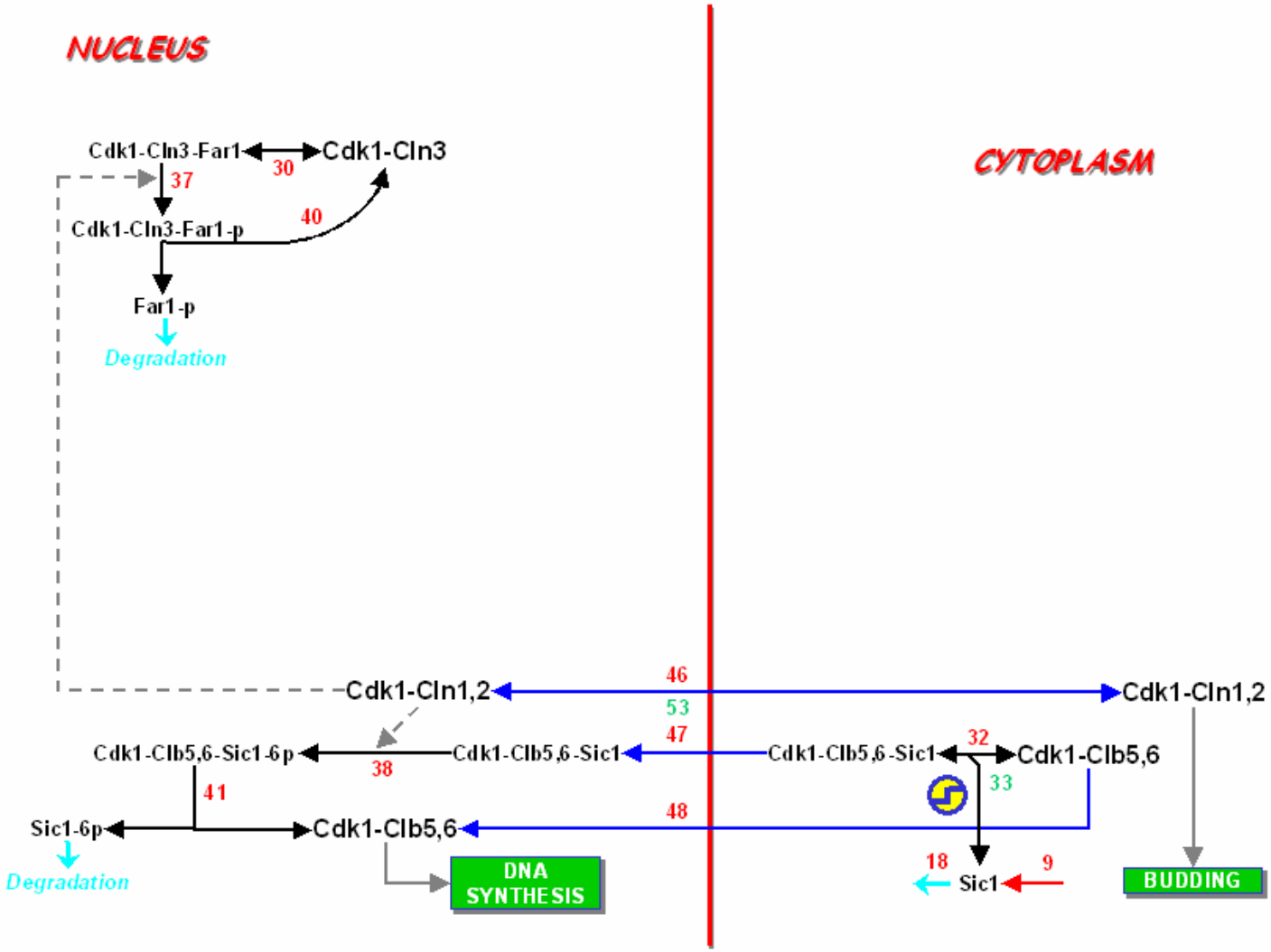

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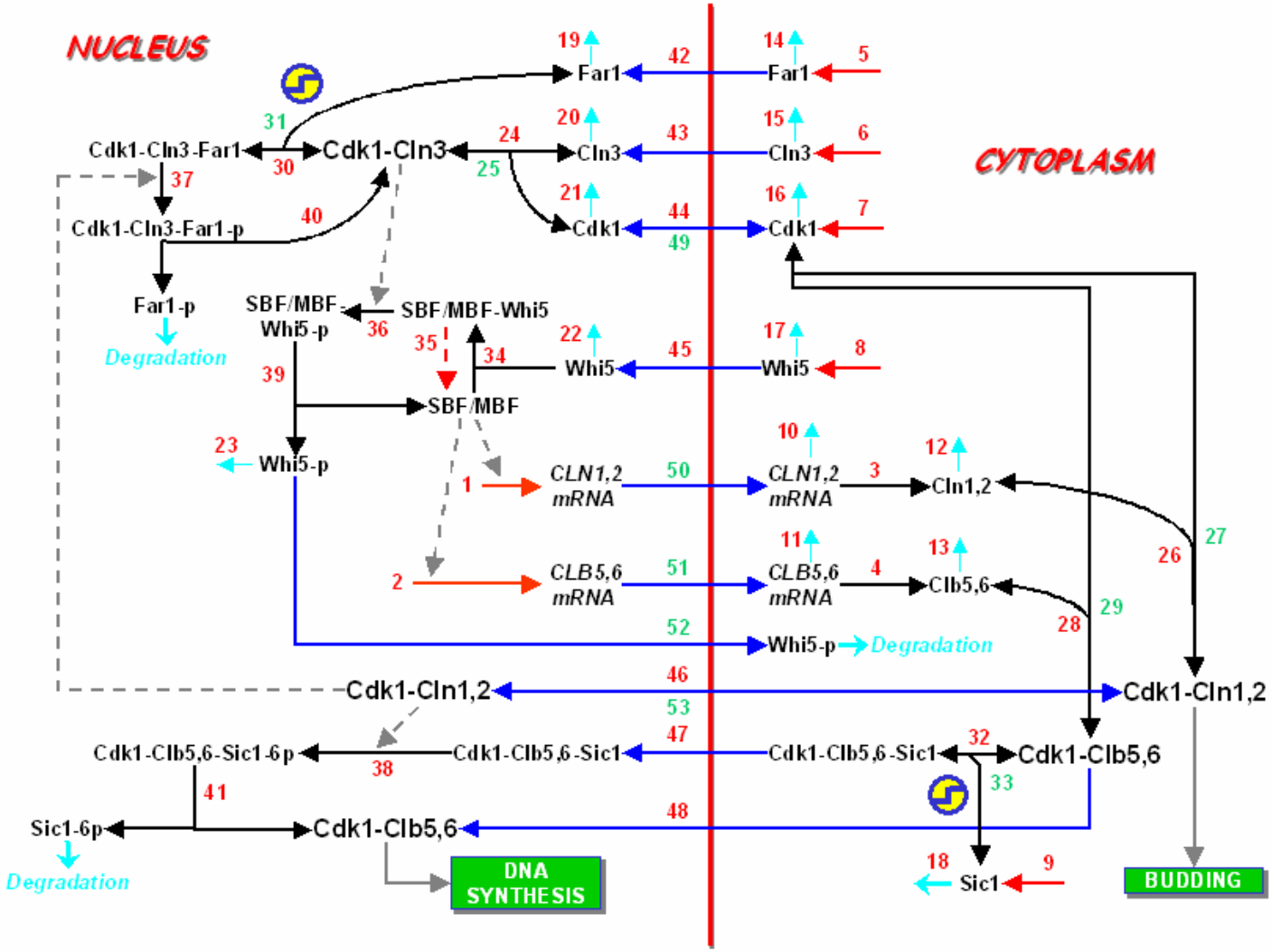


Barberis et al, 2006, submitted

EQUATIONS AND PARAMETERS OF THE MODEL

- **The model has been implemented by a set of ordinary differential equations (ODEs), that describe the temporal change of the concentrations of the involved proteins and complexes.**

- **The model considers the localization of components in different cell compartments (cytoplasm or nucleus) as well as the cell size growth during the G_1 phase.**

- **Parameter identification has been done by text mining for kinetic constants, by mathematical fitting of simulated versus experimental time series, by utilization of available experimental data as input quantities, and by parameter values utilized in literature models.**

- **The best sets of parameters has been used throughout all simulations of small elutriated cells.**

(see poster by Barberis et al for details)

EQUATIONS OF THE MODEL

mRNAs and transcription factors

$d[mcln2nuc]/dt = v13 - v14 + v44 - (d[vol_n]/dt) / vol_n[t] * [mcln2nuc][t]$

$d[mcln2cyt]/dt = v14 * k55 - v27 - (d[vol_c]/dt) / vol_c[t] * [mcln2cyt][t]$

$d[mclb5nuc]/dt = v12 - v15 + v45 - (d[vol_n]/dt) / vol_n[t] * [mclb5nuc][t]$

$d[mclb5cyt]/dt = v15 * k55 - v28 - (d[vol_c]/dt) / vol_c[t] * [mclb5cyt][t]$

$d[sbfnuc]/dt = v40 - v26 + v52 - (d[vol_n]/dt) / vol_n[t] * [sbfnuc][t]$

Inhibitors of kinase complexes

$d[far1nuc]/dt = v4 / k55 - v8 + v46 - v30 - (d[vol_n]/dt) / vol_n[t] * [far1nuc][t]$

