Modular Logical Modelling of the Budding Yeast Cell Cycle

Supplementary materials

Adrien Fauré, Aurélien Naldi, Fabrice Lopez, Claudine Chaouiya, Andrea Ciliberto, Denis Thieffry

Detailed descriptions of the models

This section covers the descriptions of the three individual modules (core, MCP and exit) and of the coupled model.

For each model, we provide:

- A graphical representation of the module, in terms of a regulatory graph, along with textual annotations and bibliographical references (links to MedLine).
- A table listing all the regulatory components considered, along with the associated logical function, textual annotations, bibliographical references and links to external databases.
- A table describing a wild-type simulation.
- A table listing different perturbations (gene loss-of-functions, ectopic expression, etc.). For the sake of clarity, detailed description of the perturbations have not been included; it can be found within the models files.

The logical functions are written in the syntax used by GINsim. The symbols “&”, “|” and “!” represent logical AND, OR and NOT, respectively. When different functions are associated with the same activity level, the rule is defined by the union of the functions

Description of the model "core_model"

Annotation

- Chen et al.'s web site
- Chen et al., 2004

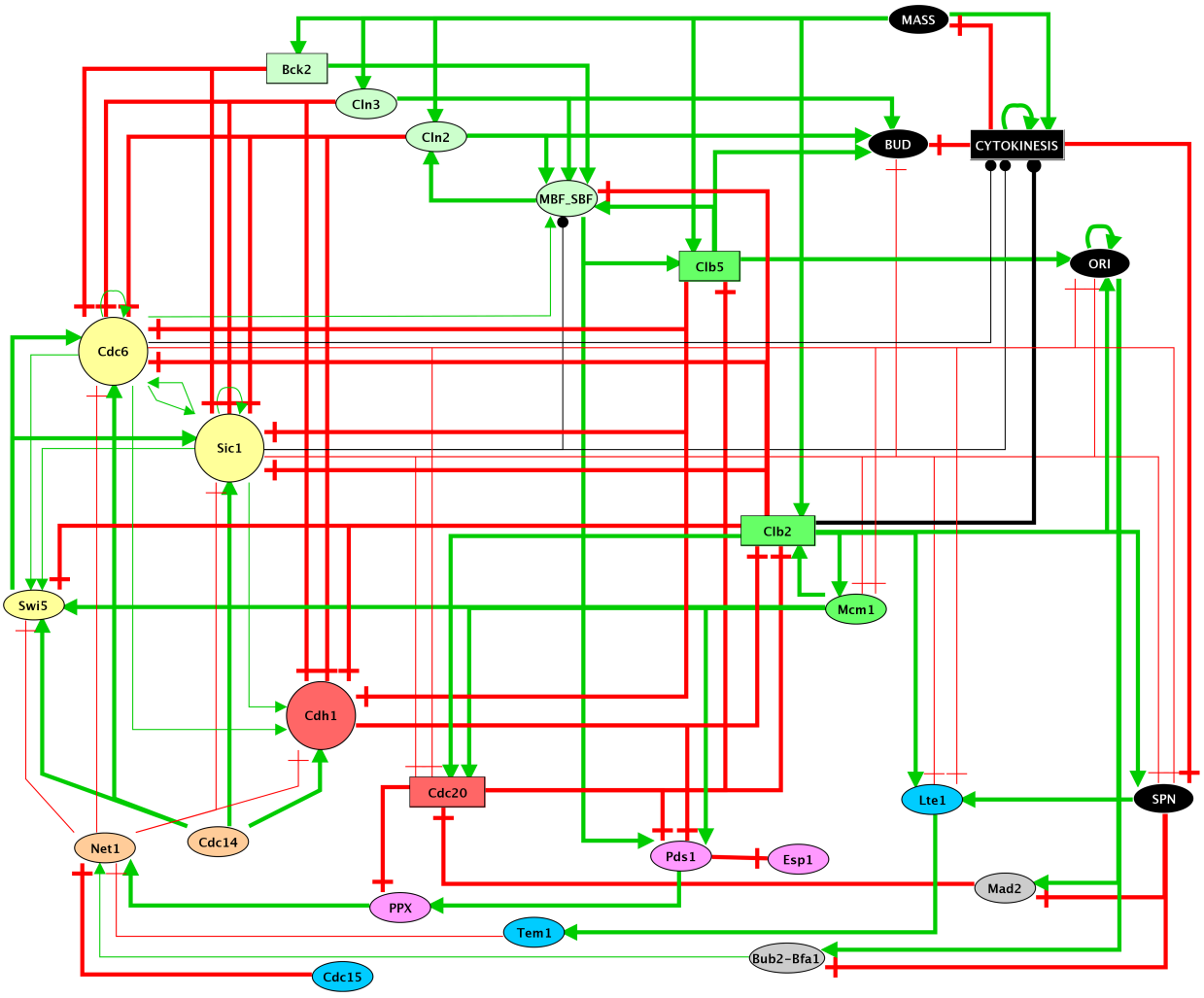
This logical model for the core cell cycle engine of the budding yeast is based on the differential published by Chen et al (2004).
This model is based on the antagonism between mitotic cyclins (Clb2 and Clb5) and G1 stabilizers (Cdh1 and the CKI). When mass increases above a certain threshold, G1 cyclins accumulate high enough to inhibit the G1 stabilizers, and thus indirectly activate the mitotic cyclins, thereby promoting entry into the S phase and mitosis. Clb2 triggers exit from mitosis and its own destruction, by activating Cdc20, and indirectly activates the G1 stabilizers through the release of the phosphatase Cdc14.
In addition, the model integrates a checkpoint mechanism that monitors DNA replication and spindle formation.

Using well defined logical rules for the updating of each considered regulatory node, the simulation of this model qualitatively recapitulates the wild-type sequence of events the cell has to go through to be considered viable.
Over a hundred of different mutants (gene knock-outs, partial loss-of-functions, temperature-sensitive mutations, over- or ectopic expressions) or perturbations (e.g. culture in galactose- or glucose-rich medium) have been analysed experimentally and reported in the literature.

Over 130 of these mutants have been successfully accounted for by Chen et al.'s model. In the present article, we set out to reproduce these results with our logical version of the model. Consistency of the mutant phenotypes is evaluated qualitatively in term of viability (cf infra), or arrest in a particular phase of the cell cycle. Dynamical analysis of most of the individual or multiple leads to results consistent with reported phenotypes, as well as with the results published by Chen et al..

Cell viability rule in terms of sequence of events: firing of the origins of replication (ORI goes up), spindle alignment (SPN goes up), separase activation (or inhibition of the securin, Pds1 goes down), division (MITOSIS goes up to level 2) after the formation of a bud (BUD must have reached value 1, although it goes down afterwards before cell division), and origin relicensing (ORI goes down).

Cell cycle phases:
G0/G1: low Clb5 and Clb2 activity (either <2 or sequestered by Sic1 / Cdc6)
S/G2: high Clb5 activity (i.e. not sequestered by the CKI), low Clb2.
M: high Clb2 activity
prophase / metaphase: low Esp1 activity (high Pds1)
anaphase / telophase: high Esp1 activity (low Pds1)



Nodes

ID	Val	Logical function	Comment
Cln3	1	● MASS	● Chen et al.'s web site: Cln3, Bck2 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P13365 Cln3 is the G1-cyclin, initiating Start events. All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1.
Bck2	1	● MASS	● Chen et al.'s web site: Cln3, Bck2 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P33306 The protein Bck2 initiates Start events.

MBF_SBF	1	● !(Clb2 & !(Sic1 | Cdc6)) & (Bck2 | Cln3 | Cln2 | (Clb5 & !Sic1))	● Chen et al.'s web site: MBF ● Chen et al.'s web site: SBF ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/O14467 MBF and SBF are transcription factors. SBF activates the transcription of Cln2. MBF activates the transcription of Clb5.
Cln2	1	● MASS & MBF_SBF	● Chen et al.'s web site: Cln2 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P20438 The cyclin Cln2 is involved in budding. Cln2 represents both Cln1 and Cln2 in the model. All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1.
Swi5	1	● (Mcm1 & !(Clb2 & !(Sic1 | Cdc6))) | (Mcm1 & Clb2 & !(Sic1 | Cdc6) & ((Cdc14:1 & !Net1) | (Cdc14:2 & ! Net1:2)))	● Chen et al.'s web site: Swi5 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P08153 Swi 5 is transcription factor controlling Sic1 and Cdc6.
Sic1	1	● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:2)) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2 | ((Clb2 | Clb5) & (Cln3 | Bck2:2)) | (Clb5 & Clb2) | (Clb5:3 & Bck2)) ● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:2)) & Swi5 & !((Clb2:3 & !(Sic1 | Cdc6)) | (((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1)) & ((Cln2 & (Cln3 | Bck2)) | (Cln3 & Bck2))) | (((Clb2 & Clb5) | Clb2:3 | Clb5:3) & Cln2 & (Cln3 | Bck2)) | (Clb2:3 & Clb5:3)) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & Swi5 & !(((Clb5 & Clb2 & !(Sic1 | Cdc6) & Cln2 & Cln3 & Bck2) | (Clb2:3 & !(Sic1 | Cdc6))) & (Clb5 | Cln2 | (Cln3 & Bck2))) ● Cdc14:3 & !Net1:2 & !Swi5 & !(Clb5 & Clb2 & Cln2 & Cln3 & Bck2) ● Cdc14:3 & !Net1:2 & Swi5 & !(Cln3 & Bck2 & Cln2 & Clb5 & !Sic1 & Clb2 & !(Sic1 | Cdc6))	● Chen et al.'s web site: Sic1 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P38634 Sic1 is a stoichiometric inhibitor of Cdc28/Clb2 and Cdc28/Clb5. The arrows directed towards Clb5 and Clb2's targets represent sequestration.
Cdc6	1	● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:2)) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2 | ((Clb2 | Clb5) & (Cln3 | Bck2:2)) | (Clb5 & Clb2) | (Clb5:3 & Bck2)) ● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:2)) & Swi5 & !((Clb2:3 & !(Sic1 | Cdc6)) | (((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1)) & ((Cln2 & (Cln3 | Bck2)) | (Cln3 & Bck2))) | (((Clb2 & Clb5) | Clb2:3 | Clb5:3) & Cln2 & (Cln3 | Bck2)) | (Clb2:3 & Clb5:3)) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & Swi5 & !(((Clb5 & Clb2 & !(Sic1 | Cdc6) & Cln2 & Cln3 & Bck2) | (Clb2:3 & !(Sic1 | Cdc6))) & (Clb5 | Cln2 | (Cln3 & Bck2))) ● Cdc14:3 & !Net1:2 & !Swi5 & !(Clb5 & Clb2 & Cln2 & Cln3 & Bck2) ● Cdc14:3 & !Net1:2 & Swi5 & !(Cln3 & Bck2 & Cln2	● Chen et al.'s web site: Cdc6 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P09119 Cdc6 is a stoichiometric inhibitor of Cdc28/Clb2, but not of Cdc28/Clb5. Cdc6 is also a licencing factor for DNA replication. The arrows directed towards Clb2's targets represent sequestration.

