INSIGHTS INTO THE NETWORK CONTROLLING THE G_1/S TRANSITION IN BUDDING YEAST

MATTEO BARBERIS[1] barberis@molgen.mpg.de
EDDA KLIPP[1,2] klipp@molgen.mpg.de

[1] *Max-Planck-Institute for Molecular Genetics, Ihnestraße 73, 14195 Berlin, Germany*
[2] *Humboldt University Berlin, Institute for Biology, Invalidenstr. 42, 10115 Berlin, Germany*

The understanding of complex biological processes whose function requires the interaction of a large number of components is strongly improved by the construction of mathematical models able to capture the underlying regulatory wirings and to predict the dynamics of the process in a variety of conditions. Iterative rounds of simulations and experimental analysis generate models of increasing accuracy, what is called the systems biology approach. The cell cycle is one of the complex biological processes that benefit from this approach, and in particular budding yeast is an established model organism for these studies. The recent publication about the modeling of the G_1/S transition of the budding yeast cell cycle under a systems biology analysis has highlighted in particular the implications of the cell size determination that impinge the events driving DNA replication. During the life cycle of eukaryotic cells, DNA replication is restricted to a specific time window, called the S phase, and several control mechanisms ensure that each DNA sequence is replicated once, and only once, in the period from one cell division to the next. Here we extend the analysis of the G_1/S transition model by including additional aspects concerning the DNA replication process, in order to give a reasonable explanation to the experimental dynamics, as well as of specific cell cycle mutants. Moreover, we show the mathematical description of the critical cell mass (P_S) that cells have to reach to start DNA replication, which value is modulated depending on the different activation of the replication origins. The sensitivity analysis of the influence that the kinetic parameters of the G_1/S transition model have on the setting of the P_S value is also reported.

Keywords: Budding yeast; G_1/S transition; probabilistic model; DNA replication; critical cell size.

1. Introduction

The machinery of the cell cycle is one of the most relevant and fine-tuned processes in the cell, and regulates important cellular processes, e.g., DNA synthesis, budding, and cell proliferation. In many cases, defects in the cell cycle events are known to be a cause of cancer, and more precise information on the regulation of these processes is useful to plan strategies of drug discovery.

The cell cycle regulation has been deeply analyzed by using *in silico* modeling from yeast to mammalian cells [2-9]. The *in vivo* dynamics of several proteins introduced in these models were obtained from results of biochemical experiments, mainly by western blotting analysis (i.e. total protein level, degradation rates). These protein data were used for parameter estimation to refine the computational models. Both data acquisition and parameter fitting are the basic steps for creating a model, and improvements of these processes are crucial for achieving precise simulation of the models that try to represent

the experimental dynamics. The quantitative results from these experiments are very useful for creating more complex and precise models. A few kinetic parameters in these models were tuned manually based on biological knowledge available in the literature, and for the major part they were fixed arbitrarily to reproduce the phenotype of a wide range of cell cycle mutants [2,3]. The differential equations that describe the reaction of a model depend on kinetic constants, which are often not accessible to experimental determination, and must therefore be estimated by fitting the model to experimental data. However, with the increase in knowledge about the single reactions involved in a biological pathway, it becomes extremely useful to estimate the kinetic parameters and to manually introduce them into the models.

Recently, we have compiled the huge amount of experimental data available in the literature and modeled the nucleo/cytoplasmic G_1/S transition of the budding yeast *Saccharomyces cerevisiae* [1]. The model was implemented by ordinary differential equations and tested by computer simulation [1]. This map reveals the main known regulatory events that impinge the functionality of this window of the cell cycle. By using time-series quantitative data on proteins reported by Alberghina *et al.* [10] and by Rossi *et al.* [11], we performed the estimation of some critical parameters. Our model [1] is compatible with time-series data measured by western blotting, and it matches the *in vivo* data from among several cell cycle mutants reported in literature. In addition, the network of the G_1/S transition highlighted the feasible approach to determine the critical cell mass, called P_S, that cells have to reach in order to enter S phase and replicate their DNA.

On the basis of the physiological significance of the G_1/S transition network, this work presents an insight on DNA replication. The activation of the replication machinery has still to be highlighted in many of its regulatory events, but a relevant step is the phosphorylation of different substrates by the Cdk1-Clb5,6 kinase complex that induces the firing of the DNA replication origins [12,13]. In [1] we described the steps which lead to the DNA replication with a simple probabilistic model that considers the availability of the Cdk1-Clb5,6 nuclear concentration as the main input. DNA replication, the main event that drives the cell cycle after the G_1/S transition, is analyzed here from the mathematical point of view, providing an explanation for the phenotype of wild type cells grown on different media, and for selected mutants of the network. Moreover, we show the mathematical description of the P_S determination, which value is modulated depending from the different activation of the replication origins. The sensitivity analysis of the influence that the kinetic parameters of the G_1/S transition model have on the setting of the P_S value is also reported.