$d[far1cyt]/dt = v1 - v4 - v29 - (d[vol_c]/dt) / vol_c[t] * [far1cyt][t]$

$d[sic1cyt]/dt = v23 - v24 + v51 - v38 - (d[vol_c]/dt) / vol_c[t] * [sic1cyt][t]$

$d[whi5cyt]/dt = v10 - v11 - v41 - (d[vol_c]/dt) / vol_c[t] * [whi5cyt][t]$

$d[whi5nuc]/dt = v11 / k55 - v26 + v52 - v42 - (d[vol_n]/dt) / vol_n[t] * [whi5cyt][t]$

Cyclins and cyclin-dependent kinase

$d[cln3nuc]/dt = v5 / k55 - v7 + v47 - v32 - (d[vol_n]/dt) / vol_n[t] * [cln3nuc][t]$

$d[cln3cyt]/dt = v2 - v5 - v31 - (d[vol_c]/dt) / vol_c[t] * [cln3cyt][t]$

$d[clb5cyt]/dt = v17 - v19 + v50 - v37 - (d[vol_c]/dt) / vol_c[t] * [clb5cyt][t]$

$d[cln2cyt]/dt = v16 - v18 + v49 - v35 - (d[vol_c]/dt) / vol_c[t] * [cln2cyt][t]$

$d[cdk1nuc]/dt = v6 / k55 - v48 - v7 + v47 - v34 - (d[vol_n]/dt) / vol_c[t] * [cdk1nuc][t]$

$d[cdk1cyt]/dt = v3 - v6 + v48 * k55 - v18 + v49 - v19 + v50 - v33 - (d[vol_c]/dt) / vol_c[t] * [cdk1cyt][t]$

Inactive complexes

$d[sbfwhi5nuc]/dt = v26 - v52 - v36 - (d[vol_n]/dt) / vol_n t] * [sbfwhi5nuc][t]$

$d[clb5cdk1sic1cyt]/dt = v24 - v51 - v25 - (d[vol_c]/dt) / vol_c[t] * [clb5cdk1sic1cyt][t]$

$d[clb5cdk1sic1nuc]/dt = v25 / k55 - v21 - (d[voln]/dt) / vol_n[t] * [clb5cdk1sic1nuc][t]$

$d[cln3cdk1far1nuc]/dt = v8 - v46 - v39 - (d[vol_n]/dt) / vol_n[t] * [cln3cdk1far1nuc][t]$

$d[sbfwhi5pnuc]/dt = v36 - v40 - (d[vol_n]/dt) / vol_n[t] * [sbfwhi5pnuc][t]$

$d[clb5cdk1sic1pnuc]/dt = v21 - v22 - (d[vol_n]/dt) / vol_n[t] * [clb5cdk1sic1pnuc][t]$

$d[cln3cdk1far1pnuc]/dt = v39 - v9 - (d[vol_n]/dt) / vol_n[t] * [cln3cdk1far1pnuc][t]$

Cyclin-dependent kinase complexes

$d[cln2cdk1cyt]/dt = v18 - v49 - v20 + v53 * k55 - (d[vol_c]/dt) / vol_c[t] * [cln2cdk1cyt][t]$

$d[cln2cdk1nuc]/dt = v20 / k55 - v53 - (d[vol_n]/dt) / vol_n[t] * [cln2cdk1nuc][t]$

$d[clb5cdk1cyt]/dt = v19 - v50 - v24 + v51 - v43 - (d[vol_c]/dt) / vol_c[t] * [clb5cdk1cyt][t]$

$d[clb5cdk1nuc]/dt = v22 + v43 / k55 - (d[vol_n]/dt) / vol_n[t] * [clb5cdk1nuc][t]$

$d[cln3cdk1nuc]/dt = v7 - v47 - v8 + v46 + v9 - (d[vol_n]/dt) / vol_n[t] * [cln3cdk1nuc][t]$

Growth

$d[vol_c]/dt = k59 * vol_c[t]$

PARAMETERS OF THE MODEL

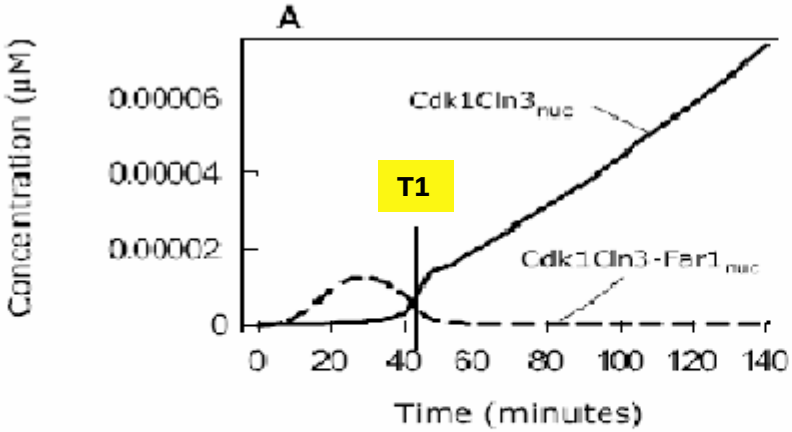
Rate constants in glucose		
k_1 = 0.03523 min^{-1}	k_{19} = 0.01 min^{-1}	k_{37} = 4363.6 $\mu M^{-1} * min^{-1}$
k_2 = 0.03523 min^{-1}	k_{20} = 0.01 min^{-1}	k_{38} = 4363.6 $\mu M^{-1} * min^{-1}$
k_3 = 0.32 min^{-1}	k_{21} = 0 min^{-1}	k_{39} = 1 min^{-1}
k_4 = 0.32 min^{-1}	k_{22} = 0.01 min^{-1}	k_{40} = 1 min^{-1}
k_5 = 0.000042 $\mu M^{-1} * min^{-1}$	k_{23} = 0.01 min^{-1}	k_{41} = 1 min^{-1}
k_6 = 0.00001 $\mu M^{-1} * min^{-1}$	k_{24} = 2.82 $\mu M^{-1} * min^{-1}$	k_{42} = 0.005 min^{-1}
k_7 = 0.01 $\mu M^{-1} * min^{-1}$	k_{25} = 0.55 min^{-1}	k_{43} = 0.005 min^{-1}
k_8 = 0.00004 $\mu M^{-1} * min^{-1}$	k_{26} = 2.82 $\mu M^{-1} * min^{-1}$	k_{44} = 0.005 min^{-1}
k_9 = 0.00005 $\mu M^{-1} * min^{-1}$	k_{27} = 0.55 min^{-1}	k_{45} = 0.005 min^{-1}
k_{10} = 0.12 min^{-1}	k_{28} = 2.82 $\mu M^{-1} * min^{-1}$	k_{46} = 0.1 min^{-1}
k_{11} = 0.12 min^{-1}	k_{29} = 0.55 min^{-1}	k_{47} = 1 min^{-1}
k_{12} = 0.1 min^{-1}	k_{30} = 42300 $\mu M^{-1} * min^{-1}$	k_{48} = 0.012 min^{-1}
k_{13} = 0.35 min^{-1}	k_{31} = 0.55 min^{-1}	k_{49} = 0.001 min^{-1}
k_{14} = 0.01 min^{-1}	k_{32} = 84.6 $\mu M^{-1} * min^{-1}$	k_{50} = 0.6 min^{-1}
k_{15} = 0.01 min^{-1}	k_{33} = 0.55 min^{-1}	k_{51} = 0.6 min^{-1}
k_{16} = 0.03 min^{-1}	k_{34} = 8.46 $\mu M^{-1} * min^{-1}$	k_{52} = 0.005 min^{-1}
k_{17} = 0.01 min^{-1}	k_{35} = 0.0005 min^{-1}	k_{53} = 0.001 min^{-1}
k_{18} = 0.0008 min^{-1}	k_{36} = 4363.6 $\mu M^{-1} * min^{-1}$	k_{growth} = 0.0051 min^{-1}