		& Clb5 & !Sic1 & Clb2 & !(Sic1 | Cdc6))	
Clb5	1	● MASS & MBF_SBF & Cdc20	● Chen et al.'s web site: Clb5 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P30283 Clb5 is a B-type cyclins appearing late in G1, involved in DNA synthesis (represents both Clb5 and Clb6 in the model). All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1.
	2	● MASS & MBF_SBF & !Cdc20	
Clb2	2	● MASS & !Cdh1 & ((!Cdc20 & !Mcm1) | (Cdc20:2 & Mcm1))	● Chen et al.'s web site: Clb2 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P24869 Clb2 is aB-type cyclin essential for mitosis, present in S/G2/M phase (represents both Clb1 and Clb2 in the model). All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1. Level 1: transient level, used to monitor Clb2 degradation (activation of MITOSIS)
	3	● MASS & !Cdh1 & !Cdc20 & Mcm1	
Mcm1	1	● Clb2 & !(Sic1 | Cdc6)	● Chen et al.'s web site: Mcm1 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P11746 Mcm1 is a transcription factor controlling the expression of Clb2, Cdc20 and Swi5.
Mad2	1	● ORI & !SPN	● Chen et al.'s web site: Mad2 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P40958 Mad2 is a spindle assembly checkpoint protein. Mad stands for "Mitosis Arrest Deficient". Mad2 is a checkpoint protein that keeps Cdc20 inactive until the chromosomes are properly aligned.
Cdc20	1	● !Mad2 & Mcm1 & !(Clb2 & !(Sic1 | Cdc6))	● Chen et al.'s web site: Cdc20 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P26309 Cdc20 is the activator of the APC; protein involved in Clb2, Clb5 and Pds1 proteolysis, and is required for exit from mitosis. The APC core is considered constant throughout the cell cycle, and thus not represented.
	2	● !Mad2 & Mcm1 & Clb2 & !(Sic1 | Cdc6)	
PPX	1	● Pds1 & !Cdc20	● Chen et al.'s web site: PPX ● Chen et al., 2004 Unknown phosphatase hypothetised by Chen et al (2004).
Bub2-Bfa1	1	● ORI & !SPN	● Chen et al.'s web site: Bub2 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P26448 ● http: //www.uniprot.org/uniprot/P47113 Spindle assembly checkpoint proteins. Budding Uninhibited by Benomyl Checkpoint protein governed by spindle orientation.
Lte1	1	● (Clb2 & !(Sic1 | Cdc6)) | SPN	● Chen et al.'s web site: Lte1 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P07866 GTP-GDP exchange factor. Present in the bud, and an activator of Tem1. Located in the daughter cell. Activates Tem1 when the later enters the bud.

Tem1	1	● Lte1	● Chen et al.'s web site: Tem1 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P38987 GTP-binding protein and a component of the MEN pathway.
Cdc15	1	● (basal value)	● Chen et al.'s web site: Cdc15 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P27636 Kinase essential for late nuclear division and a component of the MEN pathway.
Net1	1	● !(((Cdc15:1 & Tem1) | Cdc15:2) & !Bub2-Bfa1) | PPX	● Chen et al.'s web site: Net1 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P47035 Nucleolar protein and a stoichiometric inhibitor of Cdc14.
Cdc14	1	● (basal value)	● Chen et al.'s web site: Cdc14 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/Q00684 Phosphatase required for exit of mitosis. Cdc14 reverses the CDK-mediated phosphorylation of Swi5, Sic1, Cdc6 and Cdh1.
Cdh1	1	● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:2)) & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | (Cln3 & Cln2)) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & !Net1:2)) & !((Clb5 & !Sic1 & Cln3 & ((Clb2 & !Cdc6) | Cln2)) | (Clb2:3 & !(Sic1 | Cdc6) & Cln3 & Cln2)) ● Cdc14:3 & !Net1:2 & !((Clb5 & !Sic1 & Cln3 & Clb2 & !Cdc6 & Cln2) | (Clb2:3 & !(Sic1 | Cdc6) & Cln3 & Cln2))	● Chen et al.'s web site: Cdh1 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P53197 Activator of APC-dependant proteolysis. Involved in Clb2 and Pds1 proteolysis. Phosphorylation by the CDKs inhibits the binding to the APC. The APC core is considered constant throughout the cell cycle, and thus not represented.
BUD	1	● (Cln2 | (Clb5 & !Sic1)) & !CYTOKINESIS	● Chen et al.'s web site: Budding ● Chen et al., 2004 Represents the formation of a new bud (BUD = 1).
ORI	1	● (Clb5 & !Sic1) | ((Clb2:2 | Clb2:3) & !(Sic1 | Cdc6)) | (ORI & (Clb5:3 | Clb2:3 | (Clb5:1 & !Sic1) | (Clb2:1 & !(Sic1 | Cdc6))))	● Chen et al.'s web site: DNA replication ● Chen et al., 2004 Origin of replication. Represents the onset of DNA synthesis (ORI=1). Activates the spindle assembly checkpoint through Mad2 and Bub2-Bfa1.
SPN	1	● Clb2 & !(Sic1 | Cdc6) & !CYTOKINESIS	● Chen et al., 2004 Spindle. Represents chromosomes alignment on the metaphase plate (SPN=1). Activation of this variable switches off the spindle assembly checkpoint through Mad2 and Bub2-Bfa1 inhibition.
Pds1	1	● (Mcm1 & MBF_SBF & !Cdc20) | ((Mcm1 | MBF_SBF) & !Cdh1 & !Cdc20)	● Chen et al.'s web site: Pds1 ● Chen et al., 2004 ● http: //www.uniprot.org/uniprot/P40316 Stoichiometric inhibitor of Esp1 that prevents sister chromatid separation. Degradation of Pds1 is necessary for release of Cdc14 from the nucleolus.
Esp1	1	● !Pds1	● Chen et al.'s web site: Esp1 ● Chen et al., 2004 Esp1 (separase) is a caspase, responsible for the cleavage of cohesin subunit that holds the sister chromatids together. Esp1 is sequestered and kept inactive by Pds1.

MASS	1	● !CYTOKINESIS	● Chen et al., 2004 Represents the mass of the cell. Boolean in this model. It is assumed that mass increase inpacts the cell cycle by increasing Cyclins concentration in the nucleus. In the wild-type, MASS has to cross the first threshold to initiate the cycle. Reset to zero when cytokinesis occurs. Priorities are used to enforce the immediacy of the transition
CYTOKINESIS	1	● MASS & Clb2:2 & !(Sic1 | Cdc6)	● Chen et al., 2004 In the model of Chen et al (2004), exit from mitosis occurs when Clb2 activity goes down a certain threshold. To represent this in the logical framework, the CYTOKINESIS variable is (pre-)activated (level 1) by high Clb2 (Clb2 = 2 or 3). Self-activating arrow keep tracks of high Clb2 pre-activation, allowing full activation when Clb2's activity is low. (i.e., making the difference between Clb2 increase and decrease.) Presence of the CKI has the same activating effect as low Clb2, and MASS is required to make sure the variable is inactivated after cell division. When CYTOKINESIS = 2, the MASS, BUD and SPN variables are reset to zero.
	2	● MASS & ((Clb2:1 & CYTOKINESIS) | (Clb2:2 & CYTOKINESIS & (Sic1 | Cdc6)))	

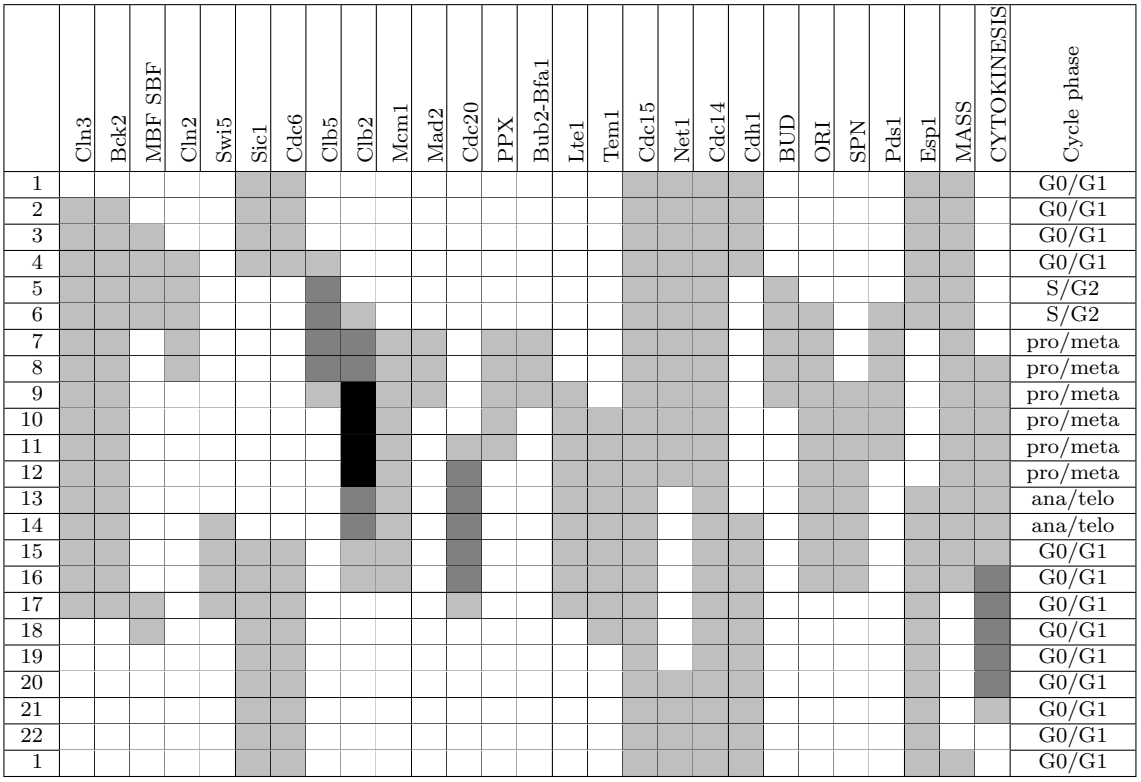
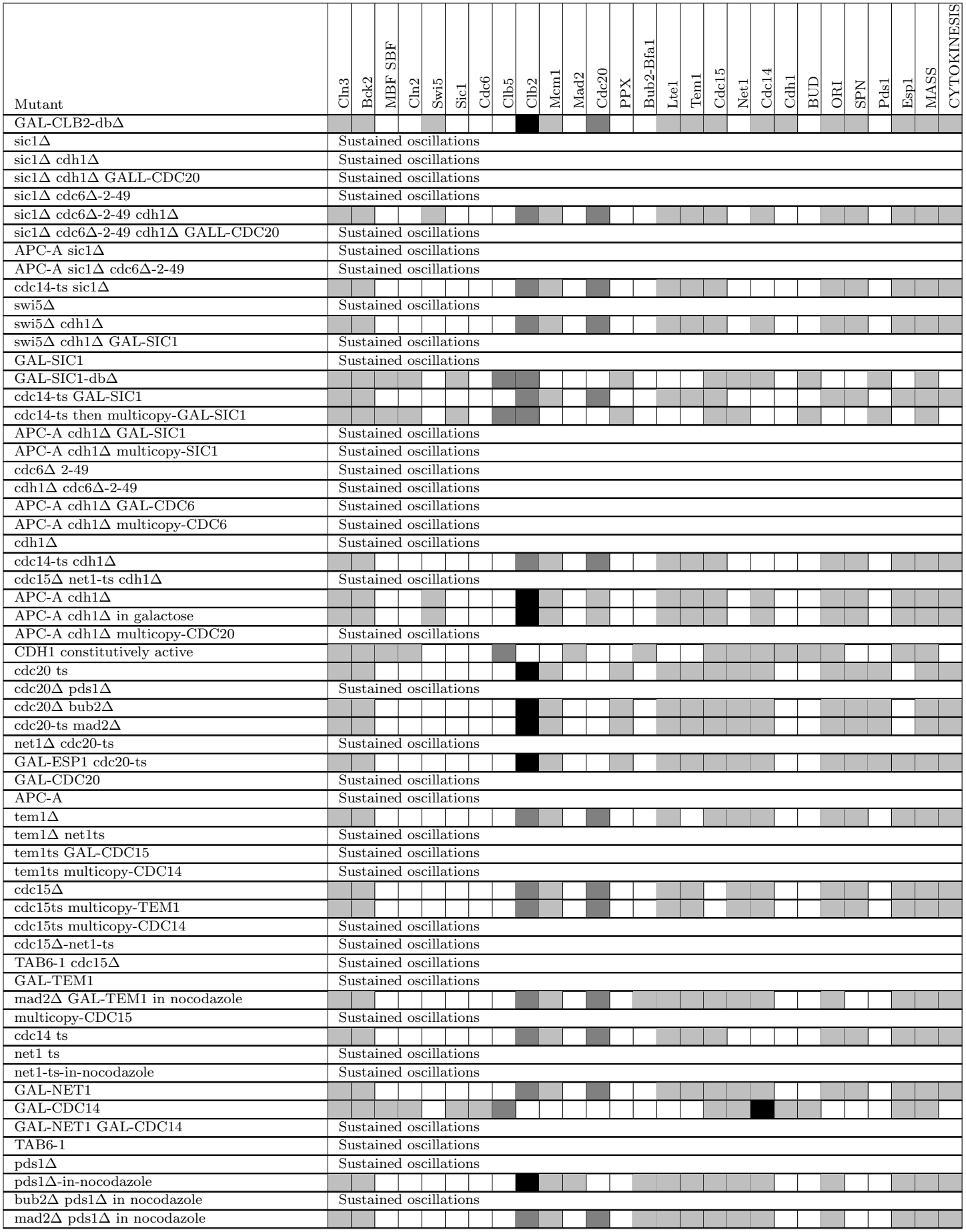


