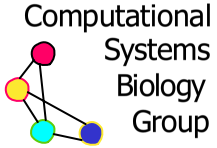

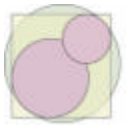

The Yeast Systems Biology Network YSBN

FEBS-SysBio 2007, Gosau – March 10-16, 2007

Cell Size at S Phase Initiation: an Emergent Property of the G_1/S Network

Matteo Barberis

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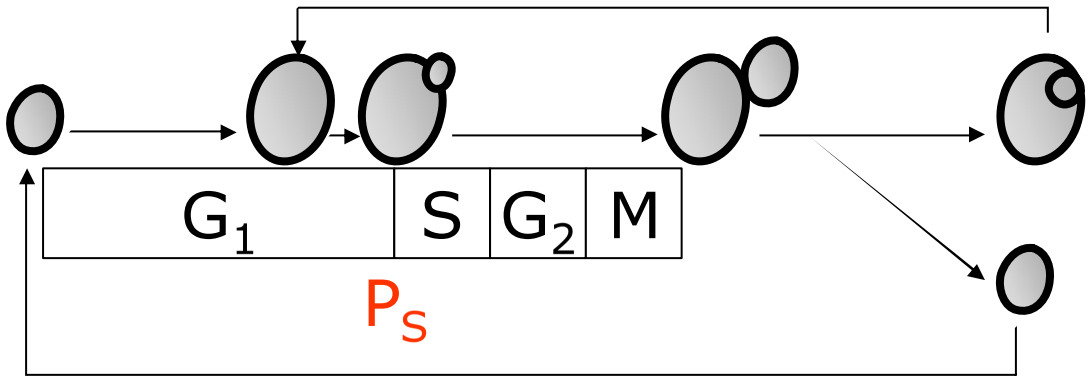
Dept. Biotechnology and Biosciences, Univ. Milano-Bicocca, Milan, Italy

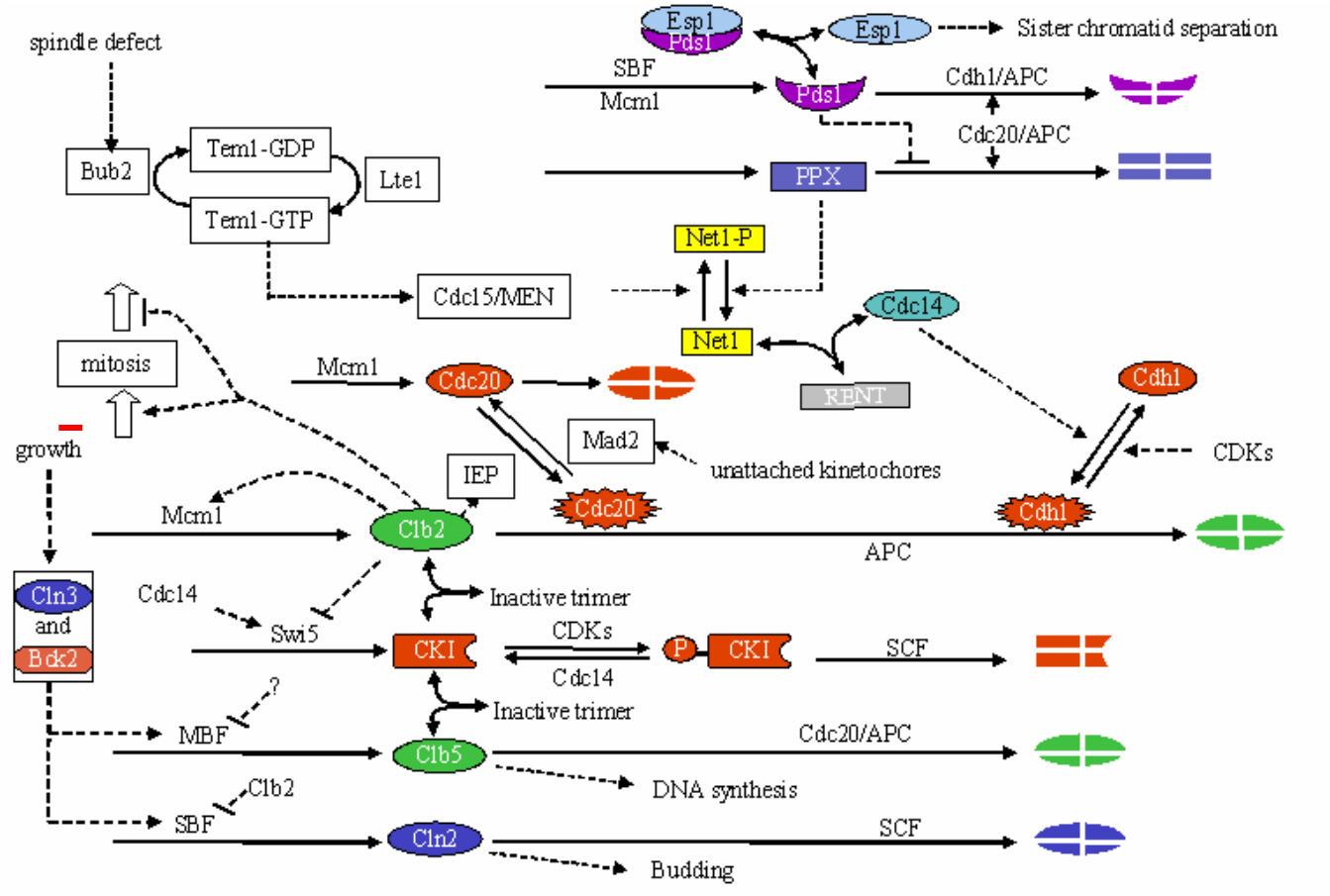
The Yeast Cell Cycle Control

In budding yeast *Saccharomyces cerevisiae* :

- The coordination between cell growth and cell division is a universal but poorly understood feature of the cell cycle
- The main regulatory event(s) takes place at START, when cells must reach a critical cell size (P_S) to enter into S phase