2. DNA replication at the G_1/S transition

2.1. *Hybrid model for DNA replication events*

The model presented by Barberis *et al.* [1] considers the analysis of the G_1/S transition events for a single cell, in a way to represent the experimental dynamics derived from single elutriated cells [10,11]. However, the real representation of the dynamics of the yeast cell cycle has to consider the behaviour of a cell population. The critical cell size P_S is a quantitative parameter known to characterize each exponentially growing population. Its value can be estimated based on the average protein content (a measure of cell size). DNA replication starts only when the cells reach the P_S value, the value of which changes depending from the nutritional medium where the cells are growing [14,15]. Thus, to estimate the P_S value, it is necessary to find a way to model the DNA replication process. To this purpose, we constructed a hybrid model of the firing of DNA replication origins, where the probabilistic model uses as input the output of the network of the G_1/S transition, the nuclear concentration of the Cdk1-Clb5,6 complex [1].

The DNA replication machinery is a highly complex process [12,13], and many details have still to be highlighted. Thus, the representation of the process cannot be absolutely defined. However, it is possible to make some acceptable assumptions - compatible with the reported literature - to simulate the effect of the G_1/S cascade on the late events of the cell cycle. We consider as relevant step of the DNA replication the phosphorylation of different substrates by Cdk1-Clb5,6 that induces the firing of the replication origins [12,13]. In addition, the probabilistic description of the process involves some approximations on the basis of the scheme reported in Fig. 1 that shows the consecutive steps of the DNA replication initiation. The proteins reported in the scheme (Cdt1 and Cdc6) represent only examples of known actors involved in the process [13], but such molecular details are outside of the scope of the model.

The firing of the DNA replication origins is modeled as a three-step process. As reported in Fig. 1, the first step includes the events that occur from the free replication origins to the formation of the pre-replicative complex (pre-RC) (see [13] for molecular details). The distance between the DNA replication origins is fixed. The time for the formation of the pre-RC complex at each of the replication origins is taken from a normal distribution with mean of 15 minutes, and a standard deviation of 2 minutes. The number of the replication origins is fixed to 440, as reported from [13,16,17]. The second step is dependent on the nuclear Cdk1-Clb5,6 complex, output of the model of the G_1/S transition [1]. In fact, we correlate the concentration of this complex to the onset of DNA replication.

The probability of the activation of the replication origin by Cdk1-Clb5,6 at a certain time is determined by the concentration of this complex at that time. In this case, we consider the period of this step that is necessary for Cdk1-Clb5,6 to exceed a value taken from a normal distribution with a mean of 0.03 μM, and standard deviation of 0.01 μM. Moreover, we consider an additional time due to the fact that as soon as Cdk1-Clb5,6 is

available, specific substrates are phosphorylated by the complex for their release from each replication origin. Finally, the third step shows the activation (firing) of the replication origins. The time for each replication origin to reach the fired state is taken from a normal distribution with a mean of 1 minute, and a standard deviation of 0.01 minute. When a replication origin has fired, then DNA replication proceeds bidirectionally from multiple replication origins, as experimentally reported [18,19].

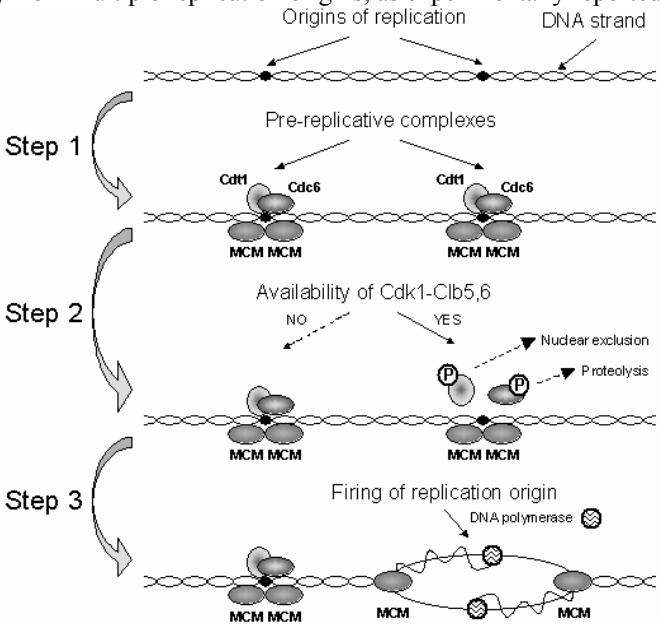

Fig. 1. Schematic representation of relevant events in the firing of the origins of DNA replication. After pre-replication complexes have assembled on the replication origins, phosphorylation of specific targets takes place as a function of the availability of Cdk1-Clb5,6. Origin firing and DNA replication then start bidirectionally.