Figure 1: Simulation of the core model in the wild-type condition. The model is simulated using a set of synchronous priority classes (See model file for details) starting from the initial state corresponding to the first row of the table. Successive rows give the successive states obtained in the simulation. Colour code: white =0, lightgray=1, gray=2, black=3.

Mutant	Cln3	Bck2	MBF SBF	Cln2	Swi5	Sic1	Cdc6	Clb5	Clb2	Mcm1	Mad2	Cdc20	PPX	Bub2-Bfa1	Lte1	Tem1	Cdc15	Net1	Cdc14	Cdh1	BUD	ORI	SPN	Pds1	Esp1	MASS	CYTOKINESIS
cln1Δ cln2Δ	Sustained oscillations																										
cln1Δ cln2Δ sic1Δ	Sustained oscillations																										
cln1Δ cln2Δ cdh1Δ	Sustained oscillations																										
cln1Δ cln2Δ bck2Δ	Sustained oscillations																										
cln1Δ cln2Δ clb5Δ clb6Δ																											
cln1Δ cln2Δ cln3Δ																											
cln1Δ cln2Δ cln3Δ GAL-CLN2	Sustained oscillations																										
cln1Δ cln2Δ cln3Δ GAL-CLN3	Sustained oscillations																										
cln1Δ cln2Δ cln3Δ sic1Δ	Sustained oscillations																										
cln1Δ cln2Δ cln3Δ cdh1Δ	Sustained oscillations																										
cln1Δ cln2Δ cln3Δ multicopy-CLB5	Sustained oscillations																										
cln1Δ cln2Δ cln3Δ GAL-CLB5	Sustained oscillations																										
cln1Δ cln2Δ cln3Δ multicopy-BCK2	Sustained oscillations																										
cln1Δ cln2Δ cln3Δ GAL-CLB2																											
cln1Δ cln2Δ cln3Δ APC-ts																											
GAL-SIC1 cln1Δ cln2Δ																											
GAL-SIC1 GAL-CLN2 cln1Δ cln2Δ	Sustained oscillations																										
GAL-SIC1 cln1Δ cln2Δ cdh1Δ																											
GAL-SIC1 GAL-CLN2 cln1Δ cln2Δ cdh1Δ	Sustained oscillations																										
GAL-CLN2 cln1Δ cln2Δ cdh1Δ	Sustained oscillations																										
GAL-CLN2 cln1Δ cln2Δ	Sustained oscillations																										
cdc14-ts GAL-CLN2																											
cln3Δ	Sustained oscillations																										
cln3Δ bck2Δ																											
cln3Δ bck2Δ GAL-CLN2 cln1Δ cln2Δ	Sustained oscillations																										
cln3Δ bck2Δ multicopy CLN2																											
cln3Δ bck2Δ sic1Δ																											
GAL-CLN3	Sustained oscillations																										
bck2Δ	Sustained oscillations																										
multicopy BCK2	Sustained oscillations																										
clb5Δ clb6Δ	Sustained oscillations																										
TAB6-1 clb5Δ clb6Δ																											
cdc20Δ clb5Δ																											
cdc20Δ pds1Δ clb5Δ	Sustained oscillations																										
CLB2-dbΔ clb5Δ																											
CLB2-dbΔ clb5Δ on galactose																											
CLB5-dbΔ	Sustained oscillations																										
CLB5-dbΔ sic1Δ	Sustained oscillations																										
CLB5-dbΔ pds1Δ	Sustained oscillations																										
CLB5-dbΔ pds1Δ cdc20Δ	Sustained oscillations																										
GAL-CLB5	Sustained oscillations																										
GAL-CLB5 cdh1Δ	Sustained oscillations																										
GAL-CLB5 sic1Δ	Sustained oscillations																										
GAL-CLB5-dbΔ	Sustained oscillations																										
clb1Δ clb2Δ																											
CLB1 clb2Δ	Sustained oscillations																										
CLB1 clb2Δ cdh1Δ	Sustained oscillations																										
CLB1 clb2Δ pds1Δ	Sustained oscillations																										
TAB6-1 CLB1 clb2Δ	Sustained oscillations																										
CLB2-dbΔ																											
CLB2-dbΔ in galactose																											
CLB2-dbΔ multicopy-SIC1	Sustained oscillations																										
CLB2-dbΔ GAL-SIC1	Sustained oscillations																										
CLB2-dbΔ multicopy-cdc6	Sustained oscillations																										
CLB2-dbΔ GAL-CDC6	Sustained oscillations																										
GAL-CLB2	Sustained oscillations																										
multicopy GAL-CLB2																											
GAL-CLB2 sic1Δ	Sustained oscillations																										
GAL-CLB2 cdh1Δ																											
APC-A GAL-CLB2																											
swi5Δ GAL-CLB2																											


Mutant
Cln3
Bck2
MBF SBF
Cln2
Swi5
Sic1
Cdc6
Clb5
Clb2
Mcm1
Mad2
Cdc20
PPX
Bub2-Bfa1
Lte1
Tem1
Cdc15
Net1
Cdc14
Cdh1
BUD
ORI
SPN
Pds1
Esp1
MASS
CYTOKINESIS
GAL-CLB2-db∆
sic1∆
sic1∆ cdh1∆
sic1∆ cdh1∆ GALL-CDC20
sic1∆ cdc6∆-2-49
sic1∆ cdc6∆-2-49 cdh1∆
sic1∆ cdc6∆-2-49 cdh1∆ GALL-CDC20
APC-A sic1∆
APC-A sic1∆ cdc6∆-2-49
cdc14-ts sic1∆
swi5∆
swi5∆ cdh1∆
swi5∆ cdh1∆ GAL-SIC1
GAL-SIC1
GAL-SIC1-db∆
cdc14-ts GAL-SIC1
cdc14-ts then multicopy-GAL-SIC1
APC-A cdh1∆ GAL-SIC1
APC-A cdh1∆ multicopy-SIC1
cdc6∆ 2-49
cdh1∆ cdc6∆-2-49
APC-A cdh1∆ GAL-CDC6
APC-A cdh1∆ multicopy-CDC6
cdh1∆
cdc14-ts cdh1∆
cdc15∆ net1-ts cdh1∆
APC-A cdh1∆
APC-A cdh1∆ in galactose
APC-A cdh1∆ multicopy-CDC20
CDH1 constitutively active
cdc20 ts
cdc20∆ pds1∆
cdc20∆ bub2∆
cdc20-ts mad2∆
net1∆ cdc20-ts
GAL-ESP1 cdc20-ts
GAL-CDC20
APC-A
tem1∆
tem1∆ net1ts
tem1ts GAL-CDC15
tem1ts multicopy-CDC14
cdc15∆
cdc15ts multicopy-TEM1
cdc15ts multicopy-CDC14
cdc15∆-net1-ts
TAB6-1 cdc15∆
GAL-TEM1
mad2∆ GAL-TEM1 in nocodazole
multicopy-CDC15
cdc14 ts
net1 ts
net1-ts-in-nocodazole
GAL-NET1
GAL-CDC14
GAL-NET1 GAL-CDC14
TAB6-1
pds1∆
pds1∆-in-nocodazole
bub2∆ pds1∆ in nocodazole
mad2∆ pds1∆ in nocodazole
Sustained oscillations

Mutant	Cln3	Bck2	MBF SBF	Cln2	Swi5	Sic1	Cdc6	Clb5	Clb2	Mcm1	Mad2	Cdc20	PPX	Bub2-Bfa1	Lte1	Tem1	Cdc15	Net1	Cdc14	Cdh1	BUD	ORI	SPN	Pds1	Esp1	MASS	CYTOKINESIS
esp1 ts	Sustained oscillations																										
GAL-PDS1-dbΔ esp1-ts	Sustained oscillations																										
GAL-PDS1-dbΔ	Sustained oscillations																										
PDS1-dbΔ	Sustained oscillations																										
mad2Δ	Sustained oscillations																										
bub2Δ	Sustained oscillations																										
mad2Δ bub2Δ	Sustained oscillations																										
WT in nocodazole	1	1	0	0	0	0	0	0	3	1	1	0	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1
mad2Δ in nocodazole	1	1	0	0	0	0	0	0	2	1	0	2	0	1	1	1	1	1	1	0	0	1	0	0	1	1	1
bub2Δ in nocodazole	1	1	0	0	0	0	0	0	3	1	1	0	1	0	1	1	1	1	1	0	0	1	0	1	0	1	1
bub2Δ mad2Δ in nocodazole	Sustained oscillations																										
ppxΔ	Sustained oscillations																										
GAL-PPX	Sustained oscillations																										
CLB1 clb2Δ PDS1-dbΔ	Sustained oscillations																										

Table 1: Core model: stable states in the mutant conditions. Colour code: white=0, lightgray=1, gray=2, black=3. See model file for mutant definition and description

Description of the model "MorphogenesisCheckPoint"

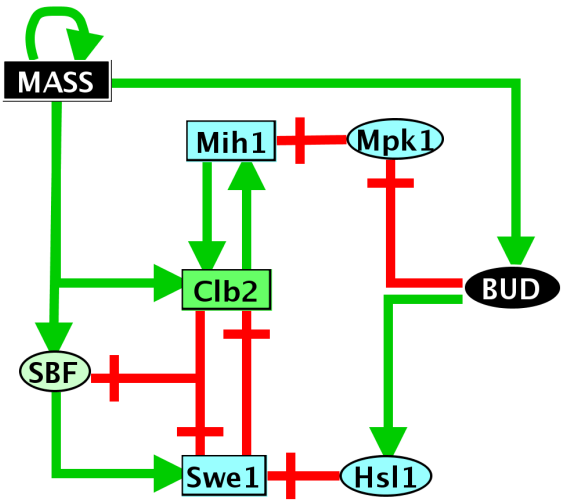
Annotation

- Ciliberto et al., 2003
- Harrison et al., 2001

Leaning on the differential model published by Ciliberto et al (2003), we have delineated a logical model for the regulatory network monitoring the formation of the bud (BUD), called the morphogenetic checkpoint (MCP).