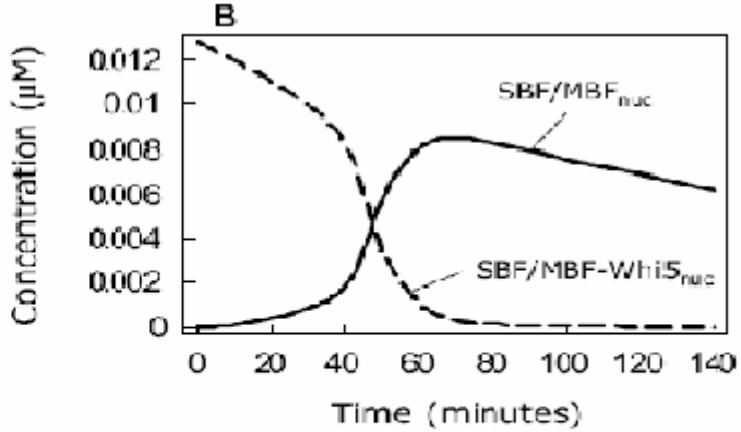
Rate constants in ethanol		
k_1 = 0.005872 min^{-1}	k_{19} = 0.01 min^{-1}	k_{37} = 4363.6 $\mu M^{-1} * min^{-1}$
k_2 = 0.005872 min^{-1}	k_{20} = 0.01 min^{-1}	k_{38} = 4363.6 $\mu M^{-1} * min^{-1}$
k_3 = 0.32 min^{-1}	k_{21} = 0 min^{-1}	k_{39} = 1 min^{-1}
k_4 = 0.32 min^{-1}	k_{22} = 0.01 min^{-1}	k_{40} = 1 min^{-1}
k_5 = 0.000019 $\mu M^{-1} * min^{-1}$	k_{23} = 0.01 min^{-1}	k_{41} = 1 min^{-1}
k_6 = 0.0000045 $\mu M^{-1} * min^{-1}$	k_{24} = 2.82 $\mu M^{-1} * min^{-1}$	k_{42} = 0.005 min^{-1}
k_7 = 0.01 $\mu M^{-1} * min^{-1}$	k_{25} = 0.55 min^{-1}	k_{43} = 0.005 min^{-1}
k_8 = 0.00004 $\mu M^{-1} * min^{-1}$	k_{26} = 2.82 $\mu M^{-1} * min^{-1}$	k_{44} = 0.005 min^{-1}
k_9 = 0.00005 $\mu M^{-1} * min^{-1}$	k_{27} = 0.55 min^{-1}	k_{45} = 0.005 min^{-1}
k_{10} = 0.12 min^{-1}	k_{28} = 2.82 $\mu M^{-1} * min^{-1}$	k_{46} = 0.1 min^{-1}
k_{11} = 0.12 min^{-1}	k_{29} = 0.55 min^{-1}	k_{47} = 1 min^{-1}
k_{12} = 0.1 min^{-1}	k_{30} = 42300 $\mu M^{-1} * min^{-1}$	k_{48} = 0.012 min^{-1}
k_{13} = 0.35 min^{-1}	k_{31} = 0.55 min^{-1}	k_{49} = 0.001 min^{-1}
k_{14} = 0.01 min^{-1}	k_{32} = 0.846 $\mu M^{-1} * min^{-1}$	k_{50} = 0.6 min^{-1}
k_{15} = 0.01 min^{-1}	k_{33} = 0.55 min^{-1}	k_{51} = 0.6 min^{-1}
k_{16} = 0.03 min^{-1}	k_{34} = 8.46 $\mu M^{-1} * min^{-1}$	k_{52} = 0.005 min^{-1}
k_{17} = 0.01 min^{-1}	k_{35} = 0.0005 min^{-1}	k_{53} = 0.001 min^{-1}
k_{18} = 0.0008 min^{-1}	k_{36} = 4363.6 $\mu M^{-1} * min^{-1}$	k_{growth} = 0.0023 min^{-1}

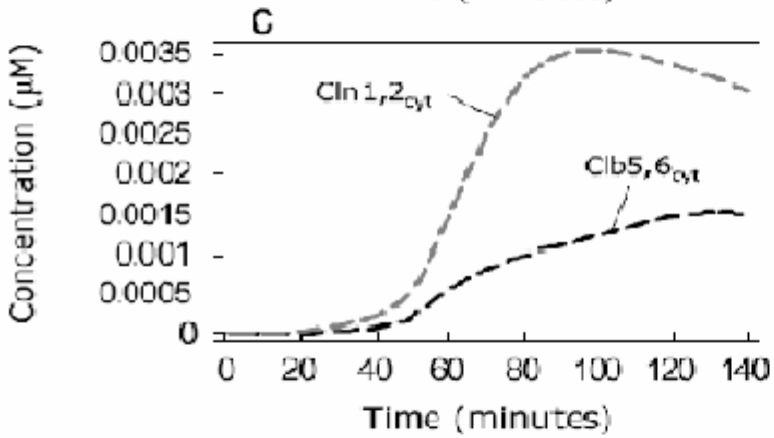
Initial concentrations (μM) in glucose	
$far1_{cyt}[0]$ = 0.0037926	$whi5_{cyt}[0]$ = 0.073564
$cln3_{cyt}[0]$ = 0.000485	$sic1_{cyt}[0]$ = 0.039234
$cdk1_{cyt}[0]$ = 0.333333	$sbfwhi5_{cyt}[0]$ = 0.025544
$cdk1_{nuc}[0]$ = 0.0074127	

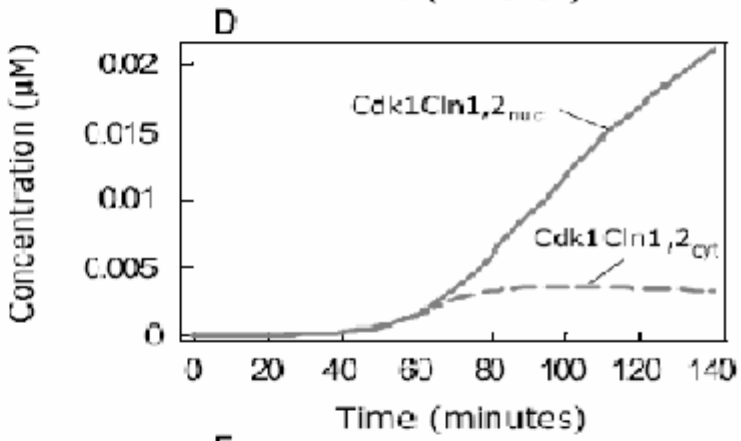
Initial concentrations (μM) in ethanol	
$far1_{cyt}[0]$ = 0.0049334	$whi5_{cyt}[0]$ = 0.073564
$cln3_{cyt}[0]$ = 0.0011916	$sic1_{cyt}[0]$ = 0.039234
$cdk1_{cyt}[0]$ = 0.333333	$sbfwhi5_{nuc}[0]$ = 0.025544
$cdk1_{nuc}[0]$ = 0.0074127	

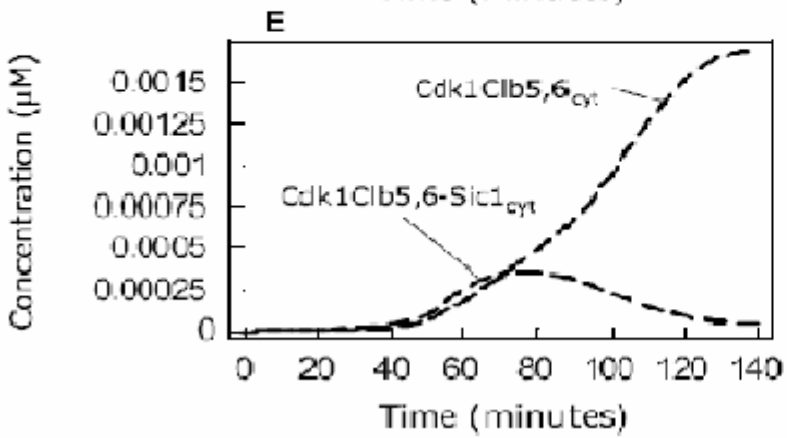
SIMULATED DYNAMICS OF G1 TO S TRANSITION