In the case that the replication reaches the neighboring origin before it fires on its own, that origin is considered as fired. To this purpose, we introduce this correction in an additional part of the code. The code includes the generation of tables with the times of the replication origins activation and the distribution of the activated replication origins in the time when each one reaches the fired state, and the corresponding graphs. Using the Mathematica software, for each origin, the state is updated in three steps (compare Fig. 1) with following duration:

t_{step1} = Random [NormalDistribution[15,2]]
v_{step2} = Random [NormalDistribution[0.03,0.01]]
t_{step2} = Time, when Cdk1-Clb5,6 concentration overcome v_{step2}
t_{step3} = Random[NormalDistribution[1,0.01]]

Each origin is activated, when either steps 1,2,3 are passed or 10 minutes after its neighboring origin was activated.

2.2. *Nutritional conditions and DNA replication*

Cell viability requires the coordination between cell growth and cell division, which in budding yeast is achieved by the attainment of a nutritionally modulated critical cell size (P_S) to trigger DNA replication [14,15]. Nutrients are the main environmental determinants that affect cell cycle progression in budding yeast (20,21,22), and it is known that higher growth rates and larger P_S are observed in rich media (23,20,21,10). Data reported in literature show that a very poor carbon source such as ethanol, or a nitrogen source limitation, yield elongation of the S phase [24,25].

In [1] simulations of the onset of DNA replication were reported for cells grown in glucose and in ethanol media. In glucose, the activation of DNA replication origins took place in a coordinated fashion roughly within a period of 70-90 min. On the contrary, in ethanol the dynamics of the G_1/S transition resulted in a longer DNA replication [1]. We considered that in ethanol-growing cells - with a growth rate about 2-fold lower compared with the glucose-growing cells - the fork rate is about one-half (and the time of origins activation is doubling) than the glucose ones. To implement this assumption, we changed in the Mathematica code the value of the time for the formation of the pre-RC complex at each of the replication origins. The value taken from a normal distribution with mean of 33.4 minutes, and a standard deviation of 2 minutes. This assumption agrees with the reported data, in which the longer S phase in yeast cells growing in poor nitrogen medium can be accounted for by a reduction in replication fork rate [25].

In Fig. 2 the simulation of the onset of DNA replication is reported for wild type cells grown either on glucose or on ethanol media. The code was run for five times to observe the variability of the probabilistic model. The effect in ethanol is a dramatic decrease of available concentrations of kinase complexes as compared to the glucose growth. In more detail, a reduced formation of the Cdk1-Cln3 (nuclear) complex as well as of the Cdk1-Cln1,2 (cytoplasmic) and Cdk1-Clb5,6 (nuclear) complexes is observed (upper panels). For wild type cells grown on glucose medium, origin firing occurs at about 80 min, when the Cdk1-Clb5,6 levels overcome the Sic1 levels [1]. As expected, by allowing efficient Cdk1-Clb5,6 nuclear import and following switch-like degradation of Sic1, a sharp spike of Cdk1-Clb5,6 activity is obtained (Fig. 2A). This sharp spike in turn allows sharp and efficient firing of replication origins (Fig. 2B). Conversely, in wild type cells grown on ethanol medium, the Cdk1-Clb5,6 complex is inefficiently imported into the nucleus over a longer period, thus resulting in sparse origin firing (Fig. 2D). In this condition, Cdk1-Clb5,6 can not activate the DNA replication process in the physiological time, thus resulting in a longer S phase (Fig. 2C) as experimentally reported [26].