This model accounts for the fact that the cell cycle is temporary blocked in G2 phase in case of budding defect. This G2 blocking can be bypassed in the presence of high Clb2 activity level, which correlates with the growth of the cell. Consequently, nuclear division occurs without cell division, thereby giving rise to dinucleate cells. To properly model this phenomenon, we have considered a second threshold for the MASS component, which denotes a mass large enough to bypass G2 arrest.

This logical model recapitulates the wild-type and knockout phenotypes considered by Ciliberto et al (2003), as well as three additional knockout mutants described in Harrison et al (2001). As this model focuses on Clb2 activation depending on the mass of the cell, its dynamics is analysed in terms of stable states for each possible value of MASS.



Nodes

ID	Val	Logical function	Comment
BUD	1	● MASS	Mass acts indirectly on BUD, through Cln2, Cln3 and Clb5 which are not represented here for the sake of simplicity. In this model mass is thus required to activate BUD. Hsl1 and Hsl7 are recruited and activated at the bud neck, where they can inactivate Swe1. When BUD formation fails, a MAPK pathway is thought to inhibit Mih1.
SBF	1	● MASS & !Clb2	Transcription factor, activated by the G1 cyclins, and thus indirectly by mass (cf yeast cell cycle core model)

			Activates the transcription of SBF activates the transcription of Swe1
Swe1	1	● SBF & ((Clb2 & !Hsl1) | (Hsl1 & ! Clb2))	Kinase. Budding yeast homologue of Wee1. Activated by the MBF and inhibited by Hsl1 and Hsl7. Inhibits Clb2 activity by phosphorylating its Cdk partner Cdc28 on tyrosine-19.
	2	● SBF & !(Hsl1 | Clb2)	
Mih1	1	● (Mpk1 & Clb2) | (!Mpk1 & !Clb2)	Phosphatase.Budding yeast homologue of Cdc25. Activates Clb2 by removing an inhibitory phosphate from Cdc28 on tyrosine-19.
	2	● !Mpk1 & Clb2	
Clb2	1	● (MASS:1 & ((Swe1:1 & !Mih1:2) | (Swe1:2 & Mih1:1))) | (MASS & Swe1:2 & !Mih1)	B-type cyclin essential for mitosis, present in S/G2/M phase (represents both Clb1 and Clb2 in the model).
	2	● (MASS:1 & (!Swe1 | Mih1:2)) | (MASS:2 & (!Swe1:2 | Mih1))	
MASS	1	● MASS:1	Represents the mass of the cell. Considered an input in this model. We assume that mass increase inpacts the cell cycle by increasing Cyclins concentration in the nucleus. In the wild-type, MASS has to cross the first threshold to initiate the cycle. MASS has to cross the second threshold to overcome the morphogenesis checkpoint inhibition of Clb2.
	2	● MASS:2	
Mpk1	1	● !BUD	Kinase. Budding yeast homologue of Wee1. Activated by the MBF and inhibited by Hsl1 and Hsl7. Inhibits Clb2 activity by phosphorylating its Cdk partner Cdc28 on tyrosine-19.
Hsl1	1	● BUD	Activation depends on bud presence. Inhibits Swe1 (mecanism is unclear.)

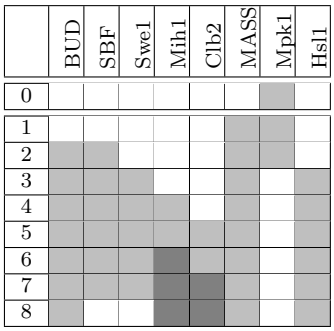


Figure 2: Simulation of the morphogenesis checkpoint model in the wild-type condition. The model is simulated using a set of synchronous priority classes (see model file for details). When MASS = 0, the model reaches a unique stable state (row 0 in the table). Increasing MASS to level 1 (row 1) triggers a cascade of transitions leading to a stable state (row 8 in the table) with active Clb2. If budding were impaired, starting from the same state 1 the simulation would lead to a stable state with inactive or low Clb2 (i.e. Clb2 = 0 or 1, see Table 2). From row 1 to 8, successive rows give the successive states obtained in the simulation. Colour code: white =0, lightgray=1, gray=2, black=3.

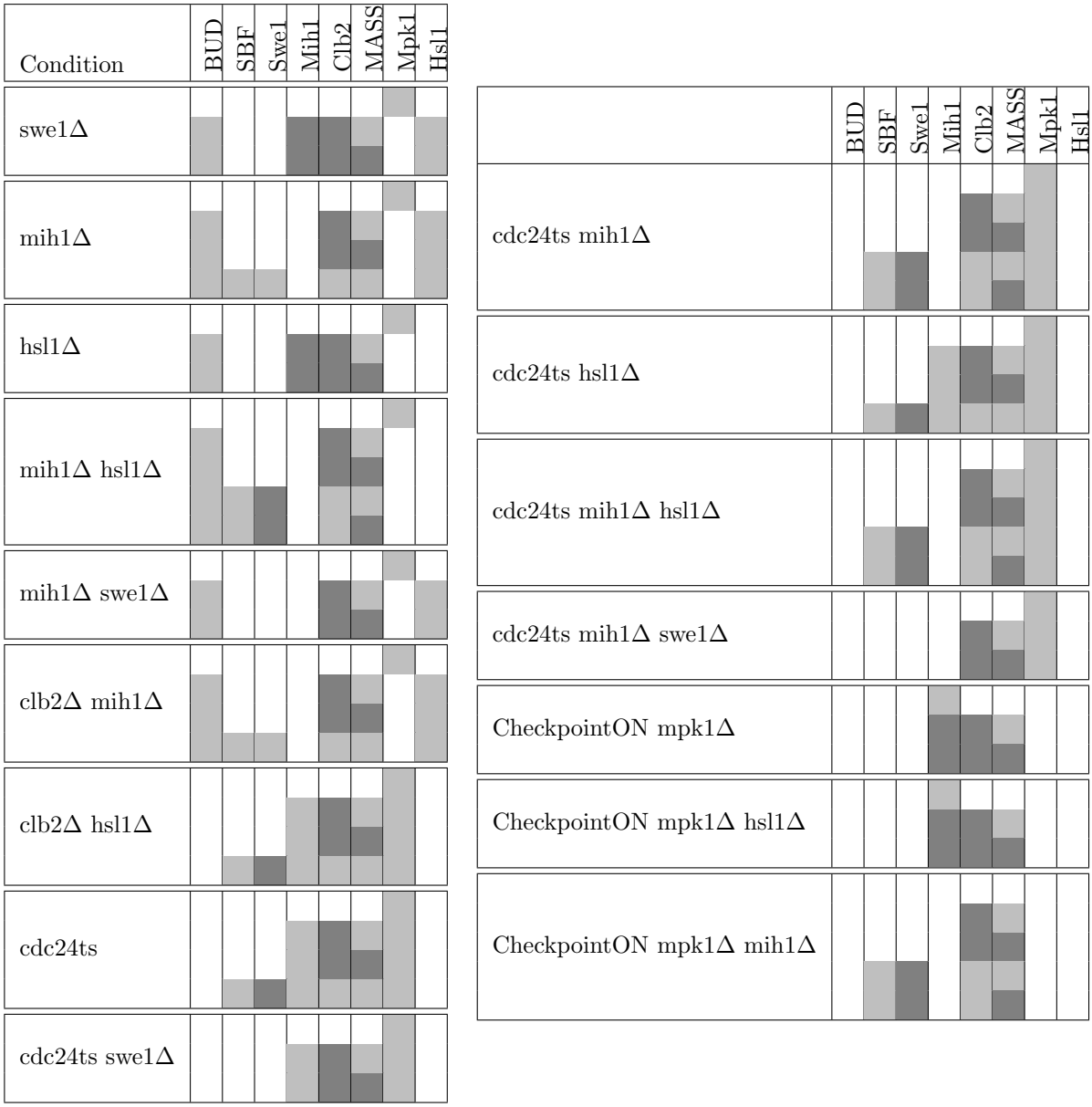


Table 2: MCP model: stable states in the mutant conditions. Reachability of the stable states can be assessed through simulation. A general observation is that, when several stable states exist for a given value of MASS, only the one with inactive or low Clb2 (i.e.e Clb2 = 0 or 1) is reachable under normal cycling conditions. More precisely, the state with high Clb2 (i.e Clb2=2) can only be reached if Clb2 is activated before the Swe1, which does not normally happen. The cdc24ts mutations correspond to an impairment of BUD formation. Colour code: white=0, lightgray=1, gray=2, black=3. See model file for mutant definition and description

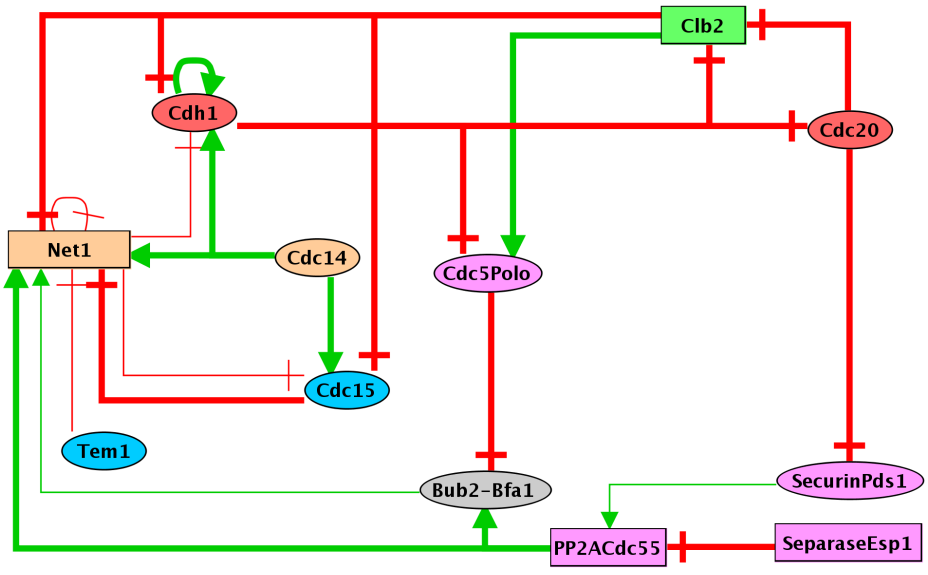
Description of the model "Exit"

Annotation

- Queralt et al., 2006

This refined model of the budding Yeast exit module is principally based on the article by Queralt et al (2006).
According to their model, when securin is degraded by Cdc20 at anaphase onset, free separase cleaves the cohesin that maintains sister chromatids together and downregulates PP2ACdc55, a phosphatase that opposes Net1 phosphorylation by Clb2.
Downregulation of PP2ACdc55 also participates in MEN activation by facilitating phosphorylation of Bfa1 by the Polo-like Cdc5 kinase.
This mechanism accounts for the two-steps release of the Cdc14 phosphatase from its competitive inhibitor Net1.
The present logical model qualitatively recapitulates the behaviours of the wild-type situation and of nine reported mutants.
In each case, we have checked whether a stable state with active Cdh1 can be reached from the state corresponding to anaphase.