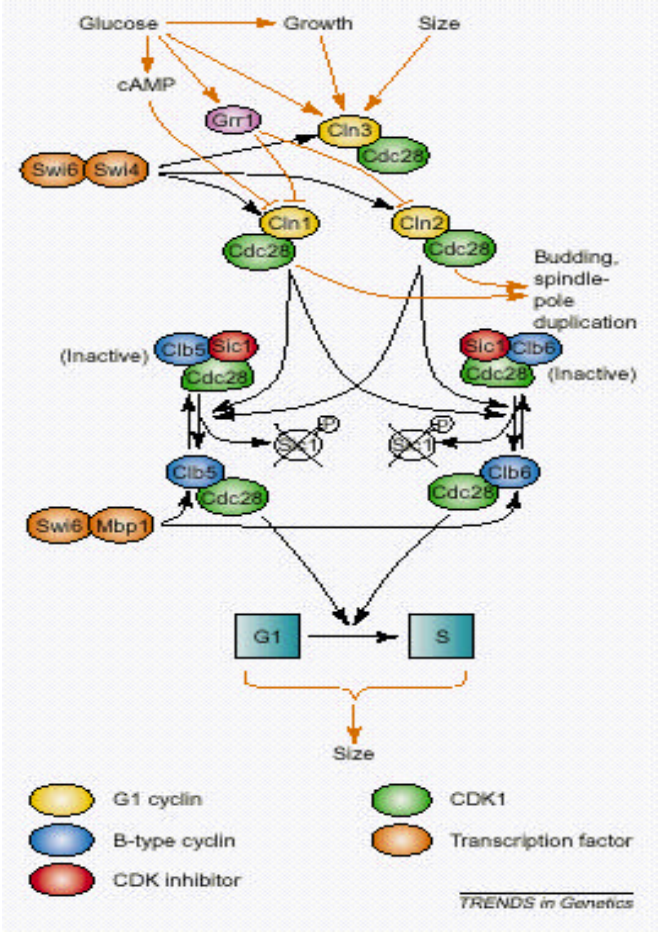


Mathematical Model: the Whole Cell Cycle

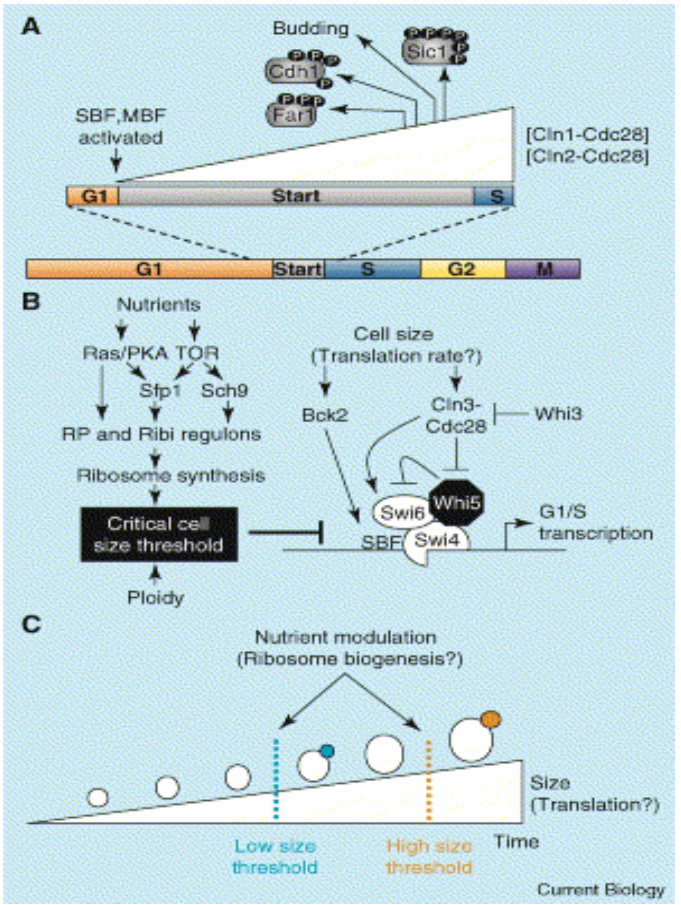


(Chen K.C., *Mol. Biol. Cell*, 2004, 15: 3841-3862)

Descriptive Models: the G_1/S Transition

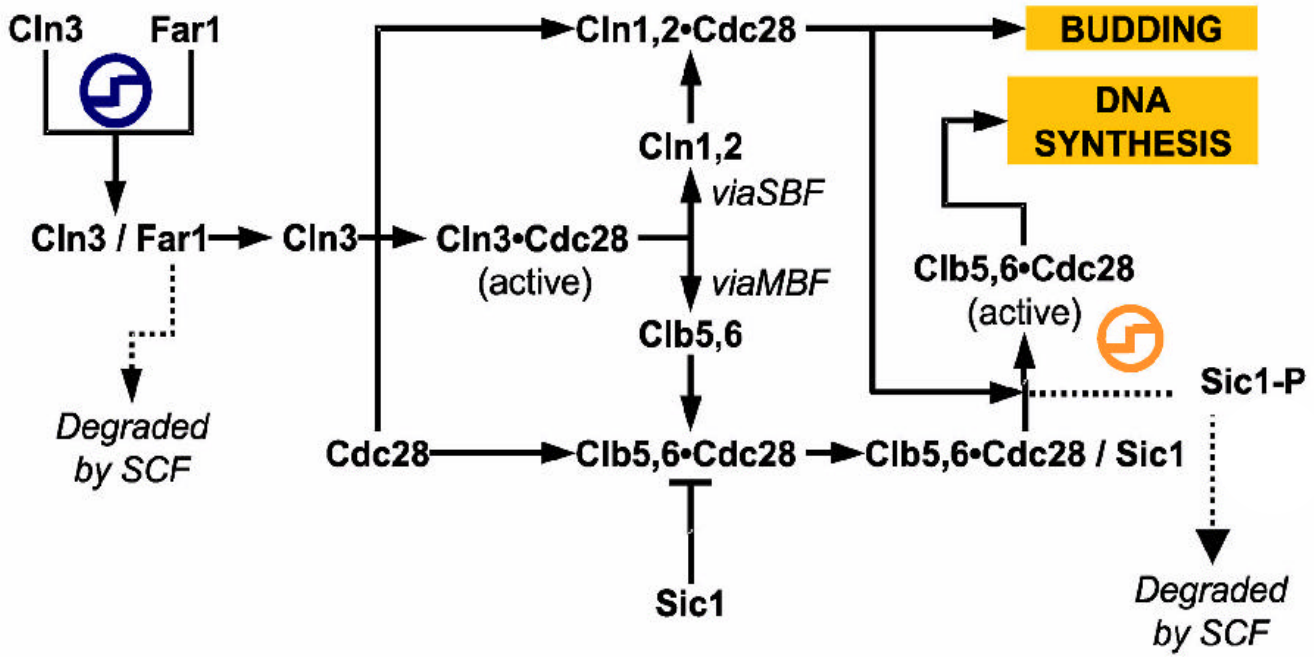


(Rupes I., *Trends Genet.*, 2002, 18: 479-485)

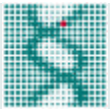


(Jorgensen P. and Tyers M., *Curr. Biol.*, 2004, 14: R1014-R1027)

Descriptive Models: the G_1/S Transition



(Alberghina L. et al., *J. Cell. Biol.*, 2004, 167: 433-443)



Outline of the work

Build the mathematical (ODE) model of the G_1/S transition based on literature data

Verify the model in different experimental conditions

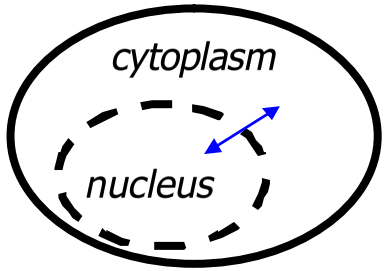