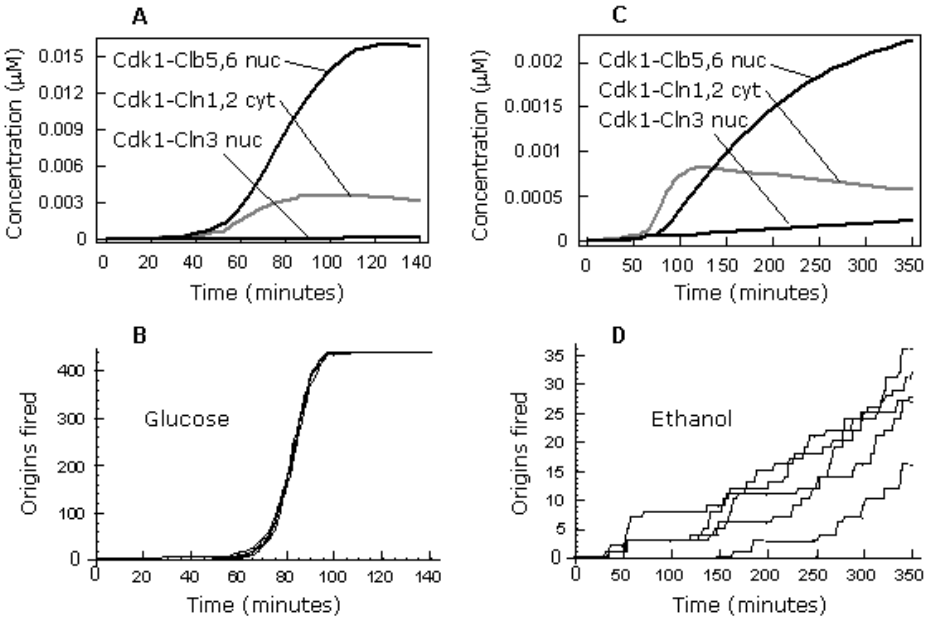

Fig. 2. Distribution of the cyclin-dependent kinase complexes in the firing of DNA replication origins. The cumulative number of fired origins was calculated basing on the probabilistic model for firing of origins in wild type cells grown on glucose (A,B) and on ethanol (C,D) media. Note different scales on the y-axis.

2.3. *Nuclear availability of Cdk1-Clb5,6 and firing of the DNA replication origins*

In the model of the DNA replication events, we related the free concentration of the nuclear Cdk1-Clb5,6 complex to the initiation of DNA replication. Now, we want to focus the attention on the event that controls the availability of Cdk1-Clb5,6, i.e. the phosphorylation of Sic1, an inhibitor of this kinase complex [27,28]. According to the current models reported in literature, Sic1 is involved in the control of DNA replication as a negative regulator of the Cdk activity, and the mathematical models of the cell cycle had of course taken into account only this function [3]. This leaves unanswered a major phenotype of the *sic1Δ* mutant, namely sparse origin firing [29].

We consider that Sic1 is also acting by promoting the Cdk1-Clb5,6 entry into the nucleus [1], based on supporting experimental data [11]. Thus, it would be expected that the Cdk1-Clb5,6 complex enters in the nucleus less efficiently in *sic1Δ* cells. Fig. 3 shows the simulation of the *sic1Δ* mutant grown on glucose media. The amount of nuclear Cdk1-Clb5,6 in wild type cells is about seven/eight times higher than in *sic1Δ* cells (see Fig. 2A for comparison). This fact induces a simulated sparse DNA firing in the mutant cell that starts earlier than in wild type, since no Sic1 degradation is required, and proceeds slowly (Fig. 3B), as experimentally observed [29]. Thus, our model gives a possible rationale explanation for the sparse origin firing observed in the *sic1Δ* mutant.

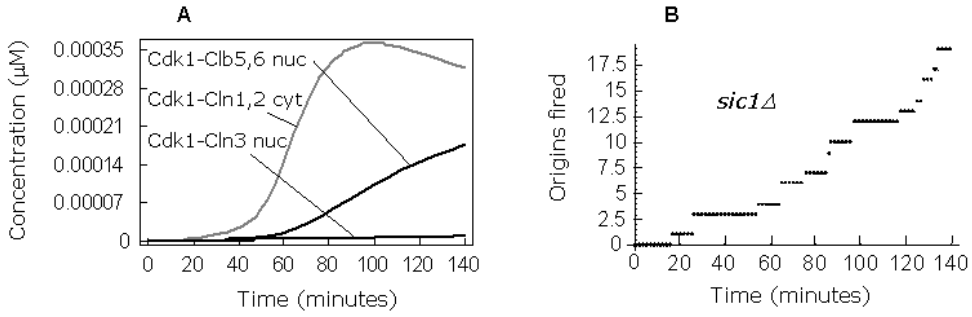

Fig. 3. Distribution of the cyclin-dependent kinase complexes in the firing of DNA replication origins for the *sic1Δ* mutant. grown on glucose medium.