In simulation we test the model for mitotic exit, starting from a state corresponding to metaphase, with high Clb2, low Cdh1 and sister chromatids attached. In the wild-type situation, this leads to a single stable state corresponding to G0/G1 phase, with low Clb2 and high Cdh1. Stable state analysis confirms that this is the only stable state in the wild-type case.



Nodes

ID	Val	Logical function	Comment
Clb2	1	● !Cdh1 & Cdc20	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P24869 B-type cyclin essential for mitosis, present in S/G2/M phase (represents both Clb1 and Clb2 in the model). Activates CDK-dependent inhibitory phosphorylation of Net1, Cdc15 and Cdh1. Two levels to account for partial inhibition by Cdc20 (full inhibition only by Cdh1).
	2	● !Cdc20 & !Cdh1	
Cdc20	1	● !Cdh1	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P26309 Activator of the APC, involved in Clb2 and Pds1 proteolysis, and required for exit from mitosis. The APC core is considered constant throughout the cell cycle, and thus not represented.
SecurinPds1	1	● !Cdc20	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P40316 Stoichiometric inhibitor of Esp1 that prevents sister chromatid separation. Degradation of Pds1 is necessary for release of Cdc14 from the nucleolus.
SeparaseEsp1	1	● (basal value)	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/Q03018 Separase protein required for sister chromatid separation.
PP2ACdc55	1	● !(!SeparaseEsp1 | (SeparaseEsp1:1 & SecurinPds1))	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/Q00362 ● http: //www.uniprot.org/uniprot/P23594 Type 2A phosphatase. Queralt et al. (2006) refer to PP2ACdc55 as a complex consisting of Tpd3 (scaffold protein), either Pph21 or Pph22 (catalytic subunit), and Cdc55 (regulatory subunit, provide substrate specificity). In metaphase, PP2ACdc55 prevents premature Net1 phosphorylation and Cdc14 activation. At anaphase onset, PP2ACdc55 activity is downregulated in a separase-dependent manner. (Queralt et al., 2006)
	2	● !SeparaseEsp1 | (SeparaseEsp1:1 & SecurinPds1)	
Cdc5Polo	1	● Clb2 & !Cdh1	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P32562 Kinase, phosphorylates Bfa1. Essential in budding yeast for Cdc14 activation. ((Jaspersen et al., 1998; Lee et al., 2001; Stegmeier and Amon, 2004), cited in Queralt et al.)
Bub2-Bfa1	1	● PP2ACdc55 | !Cdc5Polo	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P26448 ● http: //www.uniprot.org/uniprot/P47113 Spindle assembly checkpoint proteins. "Budding Uninhibited by Benomyl" Checkpoint protein governed by spindle orientation. Cdc5 phosphorylates Bfa1, causing the dissociation of the complex.
Tem1	1	● (basal value)	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P38987 GTP-binding protein and a component of the MEN pathway.
Cdc15	1	● !Clb2 | (Cdc14 & !Net1)	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P27636 Kinase essential for late nuclear division and a component of the MEN pathway.
Net1	1	● ((Cdc14 & Net1 & !Clb2 & ! PP2ACdc55) | (PP2ACdc55:1 & ((! Cdc14 & Clb2:1) | (Cdc14 &	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P47035

		Clb2:1)))) & !(Cdc15 & Tem1 & ! Bub2-Bfa1)	Nucleolar protein and a stoichiometric inhibitor of Cdc14. inhibited by phosphorylation by Cdk and Cdc5/Polo.
	2	● ((((Cdc14 & !Net1) | PP2ACdc55) & !Clb2) | PP2ACdc55:2) & !(Cdc15 & Tem1 & !Bub2-Bfa1)	
Cdc14	1	● (basal value)	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/Q00684 Phosphatase required for exit of mitosis. Cdc14 reverses the CDK-mediated phosphorylation of Net1, Cdc15 and Cdh1.
Cdh1	1	● (!Clb2:2 & Cdc14 & !Net1) | (Cdh1 & !Clb2)	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P53197 Activator of APC-dependent proteolysis; involved in Clb2 and Cdc5 proteolysis. Phosphorylation by the CDKs (Clb2) inhibits the binding to the APC. The APC core is considered constant throughout the cell cycle, and thus not represented.

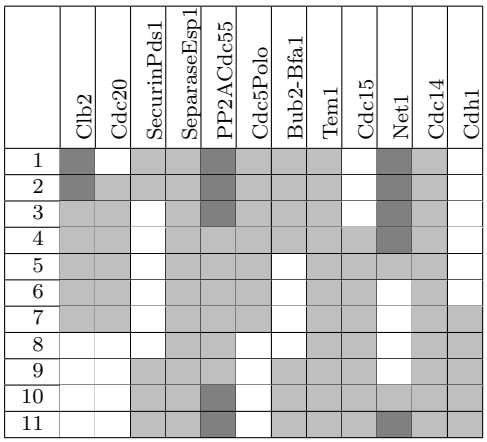


Figure 3: Simulation of the exit model in the wild-type condition. The model is simulated synchronously starting from an initial state (first row of the table) corresponding to late metaphase, just before activation of Cdc20. Successive rows give the successive states obtained in the simulation, leading to a stable state corresponding to a G0 or G1 state with low Clb2 and active Cdh1. This state is the only stable state in the wild-type condition. Colour code: white =0, lightgray=1, gray=2, black=3.

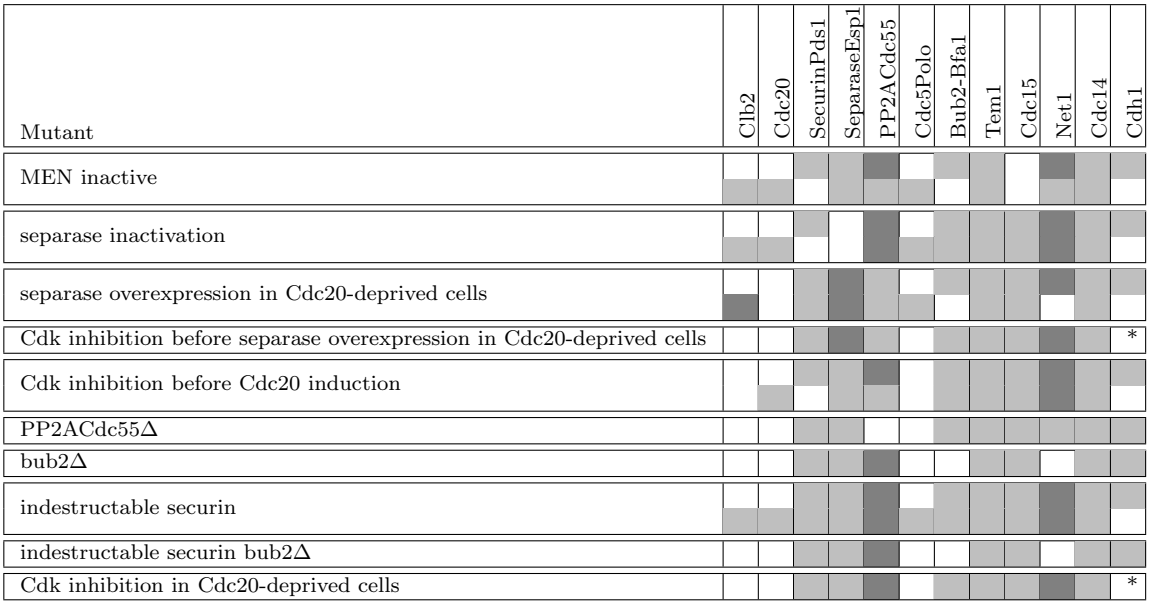


Table 3: Exit model: stable states in the mutant conditions. Reachability of the stable states can be assessed through simulation. In this model of mitotic exit, the G1 state (low Clb2, high Cdh1) is stable under all conditions. However it may not be reachable from an initial metaphase state, in which case alternative steady states appear. Colour code: white=0, lightgray=1, gray=2, black=3; star = every possible value. See model file for mutant definition and description.

Description of the model "Coupled_model"

Annotation

- http: //mpf.biol.vt.edu/research/budding_yeast_model/pp/index.php
- Chen et al., 2004
- Ciliberto et al., 2003
- Queralt et al., 2006

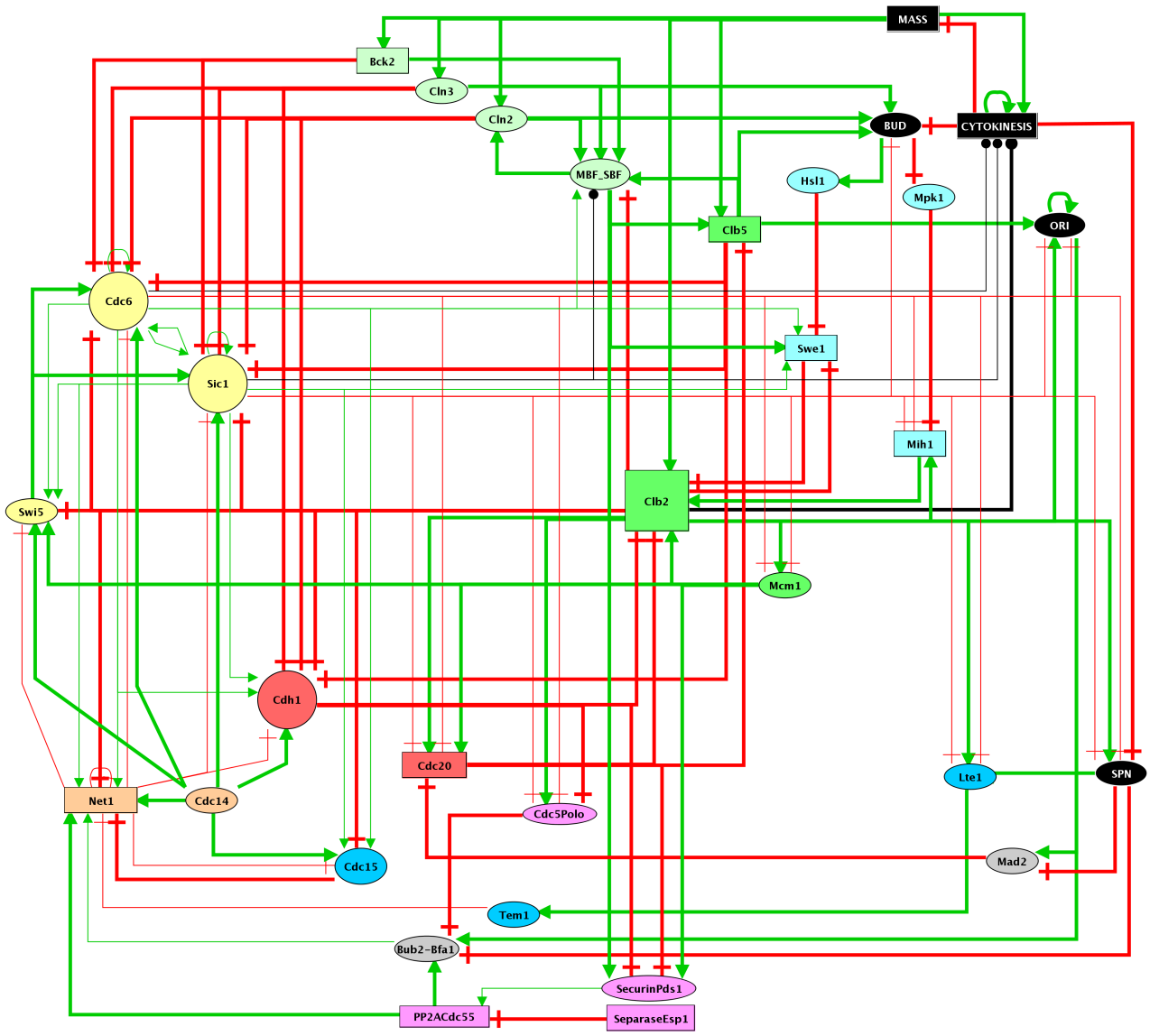
This logical model encompasses the core model (adapted from Chen et al., 2004), the morphogenesis checkpoint (MCP) module (adapted from Ciliberto et al., 2003), and a revised version of the network controlling mitotic exit (exit module, adapted from Queralt et al., 2006).