Perform a sensitivity analysis for fine tuning of parameters

Test the model by simulations of dynamics of large number of mutants

Estimate the critical cell size P_S required to enter into S phase

Model Improvements

Compartmentalization

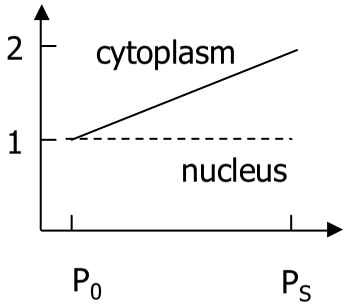


$$\frac{dm_{nuc}}{dt} = k_{transport} \cdot m_{cyt} \cdot \frac{Vol_{cyt}}{Vol_{nuc}}$$

$$\frac{dm_{cyt}}{dt} = k_{transport} \cdot m_{nuc} \cdot \frac{Vol_{nuc}}{Vol_{cyt}}$$

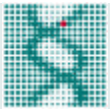
Volume Changes

Cell size growth during the G_1 phase



$$\frac{dm_{cyt}}{dt} = \sum_i v_i - \frac{dVol_{cyt}}{dt}\frac{m_{cyt}}{Vol_{cyt}}$$

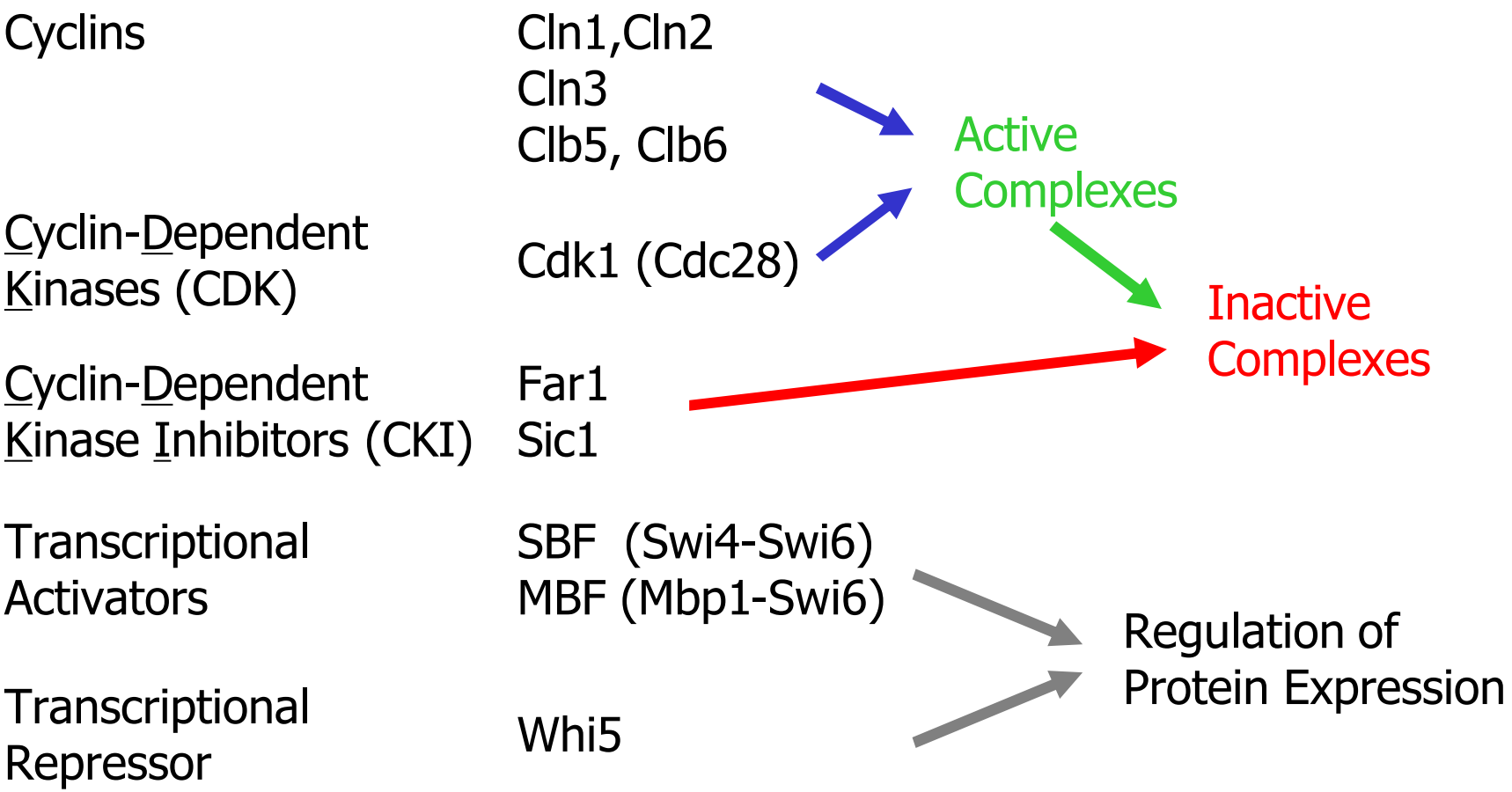
	P_0	P_S	TG_1 (exp)
Glucose	60	100	80 min
Ethanol	32	54	240 min