In order to evaluate if the model can explain the starting of DNA replication of different yeast background, we report also the results for deletion mutants or overexpression (indicated as OE- followed by the gene name). Results relating to dosage of *CLN3*, *FAR1*, and *WHI5* genes, which are central to the logic of the G_1/S network [1], are analyzed. The effects of mutations will be always compared with wild type cells. Deletion of *CLN3* gene (*cln3Δ*) prevents the nuclear Cdk1-Cln3 complex formation at the beginning of the G_1/S network, therefore preventing the activation of the SBF/MBF transcription factor. The outcome is a remarkable decrease in *CLN1,2* and *CLB5,6* transcription, and thus in formation of cytoplasmic Cdk1-Cln1,2 and nuclear Cdk1-Clb5,6 that, ultimately, cause a reduction in the number of the activated replication origins (Fig. 4A). This is in agreement with the experimental observations [30,31]. On the other side, *CLN3* overexpression (OE-*CLN3*) leads to the effect that Cln3 overcomes earlier the inhibitory activity of Far1, the force that balance the formation of the nuclear Cdk1-Cln3 complex [10]. This accelerates the formation of cytoplasmic Cdk1-Cln1,2 and nuclear Cdk1-Clb5,6, which results in an anticipated onset of DNA replication (Fig. 4B), as experimentally observed [30,31]. In *far1Δ* cells, there is no balance between the inhibitor Far1 and Cln3, and the nuclear Cdk1-Cln3 complex appears earlier, as well as the cytoplasmic Cdk1-Cln1,2 and nuclear Cdk1-Clb5,6 complexes. The result is a slightly earlier entrance into S phase (Fig. 4C) compared to wild type cells (see Fig. 2B and Ref. [10]). In contrast, in an overexpression of *FAR1* (OE-*FAR1*) the formation of nuclear Cdk1-Cln3 complex occurs to a clearly lower extent. This effect propagates down to the formation of cytoplasmic Cdk1-Cln1,2 and nuclear Cdk1-Clb5,6 and, ultimately, to a reduction in the number of the activated replication origins (Fig. 4D). The *whi5Δ* mutant lacks the initial inhibition of the SBF/MBF transcription factor, the central event of the G_1/S network, and transcription of genes for Cln1,2 and Clb5,6 is turned on immediately. The formation of Cdk/cyclin complexes is brought forward, and the mutant undergoes G_1/S transition about 40 min earlier than wild type. This results in earlier starting of DNA replication (Fig. 4E), as experimentally suggested [32]. Upon overexpression of *WHI5* gene (OE-*WHI5*), there is a stronger inhibition of the SBF/MBF

transcription factor and more nuclear Cdk1-Cln3 complex is necessary to phosphorylate Whi5 and release the inhibition. Hence, transcription of Cln1,2 and Clb5,6 is diminished resulting in reduced formation of complexes with Cdk1, and in a strong delay in the DNA replication events (Fig. 4F), as experimentally observed [32]. Overall, the simple probabilistic model connected to the G_1/S network appears to describe the starting of the DNA replication events correctly.