The coupling has been done as described in Fauré et al.

The resulting model preserve the dynamical properties of the original modules, while enabling the simulation of perturbations involving components that belong to several modules.

Cell viability rule in terms of sequence of events, as in the core model: firing of the origins of replication (ORI goes up), spindle alignment (SPN goes up), separase activation (or inhibition of the securin, Pds1 goes down), division (MITOSIS goes up to level 2) after the formation of a bud (BUD must have reached value 1, although it goes down afterwards before cell division), and origin relicensing (ORI goes down).

Cell cycle phases:
G0/G1: low Clb5 and Clb2 activity (either <2 or sequestered by Sic1 / Cdc6)
S/G2: high Clb5 activity (i.e. not sequestered by the CKI), low Clb2.
M: high Clb2 activity
prophase / metaphase: low Esp1 activity (high Pds1)
anaphase / telophase: high Esp1 activity (low Pds1) (*)
(*) In the exit module, overexpressed Esp1 (Esp1=2) can overcome Pds1 inhibition.



Nodes

ID	Val	Logical function	Comment
Cln3	1	● MASS	● Chen et al.'s web site: Cln3 ● Chen et al., 2004 G1-cyclin, initiating Start events. All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1.
Bck2	1	● MASS	● Chen et al.'s web site: Bck2 ● Chen et al., 2004 Protein initiating Start events.
MBF_SBF	1	● !(Clb2 & !(Sic1 | Cdc6)) & (Bck2 | Cln3 | Cln2 | (Clb5 & !Sic1))	● Chen et al.'s web site: MBF ● Chen et al.'s web site: SBF ● Chen et al., 2004 ● Ciliberto et al., 2003 Transcription factors. The SBF controlls activates the transcription of Cln2, and the MBF that of Clb5.
Cln2	1	● MASS & MBF_SBF	● Chen et al.'s web site: Cln2 ● Chen et al., 2004

			Cyclin involved in budding (represents both Cln1 and Cln2 in the model). All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1.
Swi5	1	● (Mcm1 & !(Clb2 & !(Sic1 | Cdc6))) | (Mcm1 & Clb2 & !(Sic1 | Cdc6) & ((Cdc14:1 & !Net1) | (Cdc14:2 & !Net1:3)))	● Chen et al.'s web site: Swi5 ● Chen et al., 2004 Transcription factor for Sic1 and Cdc6.
Sic1	1	● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:3)) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2 | ((Clb2 | Clb5) & (Cln3 | Bck2:2)) | (Clb5 & Clb2) | (Clb5:3 & Bck2)) ● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:3)) & Swi5 & !((Clb2:3 & !(Sic1 | Cdc6)) | (((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1)) & ((Cln2 & (Cln3 | Bck2)) | (Cln3 & Bck2))) | (((Clb2 & Clb5) | Clb2:3 | Clb5:3) & Cln2 & (Cln3 | Bck2)) | (Clb2:3 & Clb5:3)) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & Swi5 & !(((Clb5 & Clb2 & !(Sic1 | Cdc6) & Cln2 & Cln3 & Bck2) | (Clb2:3 & !(Sic1 | Cdc6))) & (Clb5 | Cln2 | (Cln3 & Bck2))) ● Cdc14:3 & !Net1:3 & !Swi5 & !(Clb5 & Clb2 & Cln2 & Cln3 & Bck2) ● Cdc14:3 & !Net1:3 & Swi5 & !(Cln3 & Bck2 & Cln2 & Clb5 & !Sic1 & Clb2 & !(Sic1 | Cdc6))	● Chen et al.'s web site: Sic1 ● Chen et al., 2004 Stoichiometric inhibitor of Cdc28/Clb2 and Cdc28/Clb5. Arrows directed towards Clb5 and Clb2's targets represent sequestration..
Cdc6	1	● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:3)) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2 | ((Clb2 | Clb5) & (Cln3 | Bck2:2)) | (Clb5 & Clb2) | (Clb5:3 & Bck2)) ● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:3)) & Swi5 & !((Clb2:3 & !(Sic1 | Cdc6)) | (((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1)) & ((Cln2 & (Cln3 | Bck2)) | (Cln3 & Bck2))) | (((Clb2 & Clb5) | Clb2:3 | Clb5:3) & Cln2 & (Cln3 | Bck2)) | (Clb2:3 & Clb5:3)) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & !Swi5 & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | Cln2) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & (!Net1 | Net1:1))) & Swi5 & !(((Clb5 & Clb2 & !(Sic1 | Cdc6) & Cln2 & Cln3 & Bck2) | (Clb2:3 & !(Sic1 | Cdc6))) & (Clb5 | Cln2 | (Cln3 & Bck2))) ● Cdc14:3 & !Net1:3 & !Swi5 & !(Clb5 & Clb2 & Cln2 & Cln3 & Bck2) ● Cdc14:3 & !Net1:3 & Swi5 & !(Cln3 & Bck2 & Cln2 & Clb5 & !Sic1 & Clb2 & !(Sic1 | Cdc6))	● Chen et al.'s web site: Cdc6 ● Chen et al., 2004 Stoichiometric inhibitor of Cdc28/Clb2, but not of Cdc28/Clb5. Also a licencing factor for DNA replication. Arrows directed towards Clb2's targets represent sequestration.
Clb5	1	● MASS & MBF_SBF & Cdc20	● Chen et al.'s web site: Clb5 ● Chen et al., 2004 B-type cyclins appearing late in G1, involved in DNA synthesis (represents both Clb5 and Clb6 in the model). All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1.
	2	● MASS & MBF_SBF & !Cdc20	
Mpk1	1	● !BUD	● Ciliberto et al., 2003

			Kinase. Budding yeast homologue of Wee1. Activated by the MBF and inhibited by Hsl1 and Hsl7. Inhibits Clb2 activity by phosphorylating its Cdk partner Cdc28 on tyrosine-19.
Mih1	1	● (Mpk1 & Clb2 & !(Sic1 | Cdc6)) | (!Mpk1 & (! Clb2 | Sic1 | Cdc6))	● Ciliberto et al., 2003 Phosphatase.Budding yeast homologue of Cdc25. Activates Clb2 by removing an inhibitory phosphate from Cdc28 on tyrosine-19.
	2	● !Mpk1 & Clb2 & !(Sic1 | Cdc6)	
Hsl1	1	● BUD	● Ciliberto et al., 2003 Activation depends on bud presence. Inhibits Swe1 (mecanism is unclear.)
Swe1	1	● MBF_SBF & ((Clb2 & !(Sic1 | Cdc6) & !Hsl1) | (Hsl1 & !Clb2) | Sic1 | Cdc6)	● Ciliberto et al., 2003 Kinase. Budding yeast homologue of Wee1. Activated by the MBF and inhibited by Hsl1 and Hsl7. Inhibits Clb2 activity by phosphorylating its Cdk partner Cdc28 on tyrosine-19.
	2	● MBF_SBF & !(Hsl1 | (Clb2 & !(Sic1 | Cdc6)))	
Clb2	1	● ((MASS:1 & ((Swe1:1 & !Mih1:2) | (Swe1:2 & Mih1:1))) | (MASS & Swe1:2 & !Mih1)) & ! Cdh1 & (!Cdc20 | (Cdc20:2 & Mcm1))	● Chen et al.'s web site: Clb2 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P24869 ● Ciliberto et al., 2003 B-type cyclin essential for mitosis, present in S/G2/M phase (represents both Clb1 and Clb2 in the model). All cyclins (with different efficiencies) activate CDK-dependant inhibitory phosphorylation of Sic1, Cdc6 and Cdh1. Activates CDK-dependent inhibitory phosphorylation of Net1, Cdc15 and Cdh1. Level 1: transient level, used to monitor Clb2 degradation (activation of MITOSIS)
	2	● ((MASS:1 & (!Swe1 | Mih1:2)) | (MASS:2 & (! Swe1:2 | Mih1))) & !Cdh1 & ((!Cdc20 & ! Mcm1) | (Cdc20:2 & Mcm1))	
	3	● ((MASS:1 & (!Swe1 | Mih1:2)) | (MASS:2 & (! Swe1:2 | Mih1))) & !Cdh1 & !Cdc20 & Mcm1	
Mcm1	1	● Clb2 & !(Sic1 | Cdc6)	● Chen et al.'s web site: Mcm1 ● Chen et al., 2004 Transcription factor for Clb2, Cdc20 and Swi5.
Mad2	1	● ORI & !SPN	● Chen et al.'s web site: Mad2 ● Chen et al., 2004 Spindle assembly checkpoint protein. "Mitosis Arrest Deficient" -- checkpoint protein that keeps Cdc20 inactive until the chromosomes are properly aligned.
Cdc20	1	● !Mad2 & Mcm1 & !(Clb2 & !(Sic1 | Cdc6))	● Chen et al.'s web site: Cdc20 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P26309 Activator of the APC; protein involved in Clb2, Clb5 and Pds1 proteolysis, and required for exit from mitosis. The APC core is considered constant throughout the cell cycle, and thus not represented.
	2	● !Mad2 & Mcm1 & Clb2 & !(Sic1 | Cdc6)	
Cdc5Polo	1	● Clb2 & !(Sic1 | Cdc6) & !Cdh1	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P32562 Kinase, phosphorylates Bfa1. Essential in budding yeast for Cdc14 activation. ((Jaspersen et al., 1998; Lee et al., 2001; Stegmeier and Amon, 2004), cited in Queralt et al.)
PP2ACdc55	1	● !(!SeparaseEsp1 | (SeparaseEsp1:1 & SecurinPds1))	● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/Q00362 ● http: //www.uniprot.org/uniprot/P23594 Type 2A phosphatase. Queralt et al. (2006) refer to PP2ACdc55 as a complex consisting of Tpd3 (scaffold protein), either Pph21 or Pph22 (catalytic subunit), and Cdc55 (regulatory subunit,
	2	● !SeparaseEsp1 | (SeparaseEsp1:1 & SecurinPds1)	