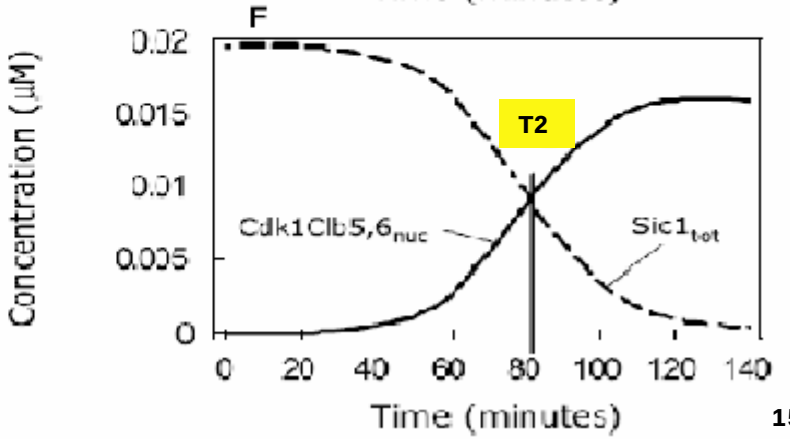




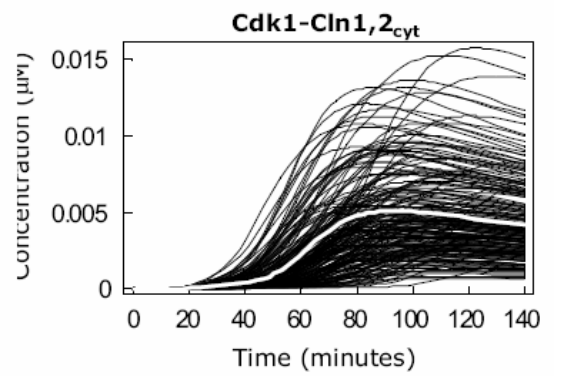




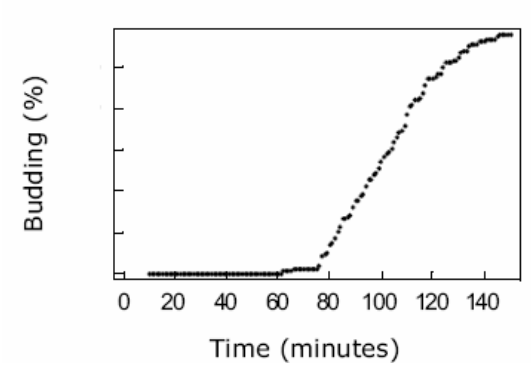




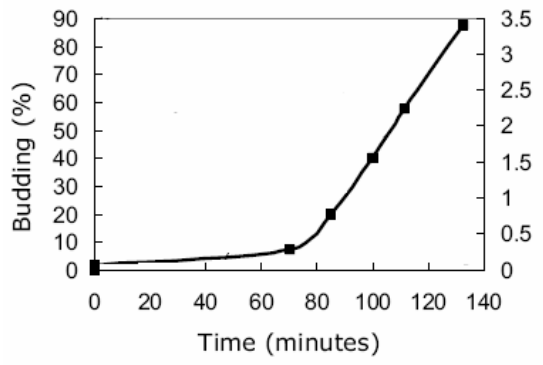
FROM INDIVIDUAL CELLS TO POPULATION
VALIDATION OF SIMULATED OVERALL DYNAMICS

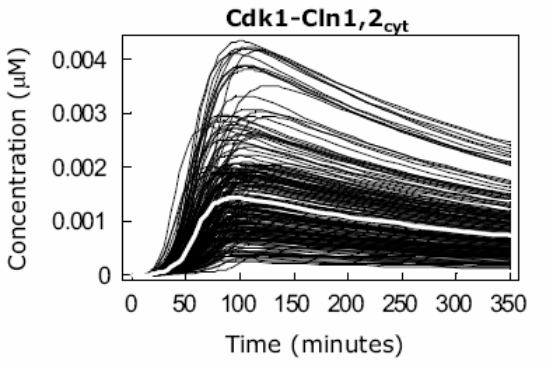


Simulated - Glucose

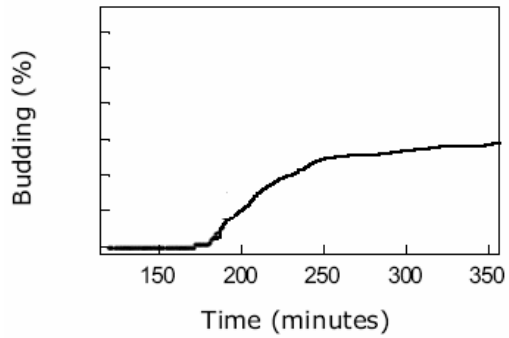


Experimental - Glucose

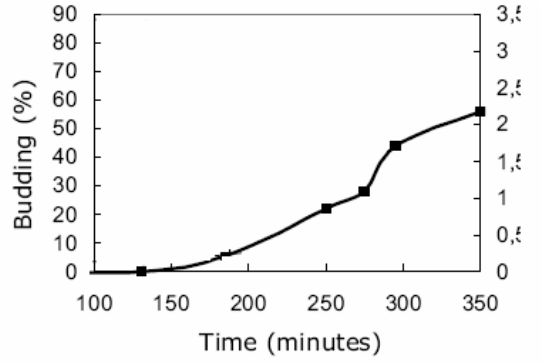




Simulated - Ethanol



Experimental - Ethanol



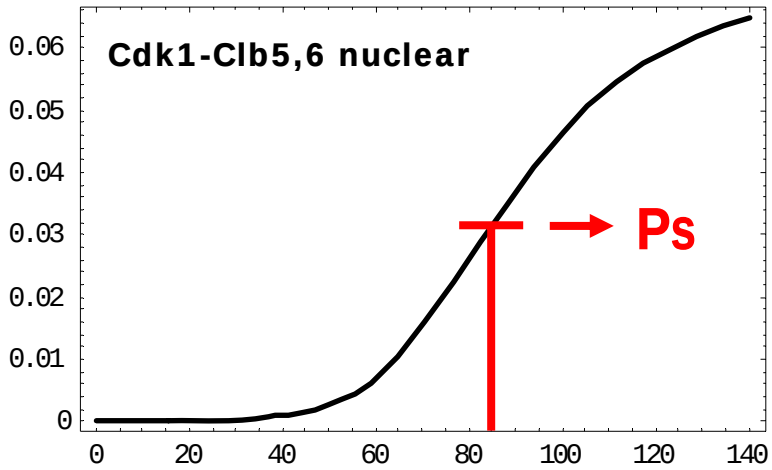
The model predicts properties (budding curve) not taken into account during model construction

Overall consistency between input data and output performance

POSITIVE TESTING OF THE MODEL WITH CELL CYCLE MUTANTS

cdk1Δ	*cln1Δ cln2Δ* CLN3 stabilized	*cln3Δ sic1Δ*
clb5Δ clb6Δ	*cln1Δ cln2Δ cln3Δ*	*far1Δ sic1Δ*
clb5Δ clb6Δ cdk1Δ	*cln1Δ cln2Δ cln3Δ sic1Δ*	sic1Δ OE-CLB5
cln1Δ cln2Δ clb5Δ clb6Δ	*cln1Δ cln2Δ cln3Δ* OE-CLB5	sic1Δ CLB5 stabilized
OE-CLB5	*cln1Δ cln2Δ cln3Δ* OE-CDK1	OE-SIC1 and stabilized
CLB5 stabilized	*cln1Δ cln2Δ cln3Δ* OE-CLN2	SIC1 stabilized
OE-CLB5 and stabilized	*cln1Δ cln2Δ cln3Δ* CLN2 stabilized	OE-SIC1 OE-CLN2
cln1Δ cln2Δ	*cln1Δ cln2Δ cln3Δ* OE-CLN3	sbfΔ mbfΔ
cln1Δ cln2Δ far1Δ	*cln1Δ cln2Δ cln3Δ* OE-CLN3 and stabilized	sbfΔ mbfΔ OE-CLN2
cln1Δ cln2Δ sic1Δ	*cln3Δ* OE-SIC1	OE-SBF
cln1Δ cln2Δ OE-SIC1	*cln3Δ* OE-WHI5	sbfΔ mbfΔ sic1Δ
cln1Δ cln2Δ OE-SIC1 OE-CLN2	CLN2 stabilized	sbfΔ mbfΔ sic1Δ OE-CLN2
cln1Δ cln2Δ OE-WHI5	CLN3 stabilized	OE-MBF
cln1Δ cln2Δ OE-CLN2	*cln3Δ far1Δ*	

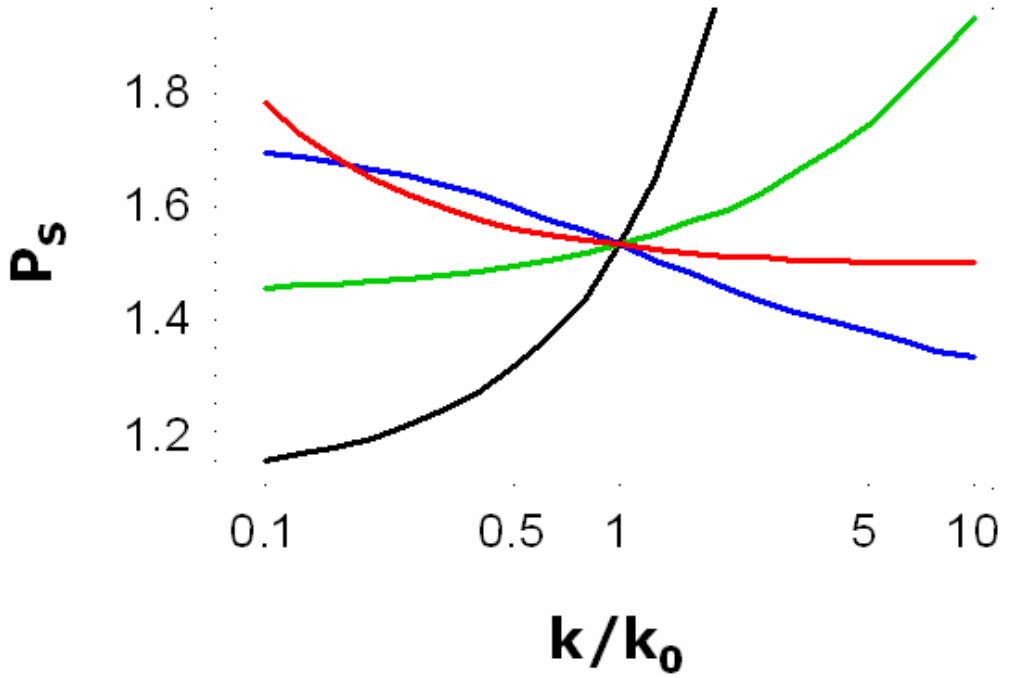
HOW TO ESTIMATE Ps



Relevant genotype	Estimated P_S
Wild type glucose	**1.54**
cln3Δ	–
OE-CLN3	**1.26**
far1Δ	**1.44**
OE-FAR1	–
whi5Δ	**1.20**
OE-WHI5	**3.31**
sic1Δ	**6.57**
OE-SIC1	**1.50**
Wild type ethanol	**1.20**