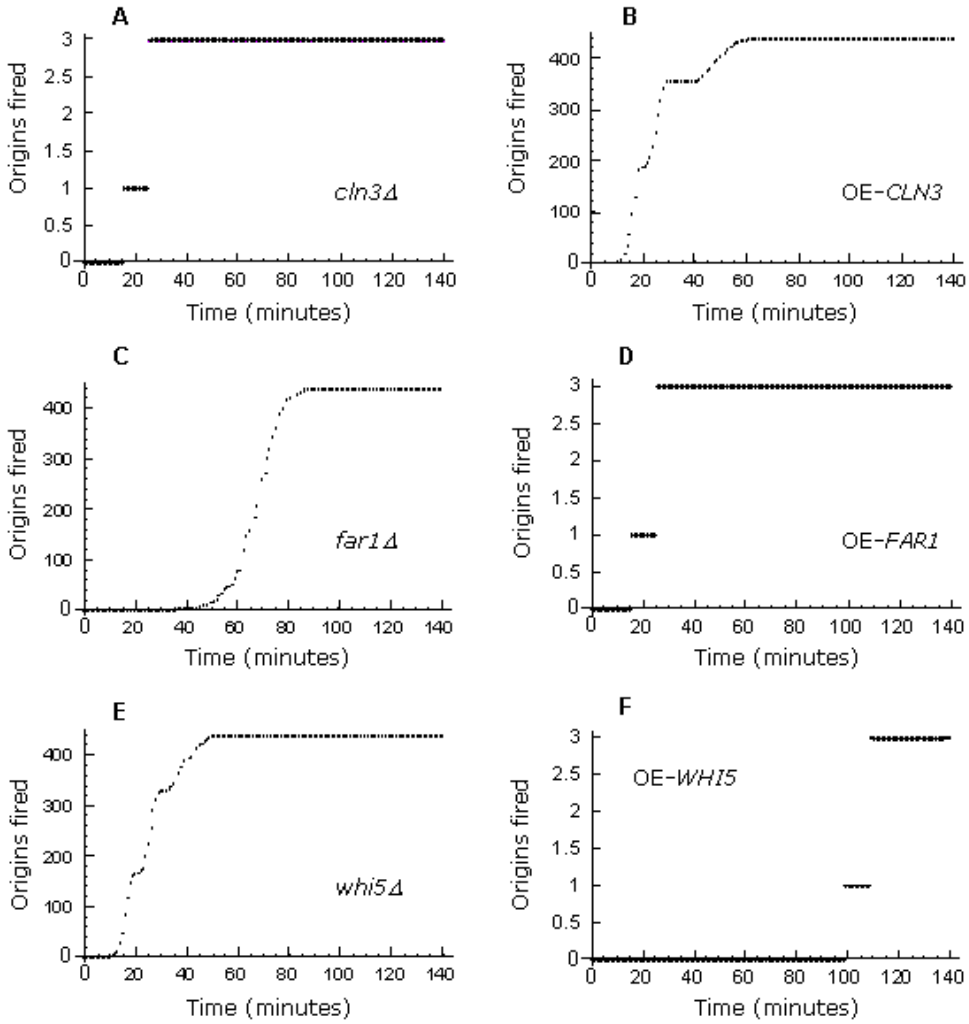

Fig. 4. Simulation of the onset of the DNA replication in glucose-growing cell populations for the deletion mutants *cln3Δ*, *far1Δ*, *whi5Δ* (A,C, and E, respectively), and for their overexpressions OE-*CLN3*, OE-*FAR1*, OE-*WHI5* (B,D, and F, respectively).

3. The critical cell size P_S and the G_1/S transition dynamics

3.1. *Derivation of the P_S value*

To estimate the critical cells size (P_S) that cells have to reach to enter S phase, we need to simulate the onset of DNA replication. In fact, P_S is defined operationally as the protein content of cells that enter S phase [33]. At this point, we estimated P_S as the cell size when 50% of the replication origins were activated in a single cell. In addition, the method permits to calculate the cell size at different % of replication origins activated.

To implement the derivation of the P_S value, we wrote a Mathematica script that calculates the value of the cell volume when a certain % of replication origins is activated. Specifically, the value of the cell volumes is calculated when the first replication origin reaches the state fired, and when 10 %, 50 %, and 90 % of the replication origins are activated, respectively.

3.2. *Fluctuations of the P_S value*

In [1] we analyzed the effects of specific parameters of the model of the G_1/S transition on the setting of P_S. Specifically, we focused on the main parameters that we established to be different from glucose to ethanol. Sensitivity analysis showed that the Ps value is mainly affected by the growth rate. Here we show the influence of each parameter of the G_1/S transition model on the setting of the critical cell mass. In Table 1 are listed the kinetic parameters, and the corresponding reactions (see [1] for the detailed explanation of the reactions), that affect significantly the P_S value (in bold face are highlighted the parameters that play a major role in changing the critical cell mass). Fig. 5 illustrates the sensitivity analysis that shows the P_S changes following the variation of the kinetic parameters from 0.1-fold to 100-fold.

As reported in Table 1, the main kinetic parameters influencing the setting of the critical cell size P_S are related to two regulatory events, which control the entrance into S phase. The first growth-dependent threshold entails the interplay of the activator Cln3 (bound to the kinase Cdk1) and the inhibitor Far1 [10,34]. The nuclear Cdk1-Cln3 activity increases proportionally to Cln3 level, and to the inverse of Far1, thus the threshold mechanism controlling the onset of S phase is operative as long as Cln3 increases faster than Far1. The Cln3/Far1 threshold indicates the reaching of a given size, when the amount of Cln3 - that increases in proportion to cell mass - overcomes Far1. Nuclear Cdk1-Cln3 phosphorylates Whi5, leading to the activation of transcription factors SBF/MBF, so opening up the pathway that leads to the onset of DNA replication. The critical cell size is reached when the cells overcome the second threshold dependent on the balance between the cyclin Clb5 (the activator) and the Cki Sic1 (the inhibitor) [1]. The analysis confirms that the coordination between the two sequential threshold mechanism (*i.e.* the Cln3/Far1 and the Clb5,6/Sic1 thresholds) regulates the critical cell mass necessary to undergo the S phase, thus effectively couples cell growth to the onset of DNA replication.