			provide substrate specificity). In metaphase, PP2ACdc55 prevents premature Net1 phosphorylation and Cdc14 activation. At anaphase onset, PP2ACdc55 activity is downregulated in a separase-dependent manner. (Queralt et al., 2006)
Bub2-Bfa1	1	● ORI & (!SPN | PP2ACdc55 | !Cdc5Polo)	● Chen et al.'s web site: Bub2 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P26448 ● http: //www.uniprot.org/uniprot/P47113 Spindle assembly checkpoint proteins. Checkpoint protein governed by spindle orientation. Cdc5 phosphorylates Bfa1, causing the dissociation of the complex.
Lte1	1	● (Clb2 & !(Sic1 | Cdc6)) | SPN	● Chen et al.'s web site: Lte1 ● Chen et al., 2004 GTP-GDP exchange factor. Present in the bud, and an activator of Tem1. Located in the daughter cell. Activates Tem1 when the later enters the bud.
Tem1	1	● Lte1	● Chen et al.'s web site: Tem1 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P38987 GTP-binding protein and a component of the MEN pathway.
Cdc15	1	● !(Clb2 & !(Sic1 | Cdc6)) | (Cdc14 & !Net1)	● Chen et al.'s web site: Cdc15 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P27636 Kinase essential for late nuclear division and a component of the MEN pathway.
Net1	1	● ((Cdc14 & Net1 & !(Clb2 & !(Sic1 | Cdc6)) & !PP2ACdc55) | (PP2ACdc55:1 & ((!Cdc14 & Clb2:2 & !(Sic1 | Cdc6)) | (Cdc14 & Clb2:2 & !(Sic1 | Cdc6))))) & !(((Cdc15:1 & Tem1) | Cdc15:2) & !Bub2-Bfa1)	● Chen et al.'s web site: Net1 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P47035 Nucleolar protein and a stoichiometric inhibitor of Cdc14. Inhibited by phosphorylation by Cdk and Cdc5/Polo. aka: Cfi1
	2	● ((((Cdc14 & !Net1) | PP2ACdc55) & !(Clb2 & !(Sic1 | Cdc6))) | PP2ACdc55:2) & !(((Cdc15:1 & Tem1) | Cdc15:2) & !Bub2-Bfa1)	
Cdc14	1	● (basal value)	● Chen et al.'s web site: Cdc14 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/Q00684 Phosphatase required for exit of mitosis. Cdc14 reverses the CDK-mediated phosphorylation of Swi5, Sic1, Cdc6 and Cdh1.
Cdh1	1	● (!Cdc14 | (Cdc14:1 & Net1) | ((Cdc14:2 | Cdc14:3) & Net1:3)) & !((Clb2 & !(Sic1 | Cdc6)) | (Clb5 & !Sic1) | (Cln3 & Cln2)) ● ((Cdc14:1 & !Net1) | (Cdc14:2 & !Net1:3)) & !((Clb5 & !Sic1 & Cln3 & ((Clb2 & !Cdc6) | Cln2)) | (Clb2:3 & !(Sic1 | Cdc6) & Cln3 & Cln2)) ● Cdc14:3 & !Net1:3 & !((Clb5 & !Sic1 & Cln3 & Clb2 & !Cdc6 & Cln2) | (Clb2:3 & !(Sic1 | Cdc6) & Cln3 & Cln2))	● Chen et al.'s web site: Cdh1 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P53197 Activator of APC-dependent proteolysis; involved in Clb2 and Pds1 proteolysis. Phosphorylation by the CDKs inhibits the binding to the APC. The APC core is considered constant throughout the cell cycle, and thus not represented.
BUD	1	● (Cln2 | (Clb5 & !Sic1)) & !CYTOKINESIS	● Chen et al.'s web site: BUD ● Chen et al., 2004

			● Ciliberto et al., 2003 Represents the formation of a new bud when BUD = 1.
ORI	1	● (Clb5 & !Sic1) | ((Clb2:2 | Clb2:3) & !(Sic1 | Cdc6)) | (ORI & (Clb5:3 | Clb2:3 | (Clb5:1 & !Sic1) | (Clb2:1 & !(Sic1 | Cdc6))))	● Chen et al.'s web site: ORI ● Chen et al., 2004 Origin of replication. Represents the onset of DNA synthesis. Activates the spindle assembly checkpoint through Mad2 and Bub2-Bfa1.
SPN	1	● Clb2 & !(Sic1 | Cdc6) & !CYTOKINESIS	● Chen et al., 2004 Spindle. Represents chromosomes alignment on the metaphase plate. Activation of this variable switches off the spindle assembly checkpoint through Mad2 and Bub2-Bfa1 inhibition.
SecurinPds1	1	● (Mcm1 & MBF_SBF & !Cdc20) | ((Mcm1 | MBF_SBF) & !Cdh1 & !Cdc20)	● Chen et al.'s web site: Pds1 ● Chen et al., 2004 ● Queralt et al., 2006 ● http: //www.uniprot.org/uniprot/P40316 Stoichiometric inhibitor of Esp1 that prevents sister chromatid separation. Degradation of Pds1 is necessary for release of Cdc14 from the nucleolus.
SeparaseEsp1	1	● (basal value)	● Chen et al.'s web site: Esp1 ● Chen et al., 2004 Esp1 (separase) is a caspase, responsible for the cleavage of cohesin subunit that holds the sister chromatids together. Esp1 is sequestered and kept inactive by Pds1.
MASS	2	● !CYTOKINESIS	● Chen et al., 2004 ● Ciliberto et al., 2003 Represents the mass of the cell. Boolean in this model. We assume that mass increase inpacts the cell cycle by increasing Cyclins concentration in the nucleus. In the wild-type, MASS has to cross the first threshold to initiate the cycle. Reset to zero when cytokinesis occurs. (Priorities are used to enforce the immediacy of the transition)
CYTOKINESIS	1	● MASS & Clb2:2 & !(Sic1 | Cdc6)	● Chen et al., 2004 In Chen et al.'s model, exit from mitosis occurs when Clb2's activity goes down a certain threshold. To represent this in the logical framework, the CYTOKINESIS variable is (pre-)activated (level 1) by high Clb2 (Clb2 = 2 or 3). Self-activating arrow keep tracks of high Clb2 pre-activation, allowing full activation when Clb2's activity is low. (i.e., making the difference between Clb2 increase and decrease.) Presence of the CKI has the same activating effect as low Clb2, and MASS is required to make sure the variable is inactivated after cell division. When CYTOKINESIS = 2, the MASS, BUD and SPN variables are reset to zero.
	2	● MASS & ((Clb2:1 & CYTOKINESIS) | (Clb2:2 & CYTOKINESIS & (Sic1 | Cdc6)))	

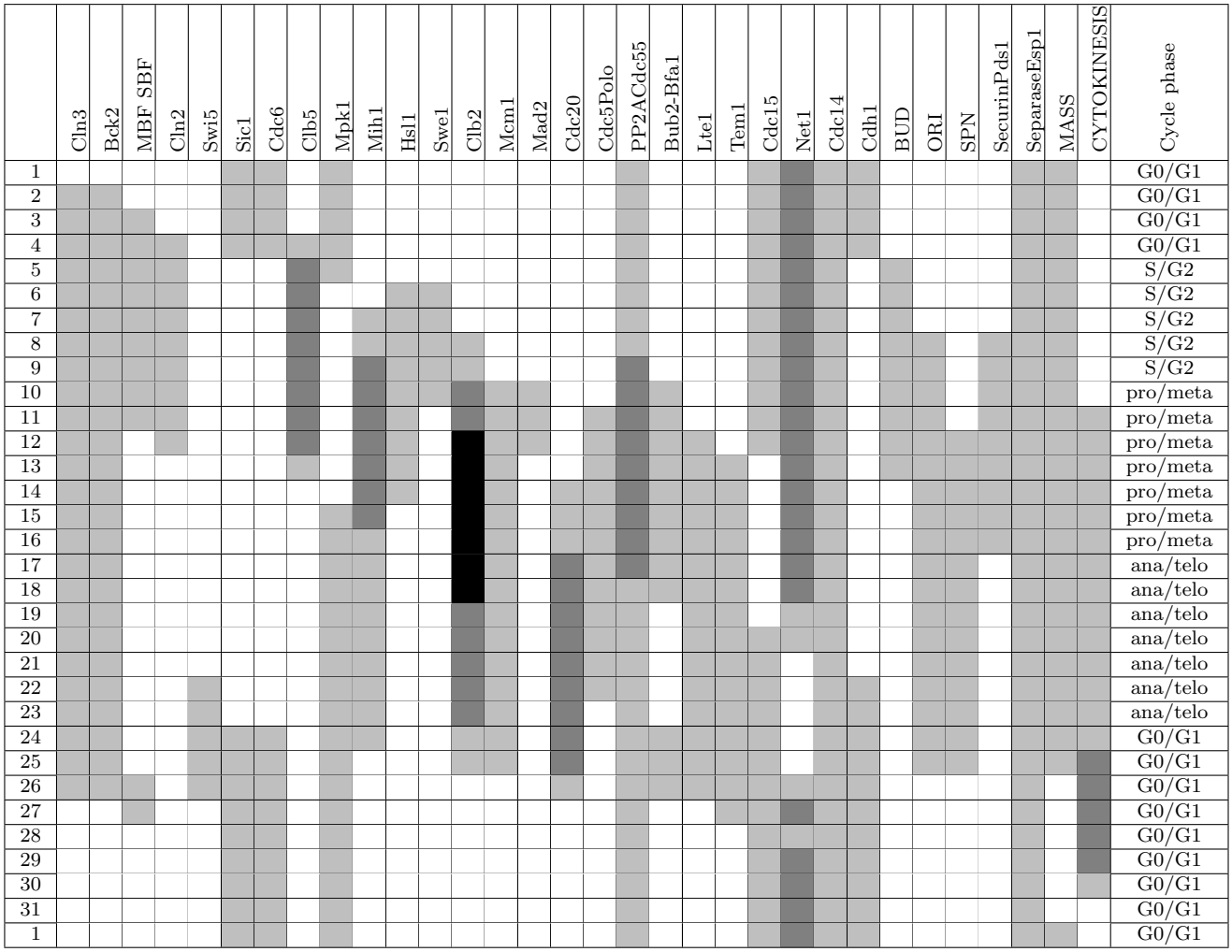


Figure 4: Simulation of the coupled model in the wild-type condition. The model is simulated using a set of synchronous priority classes (see model file for details). Starting from the initial state corresponding to the first row of the table. Successive rows give the successive states obtained in the simulation. Colour code: white =0, lightgray=1, gray=2, black=3.