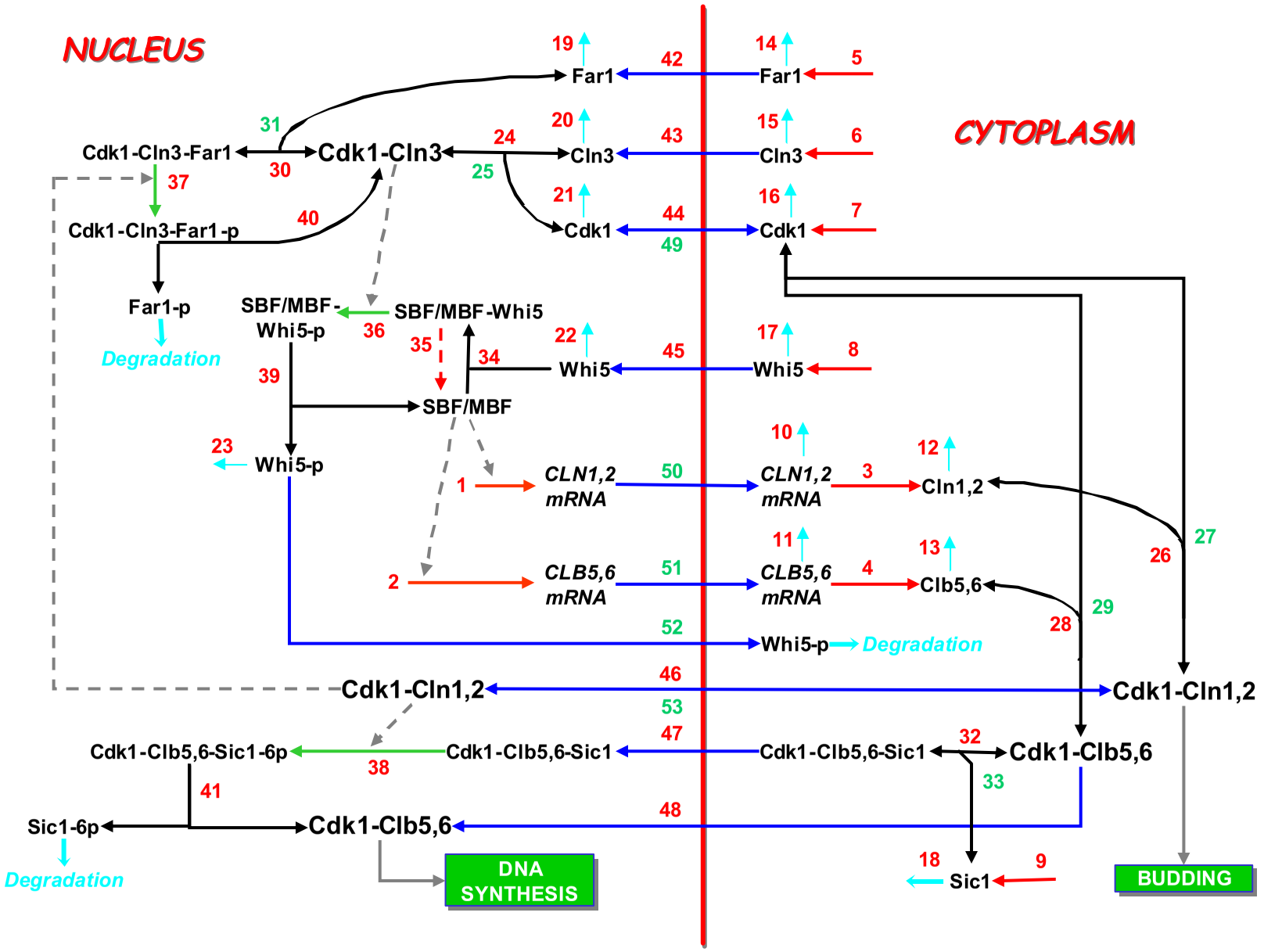


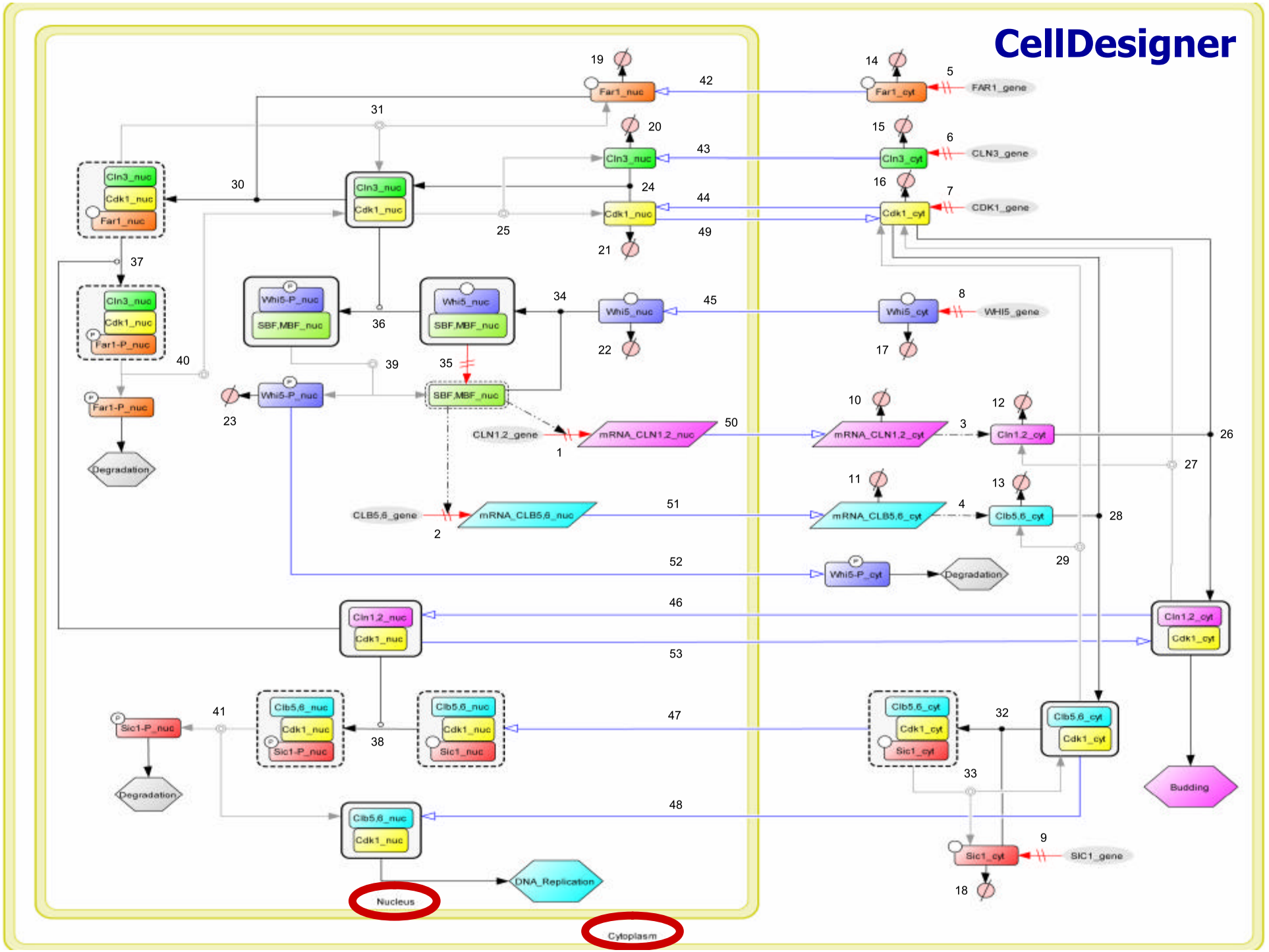
Model of the G_1/S Transition

Cyclins	Cln1,Cln2 Cln3 Clb5, Clb6
Cyclin-Dependent Kinases (CDK)	Cdk1 (Cdc28)
Cyclin-Dependent Kinase Inhibitors (CKI)	Far1 Sic1
Transcriptional Activators	SBF (Swi4-Swi6) MBF (Mbp1-Swi6)
Transcriptional Repressor	Whi5

Model of the G_1/S Transition




NUCLEUS
CYTOPLASM
Far1
Cln3
Cdk1
Whi5
Far1
Cln3
Cdk1
Whi5
Cdk1-Cln3-Far1
Cdk1-Cln3
Cdk1-Cln3-Far1-p
Far1-p
Degradation
SBF/MBF-Whi5-p
SBF/MBF-Whi5
SBF/MBF
Whi5-p
CLN1,2 mRNA
CLB5,6 mRNA
Cln1,2
Clb5,6
Whi5-p
Degradation
Cdk1-Cln1,2
Cdk1-Cln1,2
Cdk1-Clb5,6-Sic1-6p
Cdk1-Clb5,6-Sic1
Cdk1-Clb5,6-Sic1
Cdk1-Clb5,6
Sic1-6p
Degradation
Cdk1-Clb5,6
DNA SYNTHESIS
Sic1
BUDDING


CellDesigner
Cln3_nuc
Cdk1_nuc
Far1_nuc
Far1-P_nuc
Whi5-P_nuc
SBF,MBF_nuc
Whi5_nuc
Whi5-P_nuc
SBF,MBF_nuc
Far1-P_nuc
Degradation
CLN1,2_gene
mRNA_CLN1,2_nuc
CLB5,6_gene
mRNA_CLB5,6_nuc
Cln1,2_nuc
Cdk1_nuc
Sic1-P_nuc
Degradation
Clb5,6_nuc
Cdk1_nuc
Sic1-P_nuc
Clb5,6_nuc
Cdk1_nuc
Sic1_nuc
Clb5,6_nuc
Cdk1_nuc
DNA_Replication
Nucleus
Cytoplasm
Far1_nuc
Cln3_nuc
Cdk1_nuc
Cdk1_nuc
Whi5_nuc
SBF,MBF_nuc
Whi5-P_nuc
SBF,MBF_nuc
Far1_cyt
FAR1_gene
Cln3_cyt
CLN3_gene
Cdk1_cyt
CDK1_gene
Whi5_cyt
WHI5_gene
mRNA_CLN1,2_cyt
Cln1,2_cyt
mRNA_CLB5,6_cyt
Clb5,6_cyt
Whi5-P_cyt
Degradation
Cln1,2_cyt
Cdk1_cyt
Clb5,6_cyt
Cdk1_cyt
Sic1_cyt
Clb5,6_cyt
Cdk1_cyt
Budding
Sic1_cyt
SIC1_gene
19
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21
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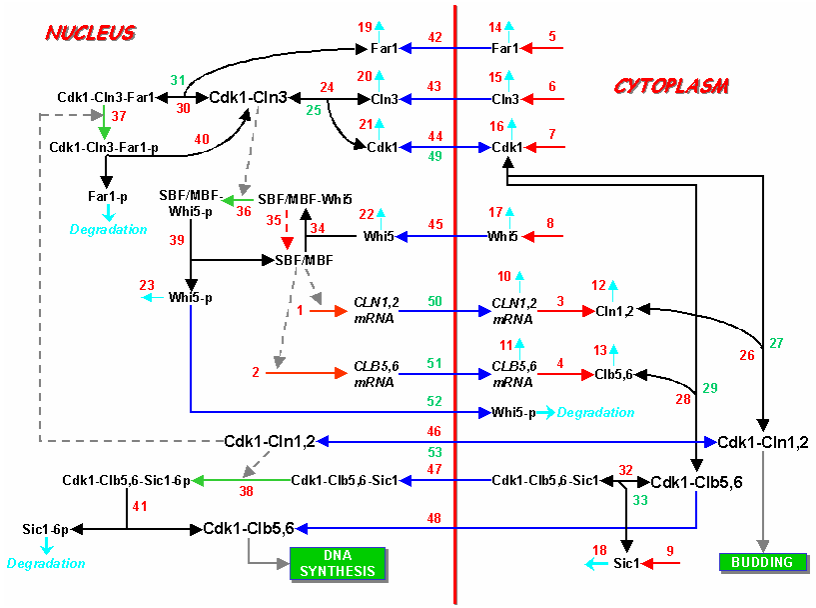
Modeling Pipeline



Collect experimental data (environmental and genetic perturbations, localizations, time courses, kinetic constants values)

Estimate model parameters

Simulate: agreement of model dynamics and experimental data?