Ps is estimated as the value of the cell size at the time when DNA replication starts

SENSITIVITY ANALYSIS OF Ps



Growth rate

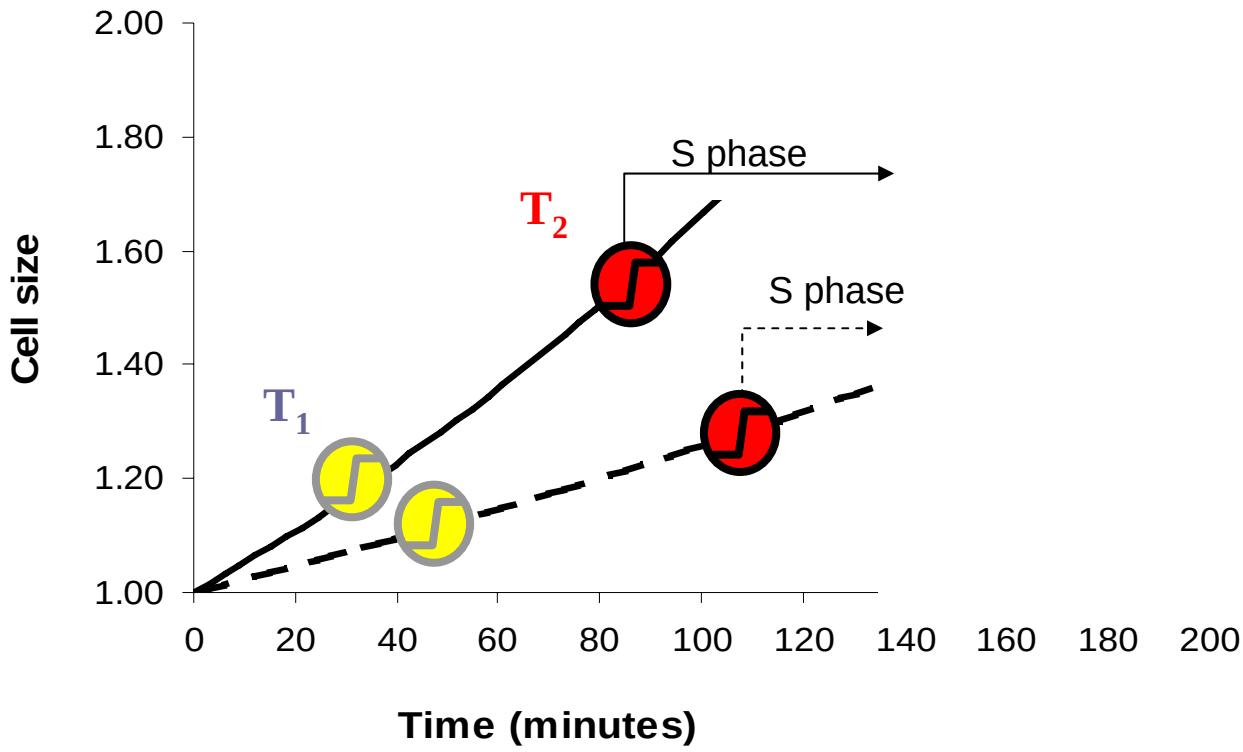
Far1 initial concentration

Cln3 initial concentration

Binding value of Sic1 to Cdk1-Clb5,6$_{cyt}$

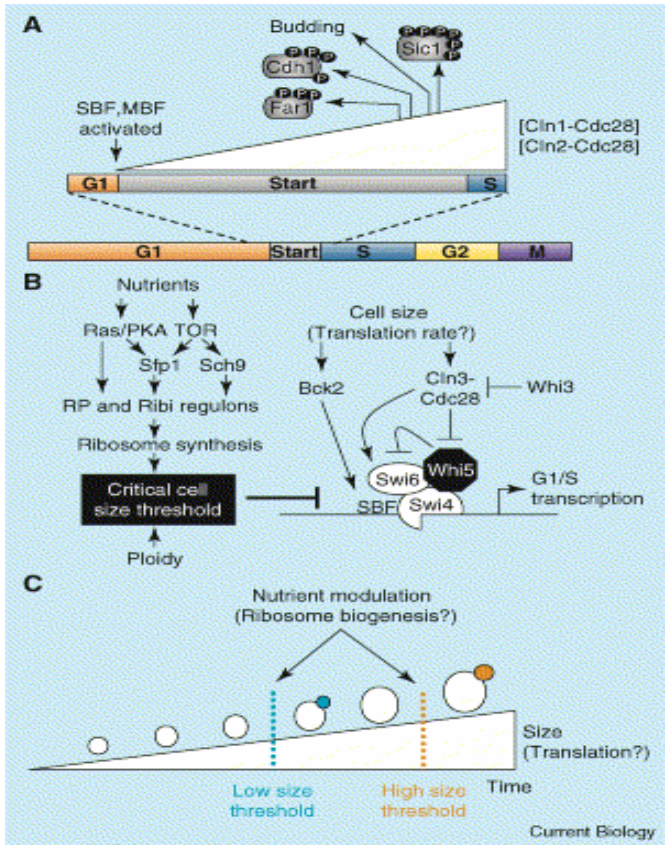
Ps is an emergent property of the entire G1 to S network

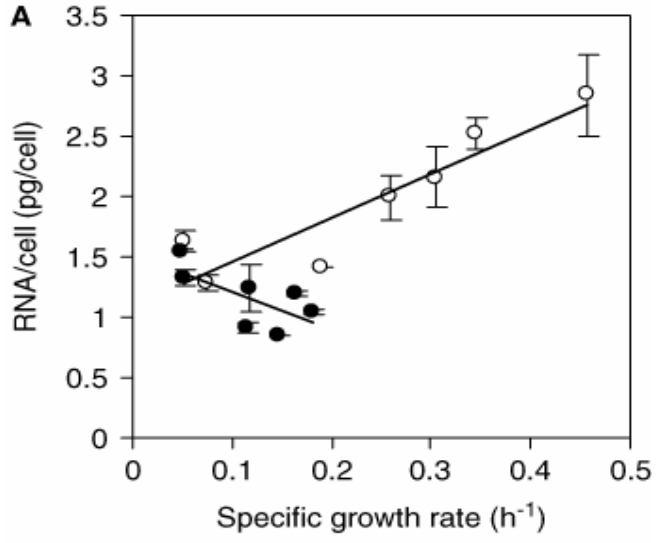
WHY GROWTH RATE MODULATES Ps



This model allows to set in a unified framework all previously proposed regulatory events for the setting of Ps

RIBOSOME BIOGENESIS AND MODULATION OF Ps: A NEW INTERPRETATION





Growth rate is linked to the level and rate of synthesis of ribosome

Our model predicts that *spf1Δ* cells in exponential growth in glucose have lower Ps than wild type since they have lower RNA biosynthesis and hence lower growth rate

Jorgensen P. and Tyers M., Curr. Biol., 14, R1014 - R1027, 2004

DISTINCTIVE FEATURES OF THE MILAN/BERLIN MODEL FOR BUDDING YEAST

- Entrance into S phase requires the overcoming of two thresholds (both involving cyclin Cdk and Cki)
  - a growth sensitive threshold involves a cyclin, whose amount in the cell is proportional to cell mass. This threshold is executed at closely similar cell sizes in fast-growing cells as well as in slow-growing ones
  - a second threshold involves the cyclin that, together with its Cdk, promotes onset of DNA replication
- Nucleo/cytoplasmic localization:

  Cki facilitates transport of cyclin/Cdk into the nucleus in a controllable way thereby affecting the dynamics of entrance into S phase
- During exponential growth the setting of Ps is sensitive to growth rate (and therefore to ribosome biosynthetic activity) for the existence of a sizable time delay between the two thresholds