Table 1. Kinetic parameters and corresponding reactions shown in Fig. 5.

Panel	Reaction	Parameter
A	Protein synthesis	$\boldsymbol{k_5}$ **(Far1$_{cyt}$)**, k_6 (Cln3$_{cyt}$), $\boldsymbol{k_7}$ **(Cdk1$_{cyt}$)** k_8 (Whi5$_{cyt}$)
B	Translation mRNA → protein SBF/MBF basal production	$\boldsymbol{k_3}$ **(*CLN1,2* mRNA$_{cyt}$ → Cln1,2$_{cyt}$)** $\boldsymbol{k_4}$ **(*CLB5,6* mRNA$_{cyt}$ → Clb5,6$_{cyt}$)** k_{35} (SBF/MBF$_{nuc}$ basal production)
C	Localization (protein nuclear import)	k_{42} (Far1$_{cyt}$), $\boldsymbol{k_{43}}$ **(Cln3$_{cyt}$)**, $\boldsymbol{k_{44}}$ **(Cdk1$_{cyt}$)** k_{45} (Whi5$_{cyt}$)
D	Localization (protein complexes nuclear import)	k_{46} (Cdk1-Cln1,2$_{cyt}$) k_{47} (Cdk1-Clb5,6-Sic1$_{cyt}$) k_{48} (Cdk1-Clb5,6$_{cyt}$)
E	Localization (protein export)	k_{49} (Cdk1$_{nuc}$), k_{50} (*CLN1,2* mRNA$_{nuc}$) k_{51} (*CLB5,6* mRNA$_{nuc}$)
F	Association	$\boldsymbol{k_{24}}$ **(Cln3$_{nuc}$ + Cdk1$_{nuc}$)** $\boldsymbol{k_{28}}$ **(Cdk1$_{cyt}$ + Clb5,6$_{cyt}$)** k_{30} (Cdk1-Cln3$_{nuc}$ + Far1$_{nuc}$) k_{34} (SBF/MBF$_{nuc}$ + Whi5$_{nuc}$)
G	Dissociation (unphosphorylated complexes)	$\boldsymbol{k_{25}}$ **(Cdk1-Cln3$_{nuc}$)**, k_{27} (Cdk1-Cln1,2$_{cyt}$) $\boldsymbol{k_{29}}$ **(Cdk1-Clb5,6$_{cyt}$)** $\boldsymbol{k_{33}}$ **(Cdk1-Clb5,6-Sic1$_{cyt}$)**
H	Dissociation (phosphorylated complexes)	k_{39} (SBF-MBF-Whi5-P$_{nuc}$) $\boldsymbol{k_{40}}$ **(Cdk1-Cln3-Far1-P$_{nuc}$)** k_{41} (Cdk1-Clb5,6-Sic1-P$_{nuc}$)
I	Catalytic activity	k_{36} (Cdk1-Cln3$_{nuc}$ on SBF-MBF-Whi5$_{nuc}$) k_{37} (Cdk1-Cln1,2$_{nuc}$ on Cdk1-Cln3-Far1$_{nuc}$)
J	Degradation (in the nucleus)	k_{19} (Far1$_{nuc}$), $\boldsymbol{k_{20}}$ **(Cln3$_{nuc}$)**
K	Degradation (mRNAs and cyclin-dependent kinase inhibitors in the cytoplasm)	k_{10} (*CLN1,2* mRNA$_{cyt}$) $\boldsymbol{k_{11}}$ **(*CLB5,6* mRNA$_{cyt}$)** k_{14} (Far1$_{cyt}$), $\boldsymbol{k_{18}}$ **(Sic1$_{cyt}$)**
L	Degradation (cyclins and Cdk in the cytoplasm)	k_{12} (Cln1,2$_{cyt}$), $\boldsymbol{k_{13}}$ **(Clb5,6$_{cyt}$)**, $\boldsymbol{k_{15}}$ **(Cln3$_{cyt}$)** $\boldsymbol{k_{16}}$ **(Cdk1$_{cyt}$)**

4. Conclusion

A significant part of regulatory events in a living cell are related to the precise functioning of the cell cycle. DNA replication is the main event that drives the cell cycle after the G_1/S transition. Here we show how a probabilistic three-steps model can describe the initiation of the DNA replication process, provides a reasonable explanation for the phenotype of wild type cells grown on different media, and for selected mutants of the network. In addition, with this model we have a valuable tool at hand to estimate the critical cell mass, P_S, which cells need to reach to duplicate the DNA. Due to the relatively simple structure of the model, and considering the nuclear concentration of the Cdk1-Clb5,6 complex as the only input [12,13], the model is very well suited for this task. Moreover, from the sensitivity analysis of the P_S value, specific kinetic parameters are recognized to be important for the fine regulation of the G_1/S transition events.