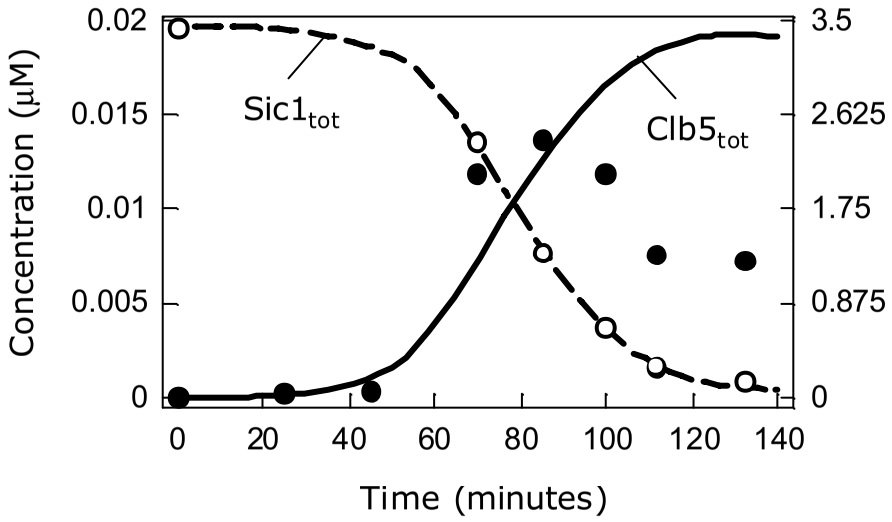
Sensitivity analysis

Model validation

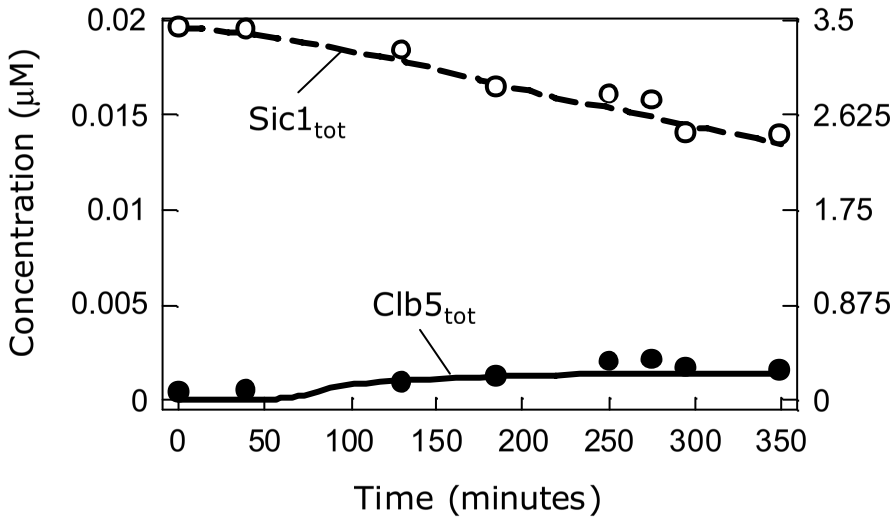
Predictions

Testing Performance of the Model

Glucose



Ethanol



Simulated dynamics of Sic1

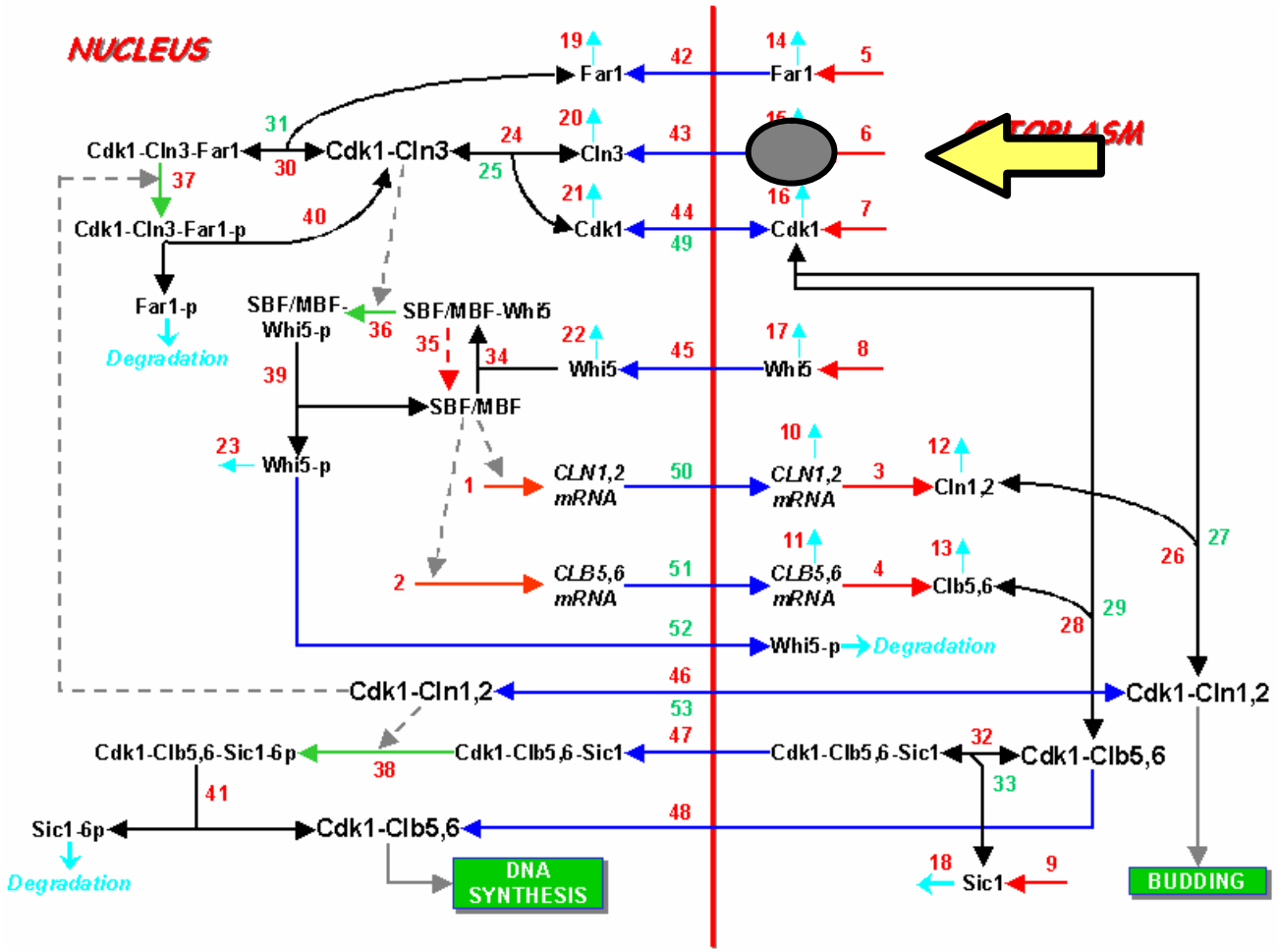
Simulated dynamics of Clb5

Experimental dynamics of Sic1

Experimental dynamics of Clb5

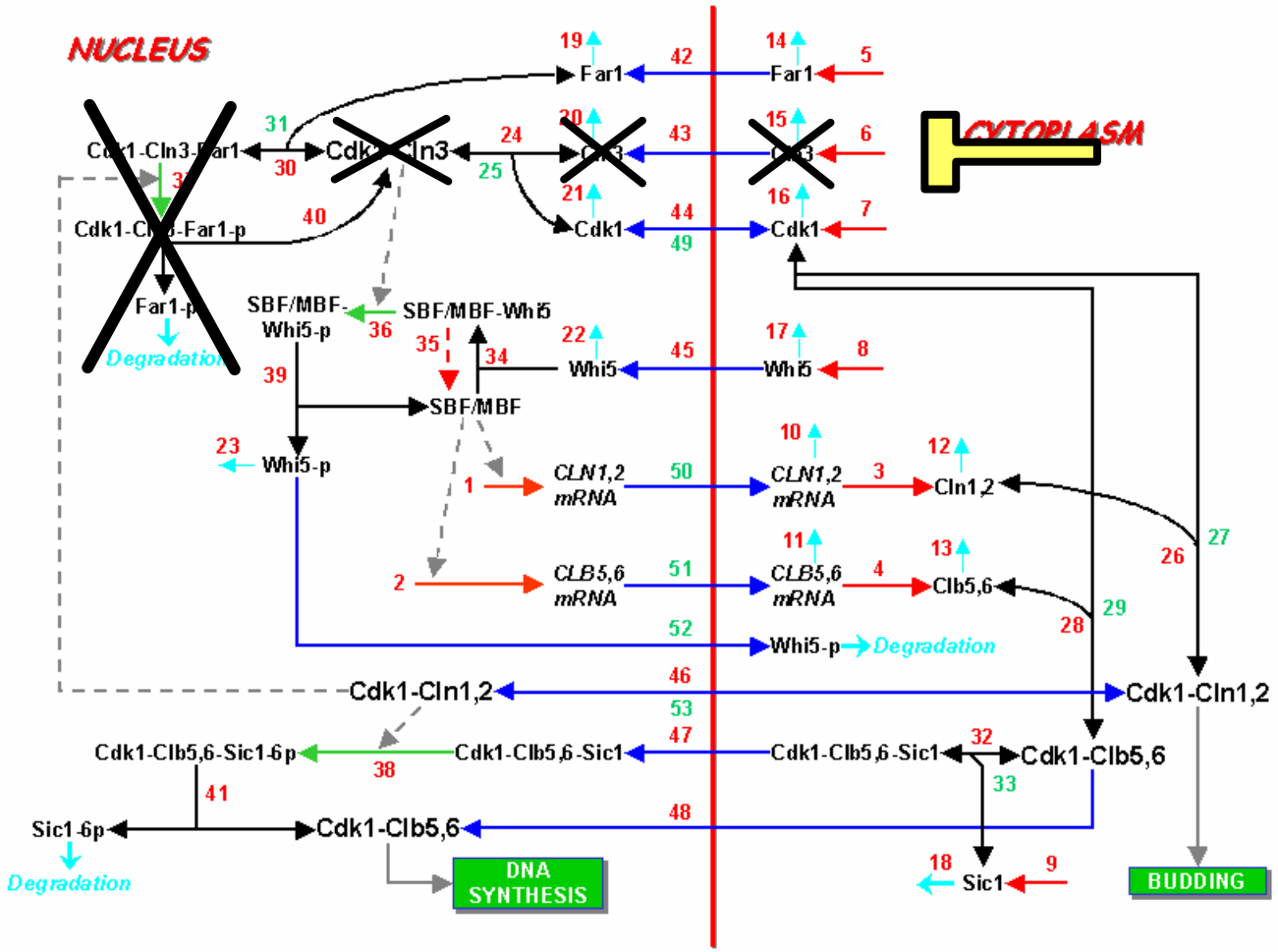
Cdk1-Clb5,6 nuc

Model Validation



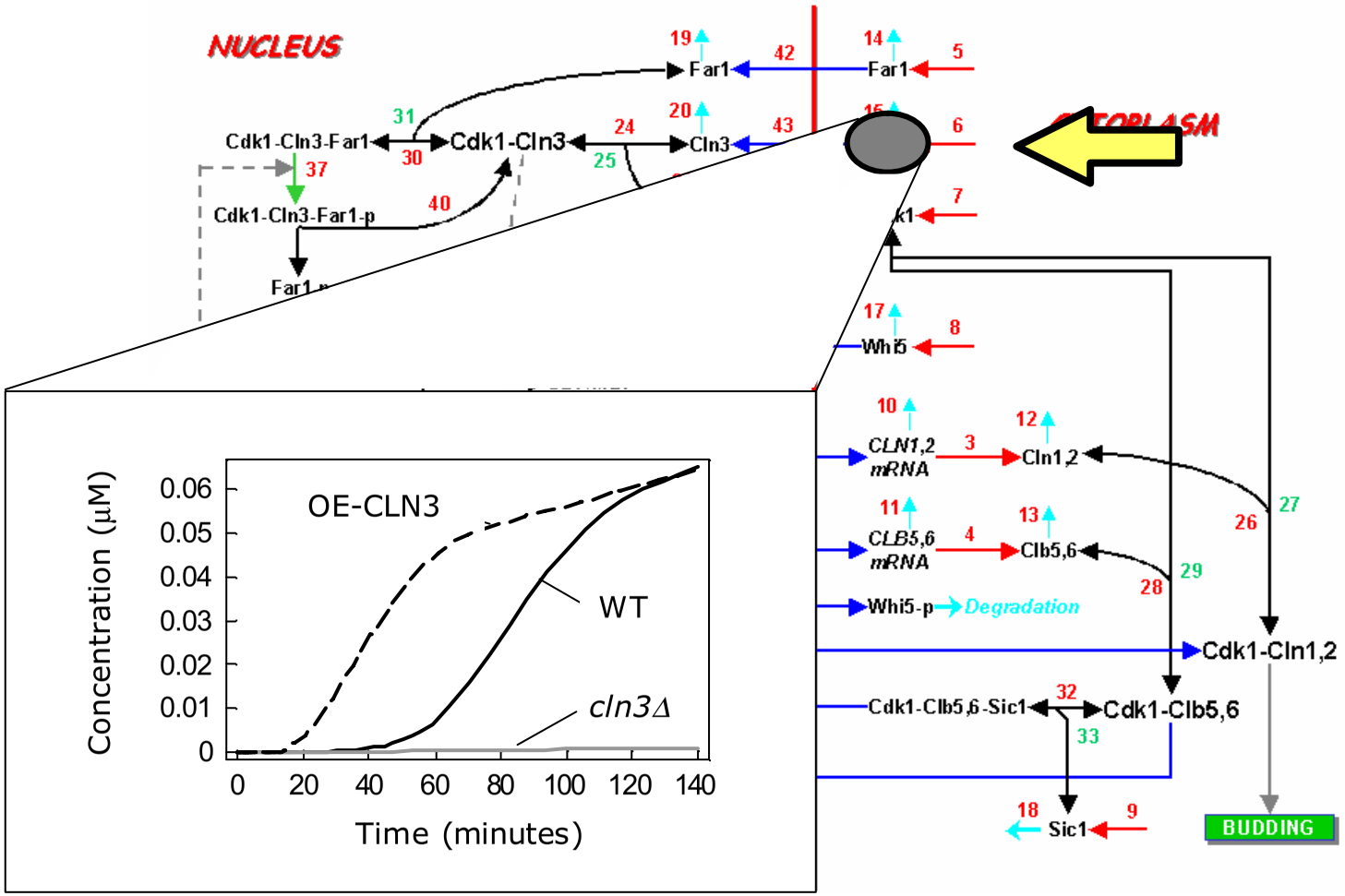
Cdk1 -Clb5,6 nuc

Model Validation



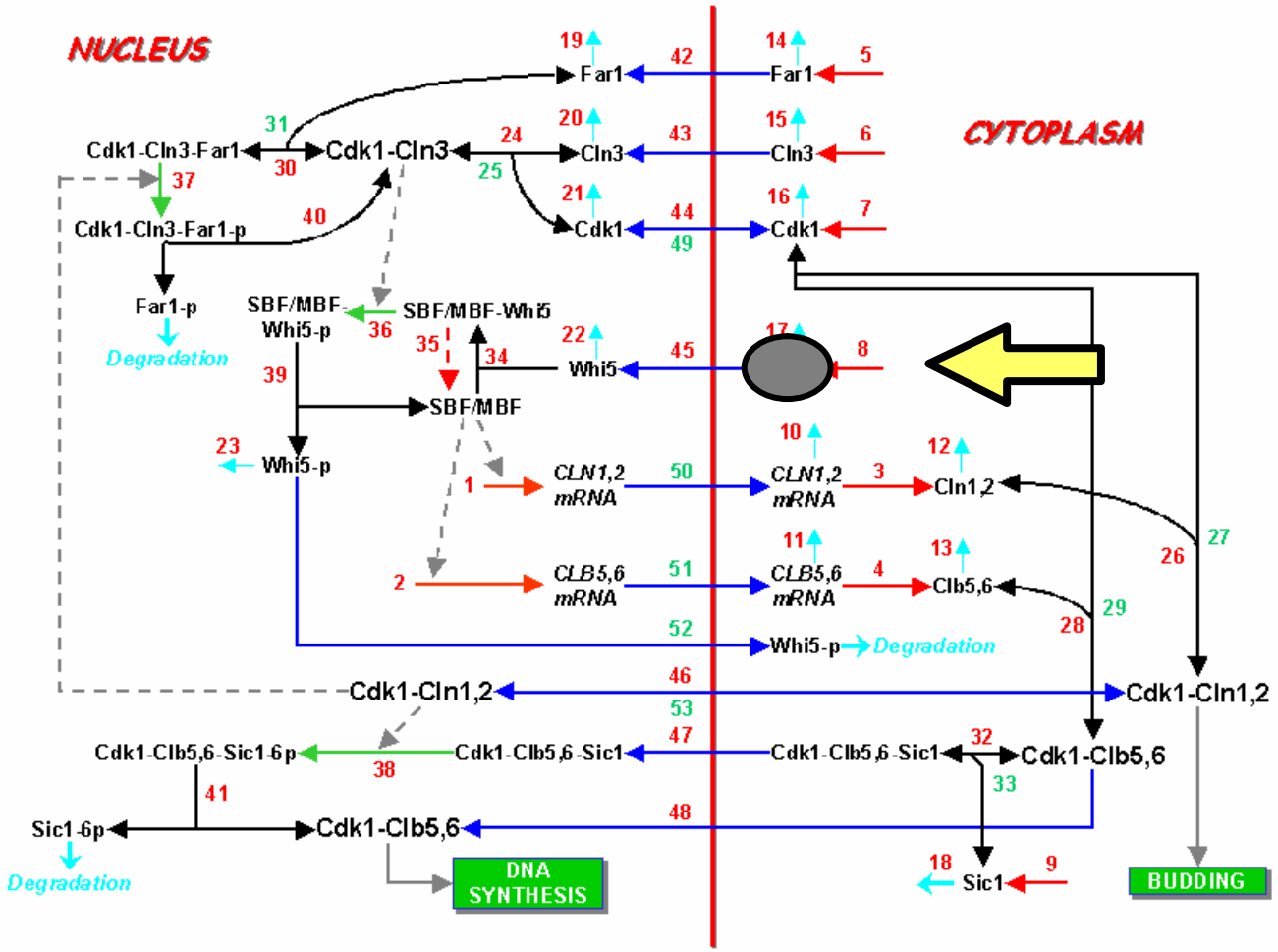
Model Validation

Cdk1 -Clb5,6 nuc



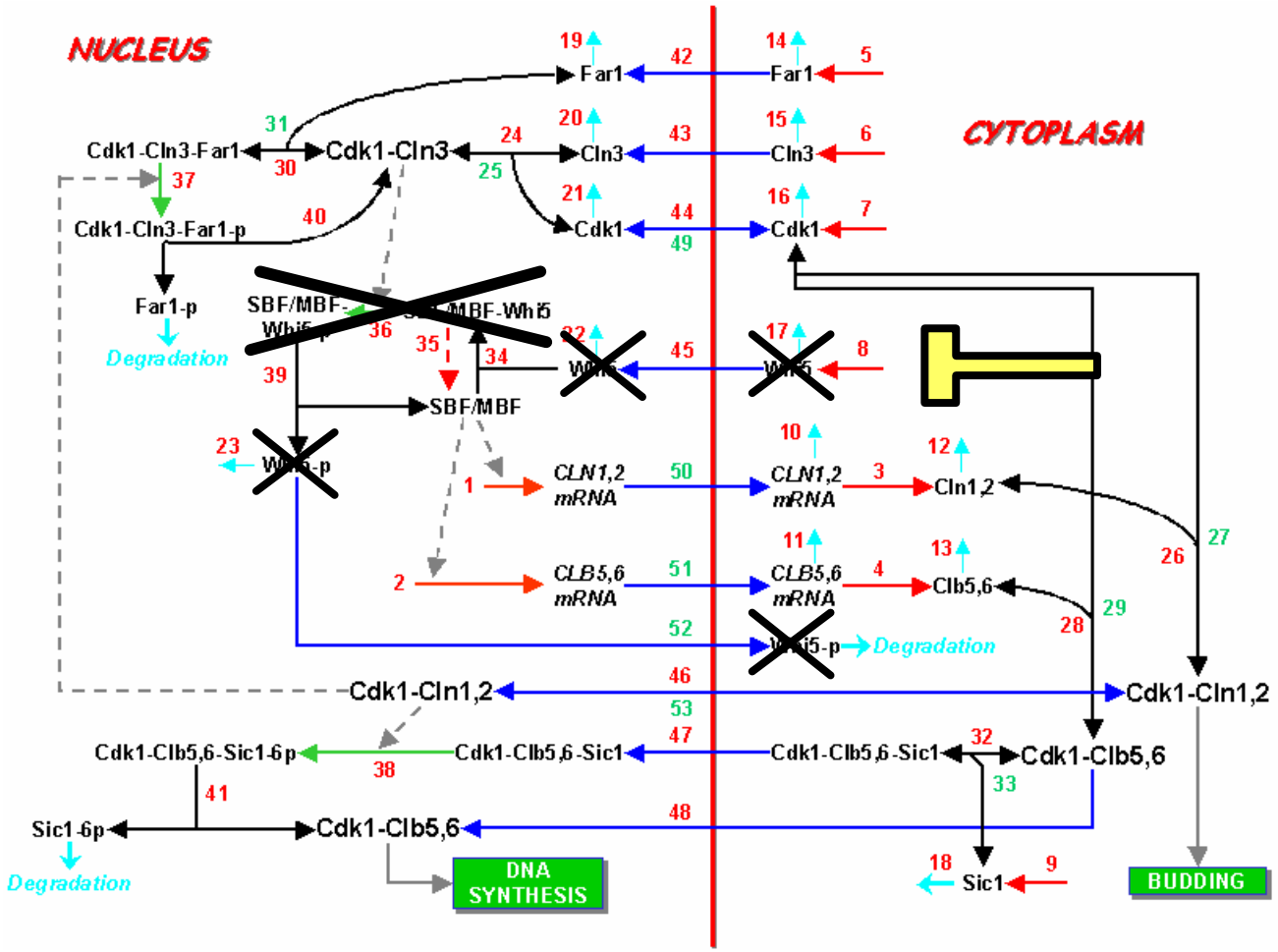
Cdk1 -Clb5,6 nuc

Model Validation



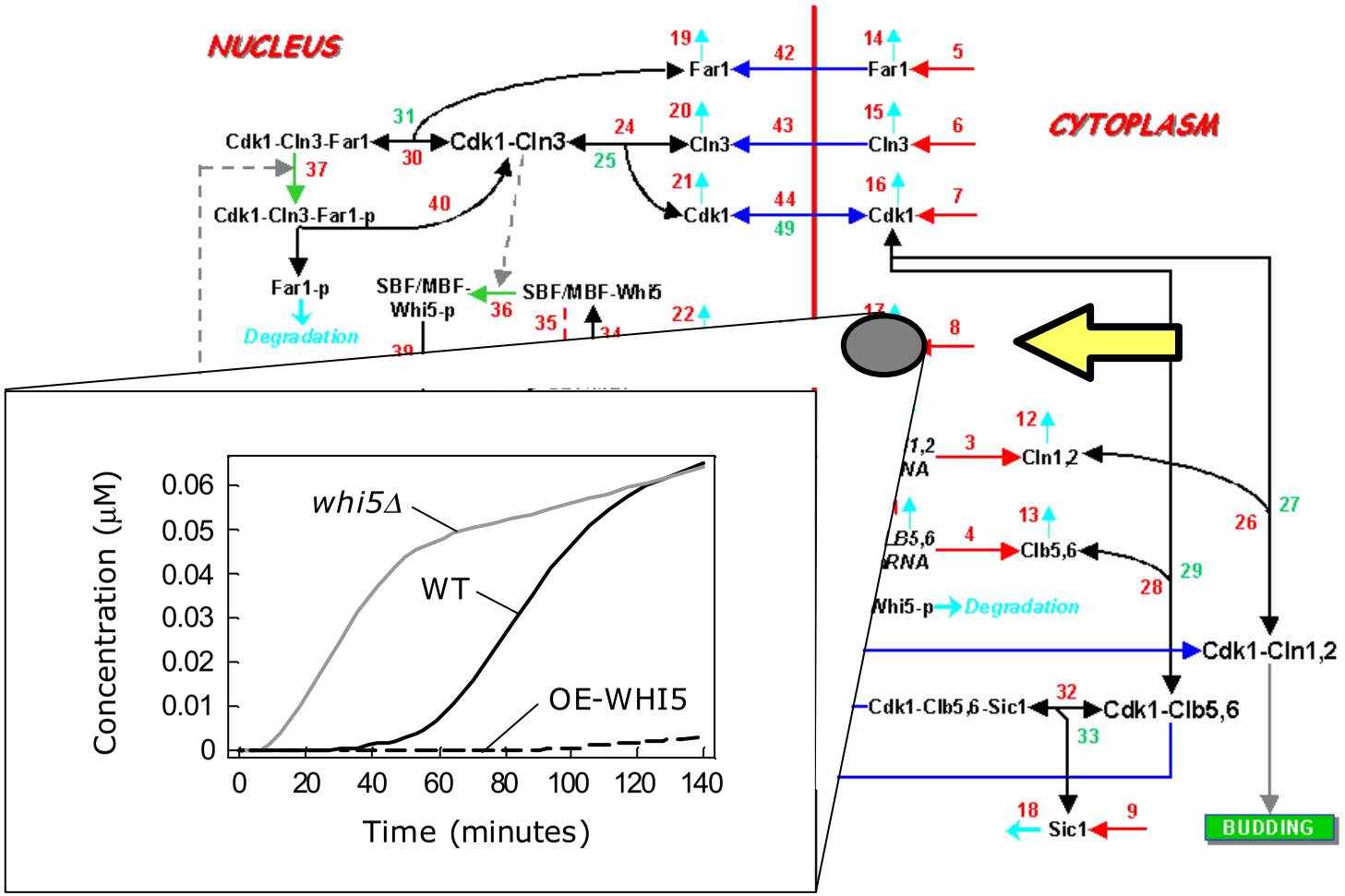