NETWORK IDENTIFICATION FOR THE G1 TO S TRANSITION IN MAMMALIAN CELLS

- We assume an evolutionary conservation of the general features of cell cycle control from yeast to mammalian cells (Nurse, 1990, 1992)

  growth-sensitive cyclin — cyclin D

  DNA replication cyclin — cyclin E

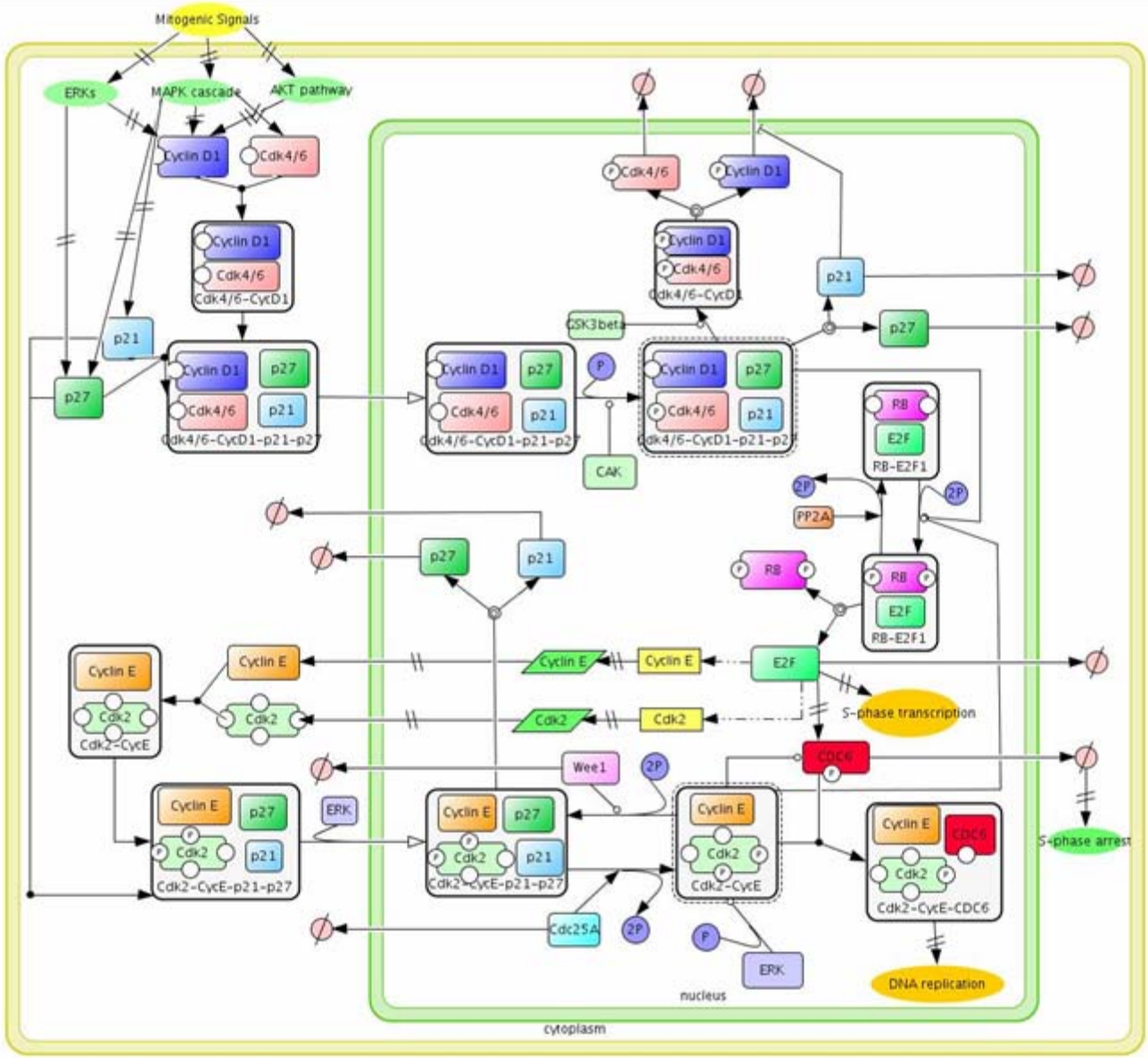
- We consider nucleo/cytoplasmic localization

  the Cki $p27^{Kip1}$ is preferentially localized in the cytoplasm of transformed cells, and correlates with tumour aggressiveness

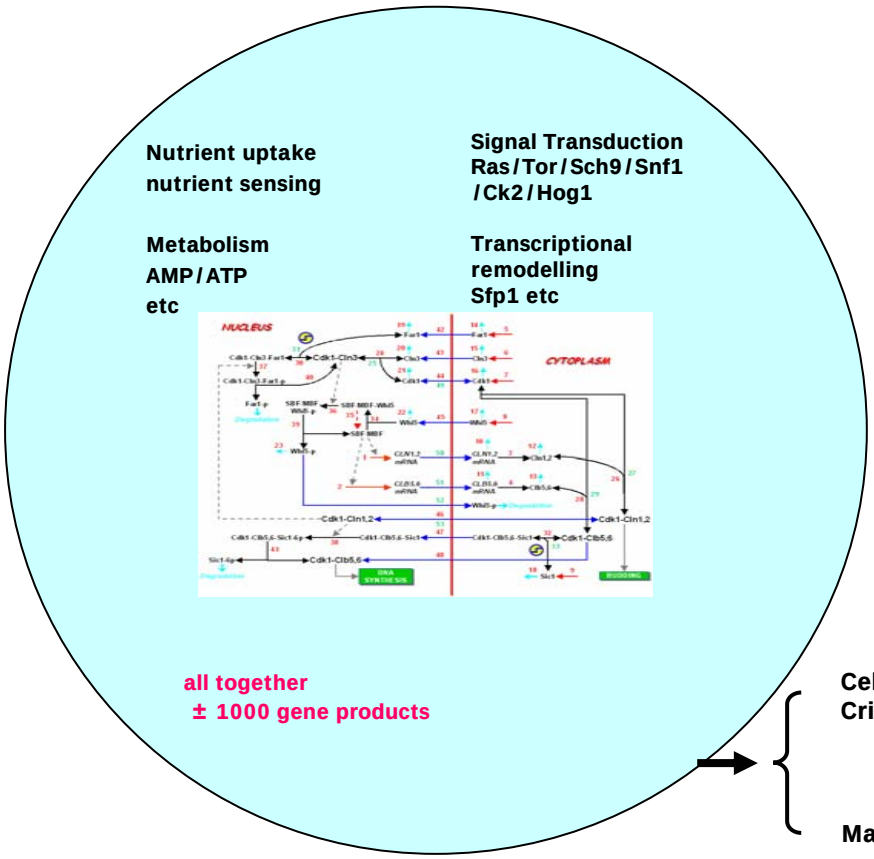
- Data mining and experimental analysis

(see poster by Milanesi/Alfieri et al)

NETWORK FOR THE G1 TO S TRANSITION IN MAMMALIAN CELLS



WHAT NEXT?



- *Links between cell signaling and cell cycle machinery in yeast*

- modulation of the Sic1 threshold in perturbed growth

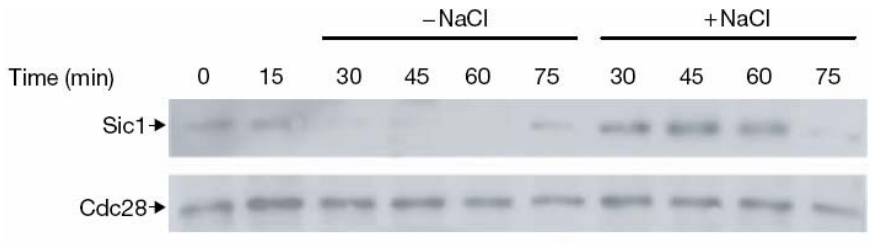
↓

phosphorylation signature of Sic1

- *Molecular model of the entire yeast cycle*
- *Molecular model of normal and transformed mammalian cell cycle*

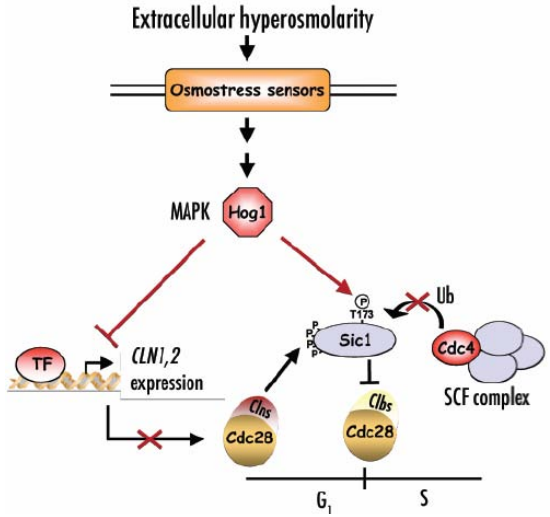
MODULATION OF THE ACTIVITY OF THE SIC1 THRESHOLD DURING PERTURBED GROWTH

OSMOSTRESS

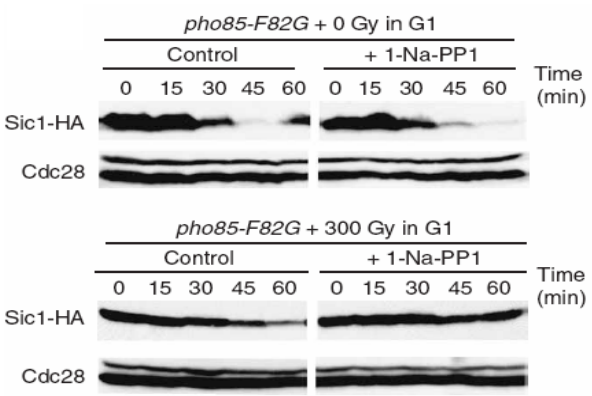


Sic1 is the molecular target for Hog1, that is required to modulate G1 to S transition in response to osmostress. Hog1 phosphorylates Sic1 promoting its stabilization and inhibition of cell-cycle progression.