Mutant	Cln3	Bck2	MBF SBF	Cln2	Swi5	Sic1	Cdc6	Clb5	Mpk1	Mih1	Hsl1	Swe1	Clb2	Mcm1	Mad2	Cdc20	Cdc5Polo	PP2ACdc55	Bub2-Bfa1	Tem1	Cdc15	Net1	Cdc14	Cdh1	BUD	ORI	SPN	SecurinPds1	SeparaseEsp1	MASS	CYTOKINESIS
cln1Δ cln2Δ	Sustained oscillations																														
cln1Δ cln2Δ sic1Δ	Sustained oscillations																														
cln1Δ cln2Δ cdh1Δ	Sustained oscillations																														
cln1Δ cln2Δ bck2Δ	Sustained oscillations																														
cln1Δ cln2Δ clb5Δ clb6Δ																															
cln1Δ cln2Δ cln3Δ																															
cln1Δ cln2Δ cln3Δ GAL-CLN2	Sustained oscillations																														
cln1Δ cln2Δ cln3Δ GAL-CLN3	Sustained oscillations																														
cln1Δ cln2Δ cln3Δ sic1Δ	Sustained oscillations																														
cln1Δ cln2Δ cln3Δ cdh1Δ	Sustained oscillations																														
cln1Δ cln2Δ cln3Δ multicopy-CLB5	Sustained oscillations																														
cln1Δ cln2Δ cln3Δ GAL-CLB5	Sustained oscillations																														
cln1Δ cln2Δ cln3Δ multicopy-BCK2	Sustained oscillations																														
LF−cln1Δ cln2Δ cln3Δ GAL-CLB2																															
cln1Δ cln2Δ cln3Δ APC-ts																															
GAL-SIC1 cln1Δ cln2Δ																															
GAL-SIC1 GAL-CLN2 cln1Δ cln2Δ	Sustained oscillations																														
GAL-SIC1 cln1Δ cln2Δ cdh1Δ																															
GAL-SIC1 GAL-CLN2 cln1Δ cln2Δ cdh1Δ	Sustained oscillations																														
GAL-CLN2 cln1Δ cln2Δ cdh1Δ	Sustained oscillations																														
GAL-CLN2 cln1Δ cln2Δ	Sustained oscillations																														
cdc14-ts GAL-CLN2																															
cln3Δ	Sustained oscillations																														
cln3Δ bck2Δ																															
cln3Δ bck2Δ GAL-CLN2 cln1Δ cln2Δ	Sustained oscillations																														
cln3Δ bck2Δ multicopy CLN2																															
cln3Δ bck2Δ sic1Δ																															
GAL-CLN3	Sustained oscillations																														
bck2Δ	Sustained oscillations																														
multicopy BCK2	Sustained oscillations																														
clb5Δ clb6Δ	Sustained oscillations																														
TAB6-1 clb5Δ clb6Δ																															
cdc20Δ clb5Δ																															
cdc20Δ pds1Δ clb5Δ	Sustained oscillations																														
CLB2-dbΔ clb5Δ																															
CLB2-dbΔ clb5Δ on galactose																															
CLB5-dbΔ	Sustained oscillations																														
CLB5-dbΔ sic1Δ	Sustained oscillations																														
CLB5-dbΔ pds1Δ	Sustained oscillations																														
CLB5-dbΔ pds1Δ cdc20Δ	Sustained oscillations																														

Mutant	Cln3	Bck2	MBF SBF	Cln2	Swi5	Sic1	Cdc6	Clb5	Mpk1	Mih1	Hsl1	Swe1	Clb2	Mcm1	Mad2	Cdc20	Cdc5Polo	PP2ACdc55	Bub2-Bfa1	Tem1	Cdc15	Net1	Cdc14	Cdh1	BUD	ORI	SPN	SecurinPds1	SeparaseEsp1	MASS	CYTOKINESIS
GAL-CLB5	Sustained oscillations																														
GAL-CLB5 cdh1Δ	Sustained oscillations																														
GAL-CLB5 sic1Δ	Sustained oscillations																														
GAL-CLB5-dbΔ	Sustained oscillations																														
clb1Δ clb2Δ																															
CLB1 clb2Δ	Sustained oscillations																														
CLB1 clb2Δ PDS1-dbΔ																															
CLB1 clb2Δ cdh1Δ	Sustained oscillations																														
CLB1 clb2Δ pds1Δ	Sustained oscillations																														
TAB6-1 CLB1 clb2Δ	Sustained oscillations																														
CLB2-dbΔ																															
CLB2-dbΔ in galactose																															
CLB2-dbΔ multicopy-SIC1	Sustained oscillations																														
CLB2-dbΔ GAL-SIC1	Sustained oscillations																														
CLB2-dbΔ multicopy-cdc6	Sustained oscillations																														
CLB2-dbΔ GAL-CDC6	Sustained oscillations																														
GAL-CLB2	Sustained oscillations																														
multicopy GAL-CLB2																															
GAL-CLB2 sic1Δ	Sustained oscillations																														
GAL-CLB2 cdh1Δ																															
APC-A GAL-CLB2																															
swi5Δ GAL-CLB2	Sustained oscillations																														
GAL-CLB2-dbΔ																															
sic1Δ	Sustained oscillations																														
sic1Δ cdh1Δ	Sustained oscillations																														
sic1Δ cdh1Δ GALL-CDC20	Sustained oscillations																														
sic1Δ cdc6Δ-2-49	Sustained oscillations																														
sic1Δ cdc6Δ-2-49 cdh1Δ																															
sic1Δ cdc6Δ-2-49 cdh1Δ GALL-CDC20	Sustained oscillations																														
APC-A sic1Δ	Sustained oscillations																														
APC-A sic1Δ cdc6Δ-2-49	Sustained oscillations																														
cdc14-ts sic1Δ																															
swi5Δ	Sustained oscillations																														
swi5Δ cdh1Δ																															
swi5Δ cdh1Δ GAL-SIC1	Sustained oscillations																														
GAL-SIC1	Sustained oscillations																														
GAL-SIC1-dbΔ																															
cdc14-ts GAL-SIC1																															
cdc14-ts then multicopy-GAL-SIC1																															
APC-A cdh1Δ GAL-SIC1	Sustained oscillations																														

Mutant	Cln3	Bck2	MBF SBF	Cln2	Swi5	Sic1	Cdc6	Clb5	Mpk1	Mih1	Hsl1	Swe1	Clb2	Mcm1	Mad2	Cdc20	Cdc5Polo	PP2ACdc55	Bub2-Bfa1	Tem1	Cdc15	Net1	Cdc14	Cdh1	BUD	ORI	SPN	SecurinPds1	SeparaseEsp1	MASS	CYTOKINESIS
APC-A cdh1Δ multicopy-SIC1	Sustained oscillations																														
cdc6Δ 2-49	Sustained oscillations																														
cdh1Δ cdc6Δ-2-49	Sustained oscillations																														
APC-A cdh1Δ GAL-CDC6	Sustained oscillations																														
APC-A cdh1Δ multicopy-CDC6	Sustained oscillations																														
cdh1Δ	Sustained oscillations																														
cdc14-ts cdh1Δ																															
cdc15Δ net1-ts cdh1Δ	Sustained oscillations																														
APC-A cdh1Δ																															
APC-A cdh1Δ in galactose																															
APC-A cdh1Δ multicopy-CDC20	Sustained oscillations																														
CDH1 constitutively active																															
cdc20 ts																															
cdc20Δ pds1Δ	Sustained oscillations																														
cdc20Δ bub2Δ																															
cdc20-ts mad2Δ																															
net1Δ cdc20-ts	Sustained oscillations																														
GAL-ESP1 cdc20-ts	Sustained oscillations																														
GAL-CDC20	Sustained oscillations																														
APC-A	Sustained oscillations																														
tem1Δ																															
tem1Δ net1ts	Sustained oscillations																														
tem1ts GAL-CDC15	Sustained oscillations																														
tem1ts multicopy-CDC14	Sustained oscillations																														
cdc15Δ																															
cdc15ts multicopy-TEM1																															
cdc15ts multicopy-CDC14	Sustained oscillations																														
cdc15Δ-net1-ts	Sustained oscillations																														
TAB6-1 cdc15Δ	Sustained oscillations																														
GAL-TEM1	Sustained oscillations																														
mad2Δ GAL-TEM1 in nocodazole																															
multicopy-CDC15	Sustained oscillations																														
cdc14 ts																															
net1 ts	Sustained oscillations																														
net1-ts-in-nocodazole	Sustained oscillations																														
GAL-NET1																															
GAL-CDC14																															
GAL-NET1 GAL-CDC14	Sustained oscillations																														
TAB6-1	Sustained oscillations																														
pds1Δ	Sustained oscillations																														

Mutant	Cln3	Bck2	MBF SBF	Cln2	Swi5	Sic1	Cdc6	Clb5	Mpk1	Mih1	Hsl1	Swe1	Clb2	Mcm1	Mad2	Cdc20	Cdc5Polo	PP2ACdc55	Bub2-Bfa1	Lte1	Tem1	Cdc15	Net1	Cdc14	Cdh1	BUD	ORI	SPN	SecurinPds1	SeparaseEsp1	MASS	CYTOKINESIS
pds1Δ-in-nocodazole	Sustained oscillations																															
bub2Δ pds1Δ in nocodazole	Sustained oscillations																															
mad2Δ pds1Δ in nocodazole																																
esp1 ts																																
GAL-PDS1-dbΔ esp1-ts																																
GAL-PDS1-dbΔ																																
PDS1-dbΔ																																
mad2Δ	Sustained oscillations																															
bub2Δ	Sustained oscillations																															
mad2Δ bub2Δ	Sustained oscillations																															
WT in nocodazole																																
mad2Δ in nocodazole																																
bub2Δ in nocodazole																																
bub2Δ mad2Δ in nocodazole	Sustained oscillations																															
MEN inactive																																
separase inactivation																																
separase overexpression in cdc20 deprived cells	Sustained oscillations																															
Cdk inhibition before separase overexpression in Cdc20-deprived cells																																
Cdk inhibition before Cdc20 induction																																
PP2ACdc55Δ	Sustained oscillations																															
indestructable securin																																
indestructable securin bub2Δ	Sustained oscillations																															
swe1Δ	Sustained oscillations																															
mih1Δ	Sustained oscillations																															
hsl1Δ	Sustained oscillations																															
mih1Δ hsl1Δ																																
mih1Δ swe1Δ	Sustained oscillations																															
cdc24ts	Sustained oscillations																															
cdc24ts swe1Δ	Sustained oscillations																															
cdc24ts mih1Δ																																
cdc24ts hsl1Δ	Sustained oscillations																															
cdc24ts mih1Δ hsl1Δ																																
cdc24ts mih1Δ swe1Δ	Sustained oscillations																															
CheckpointON mpk1Δ	Sustained oscillations																															
CheckpointON mpk1Δ hsl1Δ	Sustained oscillations																															
CheckpointON mpk1Δ mih1Δ																																

Table 4: Coupled model: stable states in the mutant conditions. Colour code: white=0, lightgray=1, gray=2, black=3. See model file for mutant definition and description.

Supplementary Material (ESI) for Molecular BioSystems
This journal is (c) The Royal Society of Chemistry, 2009