Cdk1 -Clb5,6 nuc

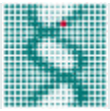
Model Validation



Model Validation

Cdk1 -Clb5,6 nuc



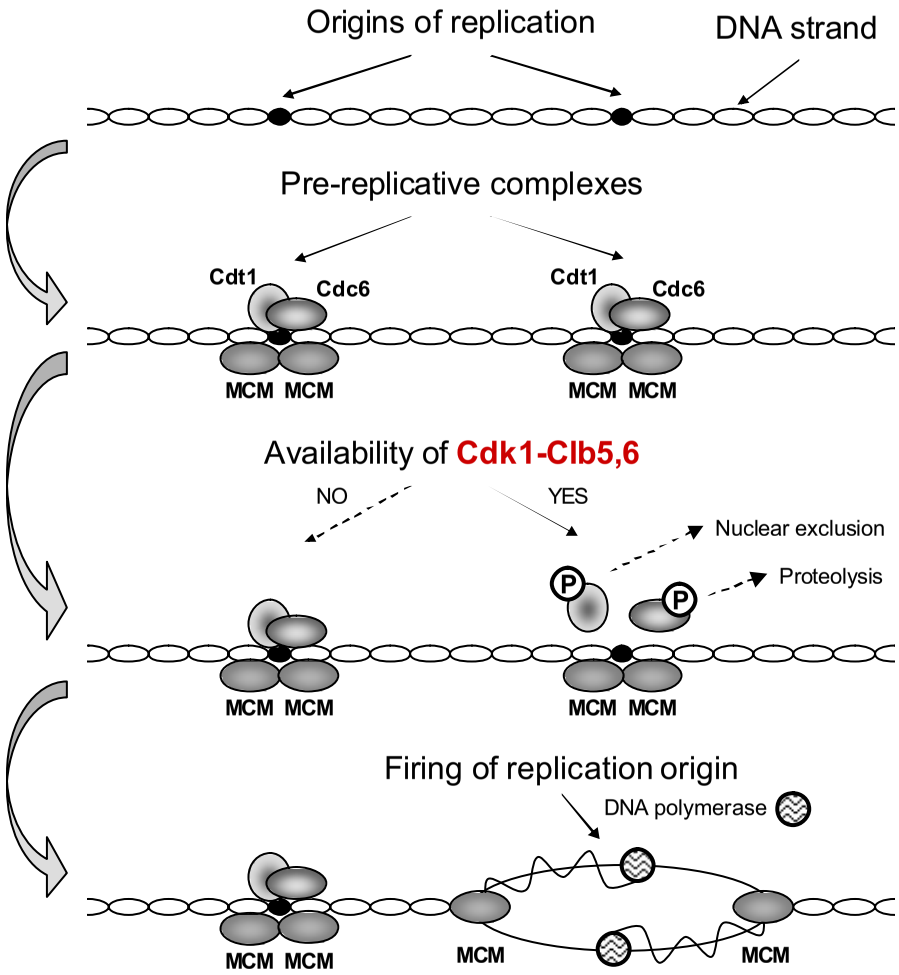


Model Validation
– Combined Deletion Mutants –

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

Setting of the Critical Cell Size

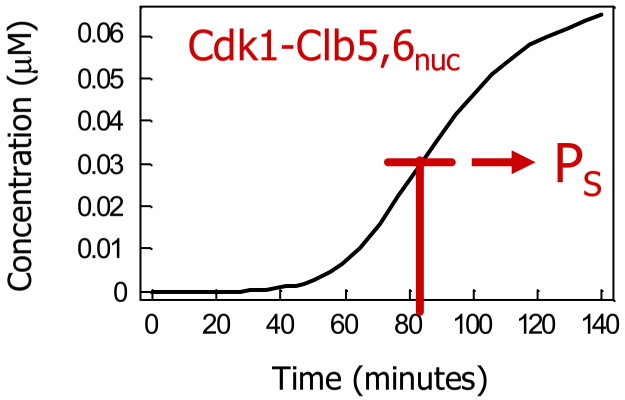
→ Since P_S is defined as the protein content of cells that enter S phase, we correlate the concentration of Cdk1-Clb5,6$_{nuc}$ with the onset of DNA replication

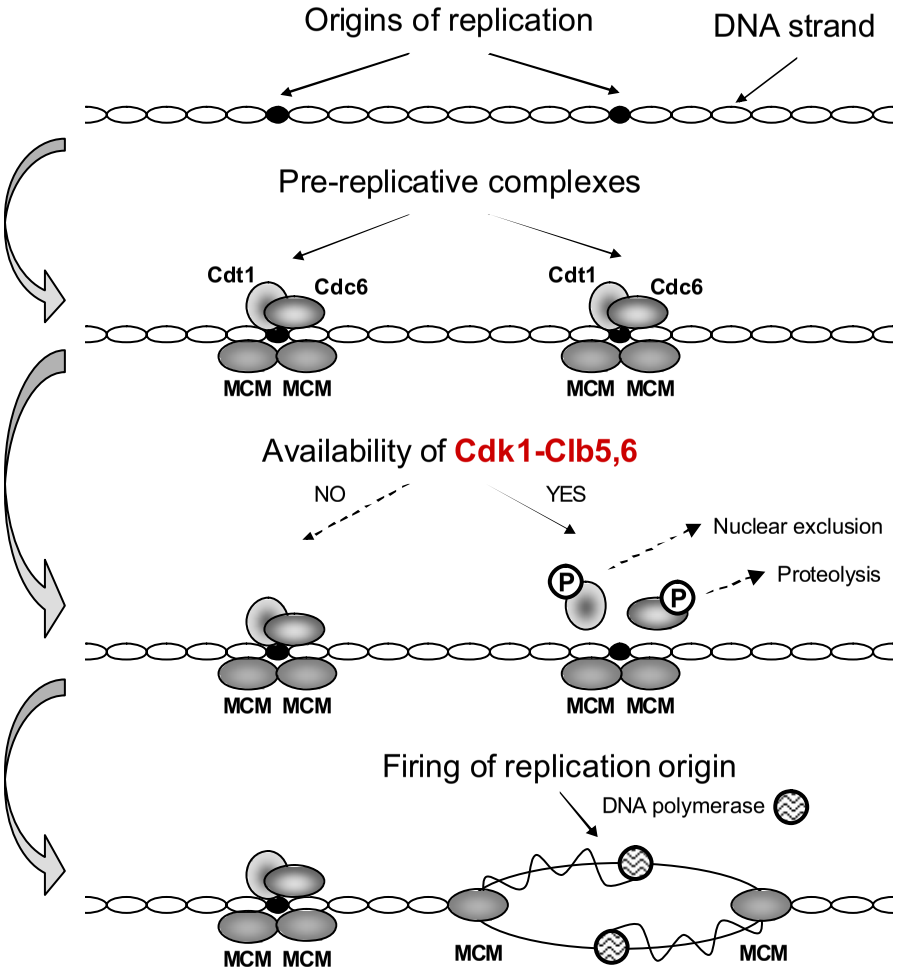


Setting of the Critical Cell Size

→ Since P_S is defined as the protein content of cells that enter S phase, we correlate the concentration of Cdk1-Clb5,6$_{nuc}$ with the onset of DNA replication