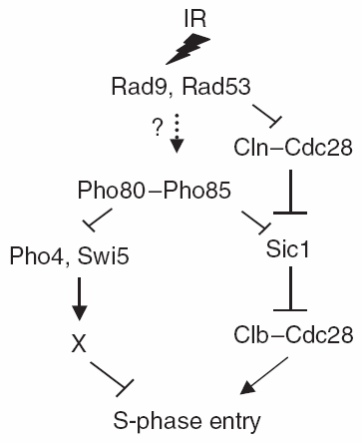
Escotè *et al.*, Nat Cell Biol 2004



DNA DAMAGE



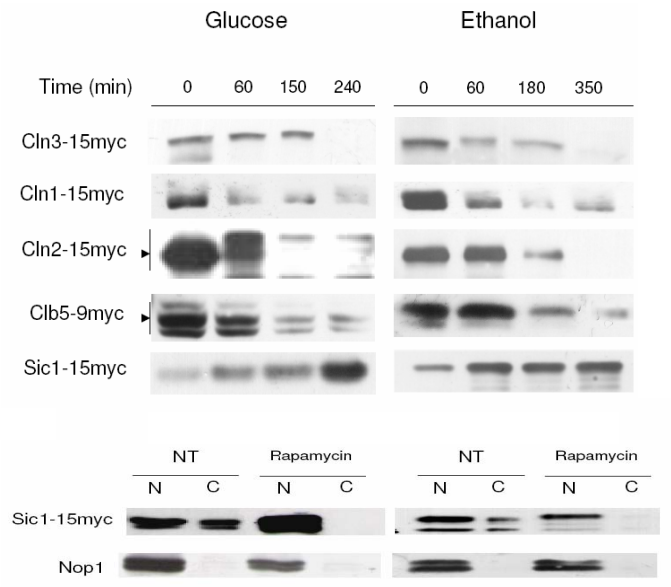
DNA damage in G1 results in a delay to enter into S phase, linked to the inhibition of Sic1 degradation by Pho85 .

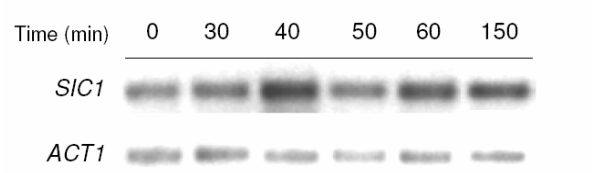


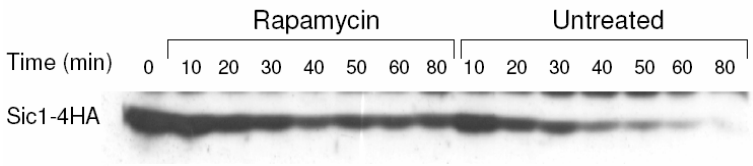
Wysocki *et al.*, Nat Struct Mol Biol. 2006

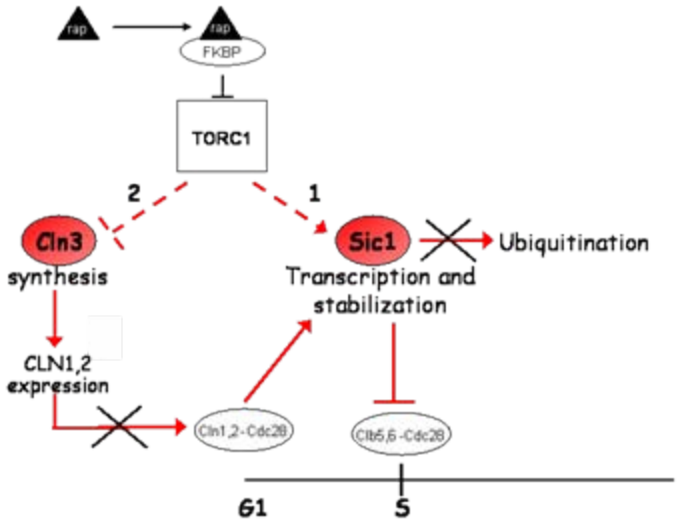
MODULATION OF THE ACTIVITY OF THE SIC1 THRESHOLD DURING PERTURBED GROWTH

TOR pathway inhibition (rapamycin)









Rapamycin causes G1 arrest by a dual mechanism that comprises down-regulation of Cln1-3 and up-regulation of Sic1. Rapamycin-mediated up-regulation of Sic1 involves an increase in mRNA and nuclear accumulation of a more stable, non ubiquitinated protein.

Zinzalla *et al.*, 2006, submitted

CONCLUSIONS

- Simulation analysis of budding yeast cell cycle model indicate that, during balanced exponential growth, the critical cell size is an emergent property of the G1 to S network sensitive to growth rate and therefore to ribosome biosynthesis

- Molecular investigations indicate that Sic1 is a relevant target of perturbed growth conditions delaying/blocking entrance into S phase. New circuits have to be modeled to account for growth perturbations. Simulations of extended molecular networks should offer new insight into cell cycle control.

ITERATIVE PROCEEDING OF SYSTEMS BIOLOGY

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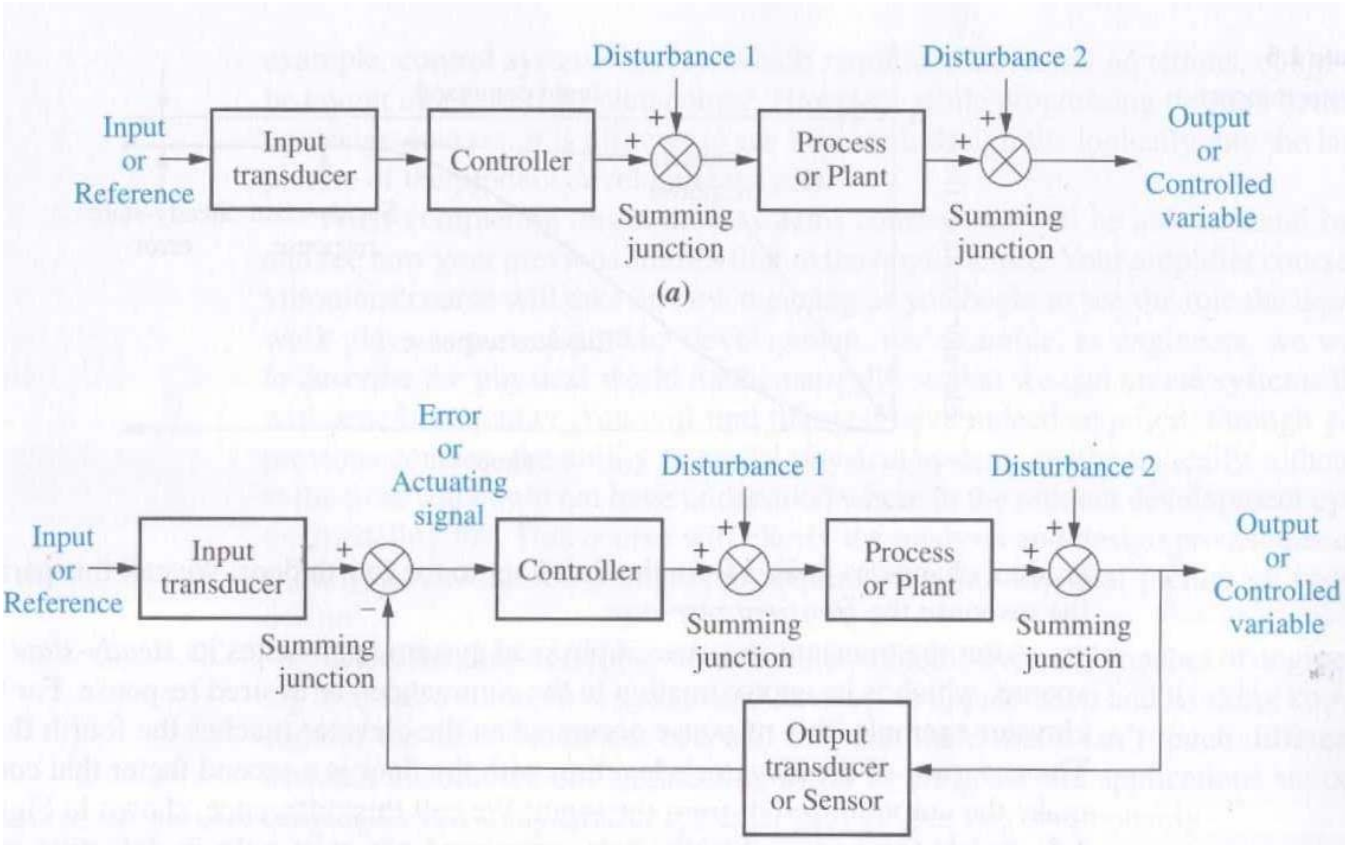
BLOCK DIAGRAMS IN SYSTEMS CONTROL