→ P_S is estimated as the value of the cell size at the time when 50 % of replication origins have fired

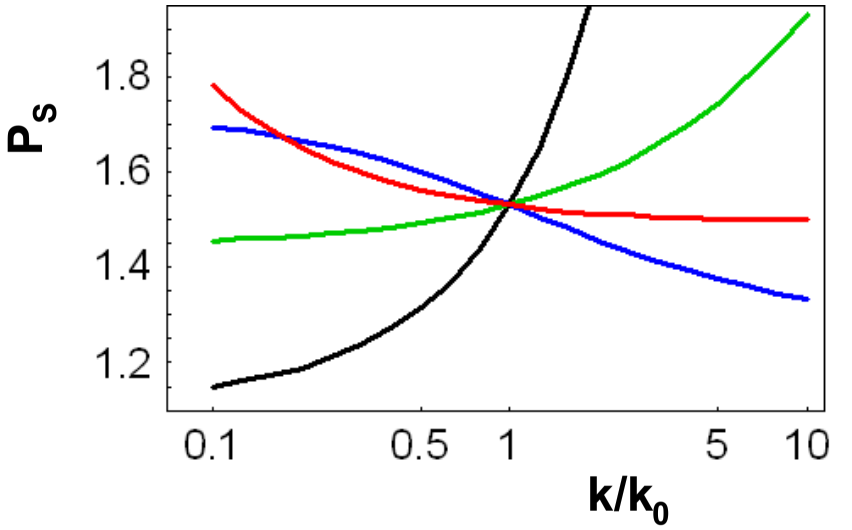




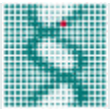
Parameters Influence on the P_S Value

Relevant genotype	Medium	Estimated P_S
Wild type	glucose	1.54
cln3Δ	glucose	–
OE-CLN3	glucose	1.26
far1Δ	glucose	1.44
OE-FAR1 2X	glucose	1.63
whi5Δ	glucose	1.20
OE-WHI5	glucose	3.31
sic1Δ	glucose	6.57
OE-SIC1	glucose	1.50
Wild type	ethanol	1.20
OE-FAR1 10X	ethanol	1.42

→ P_S is an emergent property of the G_1 to S network, and its value strongly depends on growth rate



Growth rate
Far1 level
Cln3 level
Binding constant for the Cdk1-Clb5,6/Sic1 complex formation



Conclusions

→ The model of the G_1/S transition allows for a testable representation of experimental knowledge

→ The model of the G_1/S transition allows to predict experimental behaviors revealing regulatory properties of cellular network

Barberis M, Klipp E, Vanoni M, Alberghina L (2007) *PLoS Comp. Biol.*, *in press*

Acknowledgements

Marie Curie Actions
Human Resources and Mobility Activity

EARLY STAGE TRAINING
SYSTEMS BIOLOGY

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Jannis Uhlendorf
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Systems
Biology
Group

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