N. S. Nise, Control Systems Engineering, 2004, Wiley and Sons

FAR1 GENE DOSAGE ON SELECTED TRANSCRIPTOME AND PROTEOME FINDINGS

EXPONENTIAL GROWTH IN GLUCOSE						
NAME	Rel. expression ratio mutant/wt				MOLECULAR FUNCTION	BIOLOGICAL PROCESS
	mRNA		*PROTEIN*			
	far1Δ	*FAR1^{tet}*	*far1Δ*	*FAR1^{tet}*		
PDC1	1	1	1	7,9	Pyruvate decarboxylase isozyme 1	Carbohydrate metabolism
YDL124W	1	1	1,8	2,1	NAD(P)H-dependent reductase	Carbohydrate metabolism
ADH1	1	1			Alcohol dehydrogenase I	Carbohydrate metabolism
WRS1	1	1	-2,3	-2,9	Tryptophanyl-tRNA synthetase	Cell growth and/or maintenance
CDC19	1	1	1	10	Pyruvate kinase	Glycolysis
HXK1	1	1	1	9,1	Hexokinase I	Glycolysis
PGI1	1	1			Glucose-6-phosphate isomerase	Glycolysis
ENO1	1	1	-3,3	-1,5	Enolase 1	Glycolysis
TDH3	1	1	-1,8	-1,6	Glyceraldehyde-3-phosphate dehydrogenase 3	Glycolysis
DLD3	1	-2,9	-4	-3,1	D-lactate dehydrogenase	Lactate metabolism
KRS1	1	1	1	-10	Lysyl-tRNA synthetase	Lysyl-tRNA aminoacylation
MET6	1	1			Homocysteine methyltransferase	Methionine biosynthesis
RIB4	1	1	-2,2	1	6,7-dimethyl-8-ribityllumazine synthase	Polar budding; Bud site selection
EFT1	1	1			Translation elongation factor EF2	Protein biosynthesis
RPL2	1	1	1	6,7	60S Ribosomal protein L2	Protein biosynthesis
RPL8	1	1	1	6,7	Ribosomal protein L8	Protein biosynthesis

EXPONENTIAL GROWTH IN GLUCOSE						
NAME	Rel. expression ratio mutant/wt				MOLECULAR FUNCTION	BIOLOGICAL PROCESS
	mRNA		*PROTEIN*			
	far1Δ	*FAR1^{tet}*	*far1Δ*	*FAR1^{tet}*		
RPS1A	1	1	1	6,2	Ribosomal protein S1	Protein biosynthesis
RPS4	1	1	1	6,7	40S Ribosomal protein S4	Protein biosynthesis
RPL26	1	1	1	2,4	Ribosomal protein L26	Protein biosynthesis
RPS12	1	1	1	1,8	40S Ribosomal protein S12	Protein biosynthesis
RPS18	1	1	1	2,8	40S Ribosomal protein S18	Protein biosynthesis
RPS24	1	1	1	2,8	40S Ribosomal protein S24	Protein biosynthesis; Cell growth and/or maintenance
RPS7A	1	1	1	7,9	40S Ribosomal protein S7	Protein biosynthesis; Polar budding
RPS17	1	1	1	2,8	40S Ribosomal protein S17	Protein biosynthesis; Polar budding;
RPS2	1	1	1	6,2	40S Ribosomal protein S2	Protein biosynthesis; RNA splicing
NPL3	1	-1,9	1	1,5	DNA and RNA binding protein	Protein-nucleus import rRNA-nucleus export
FUR1	1,8	1	2,2	1	Uracil phosphoribosyltransferase	Pyrimidine salvage
EGD2	1	1	-2	1	subunit of the nascent polypeptide associated complex	Regulation of transcription; Protein folding
STM1	1	1	1	6,9	Ribosome-associated protein	Telomere maintenance; Mitosis
HOM2	1	1			Aspartate-semialdehyde dehydrogenase	Threonine and Methionine biosynthesis
HOM6	1	1			Homoserine dehydrogenase	Threonine and Methionine biosynthesis
GUA1	1	1	1	-2,8	GMP synthetase	Xanthine catabolism; GMP biosynthesis

increased protein or mRNA

decreased protein or mRNA

Not changed protein or mRNA

Not detected in 2D gel protein

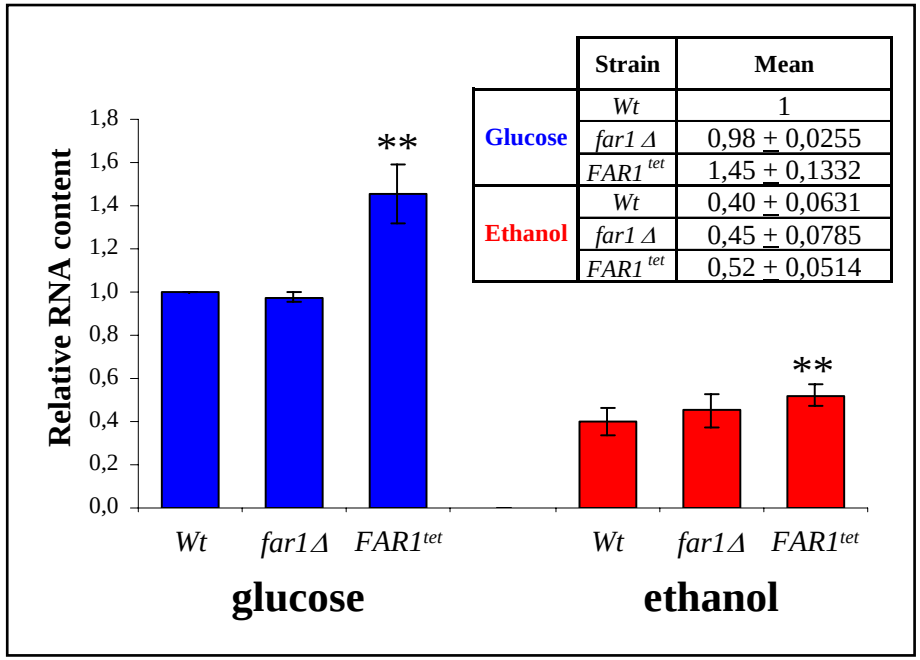
FAR1 OVEREXPRESSION INCREASES RNA CONTENT OF THE CELLS

When exponentially grown in glucose, *FAR1*^{tet} cells show a coordinate induction of the translation/stability of several ribosomal proteins

Loss of balance in ribosomal protein biogenesis could take place

An imbalance in the synthesis of the two ribosome subunits 40S and 60S can induce ribosomal protein and rRNA synthesis by an autoregulation process (Zhao *et al.*, 2003)

***FAR1*^{tet} mutant has more rRNA too?**



	Strain	Mean
Glucose	*Wt*	1
	far1 Δ	0,98 ± 0,0255
	FAR1 ^{tet}	1,45 ± 0,1332
Ethanol	*Wt*	0,40 ± 0,0631
	far1 Δ	0,45 ± 0,0785
	FAR1 ^{tet}	0,52 ± 0,0514

***FAR1*^{tet} mutant shows a statistically significant RNA level increase if compared with wild type strain, both in glucose and in ethanol supplemented media**

ASSUMPTIONS IN TYSON'S MODELS

For budding yeast

- Very few cell cycle players involved
- No nucleus/cytoplasmic localization considered
- No role for ribosome biosynthesis in setting critical cell size
- Zero-order ultrasensitive switch for transcription factors (SBF/MBF) activation. It is assumed that the activation of SBF/MBF depends upon the balance of kinase/phosphatase activities showing a sharp activation as cell grows through the critical cell size. The volume of the size at which [SBF] = ½ (to be entered as a parameter into the model) is calculated from a relation with about ten components (of no direct experimental determination) obtaining a value (1,2) easily estimated from cell mass increase in average newborn cell compared to cell entering S phase (Chen et al, Mol. Biol. Cell, 2000)
- Cln3 dosage and Ps. In order to account for the experimental maintenance of Ps value at increasing Cln3 dosage, an ad hoc saturation relation is assumed between Cln3 and cell mass (Chen et al, Mol. Biol. Cell, 2000)

For mammalian cells

- No nuclear/cytoplasmic localization is modeled
- The role of cyclin D/CDK4/p21 ^{cip1}/p27 ^{kip} in controlling entrance into S phase is played down
- The role of cyclin E/CDK2 in controlling onset of DNA replication is not considered.

"IN VIVO" ROBUSTNESS OF CELL CYCLE IS LARGER THAN THAT PREDICTED BY CHEN et al (2004) MODEL