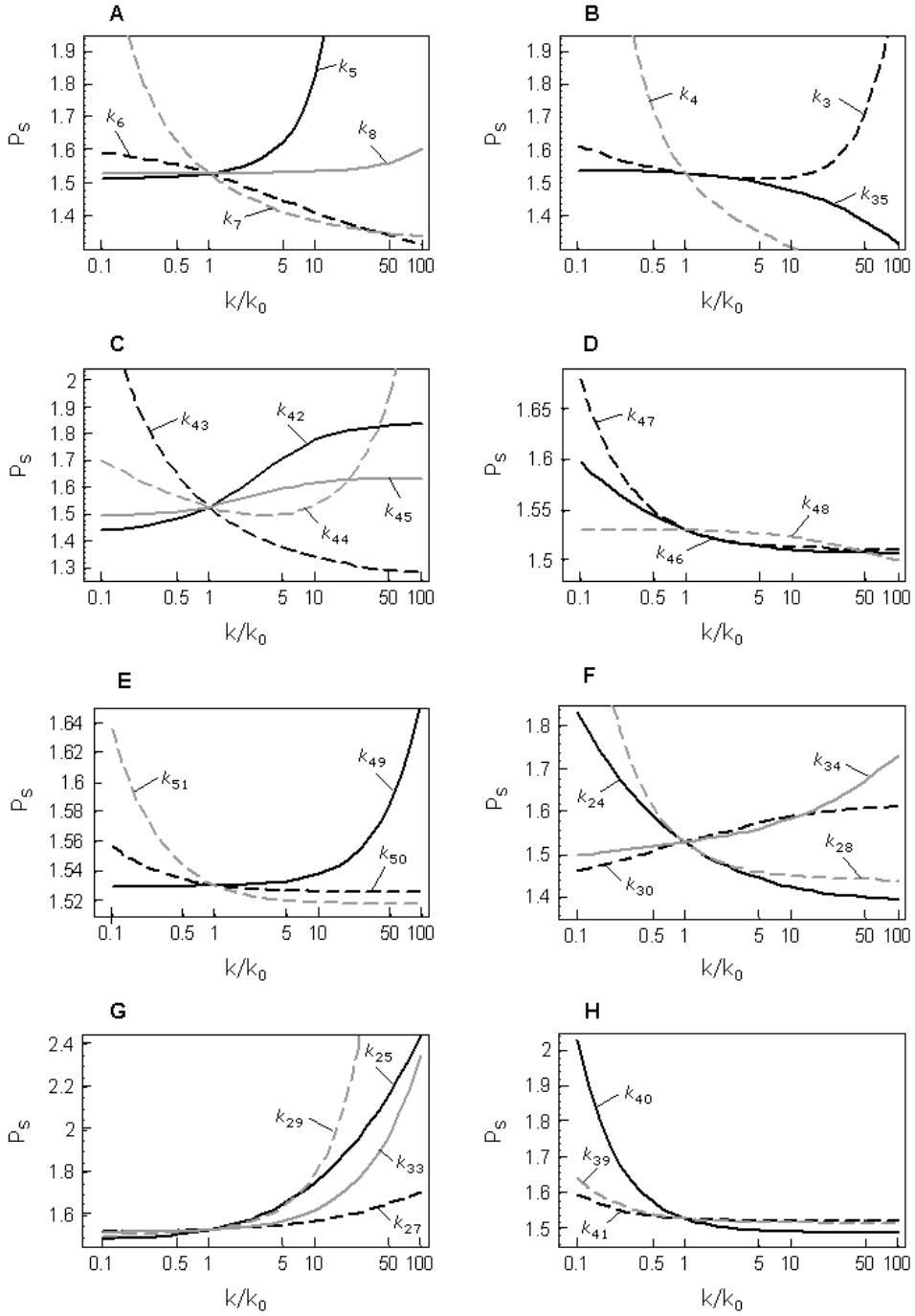

Fig. 5 (A-H). Sensitivity analysis of the P_S value. The kinetic parameters have been varied from 0.1-fold to 100-fold. See Table 1 and [1] for the details of the reactions.

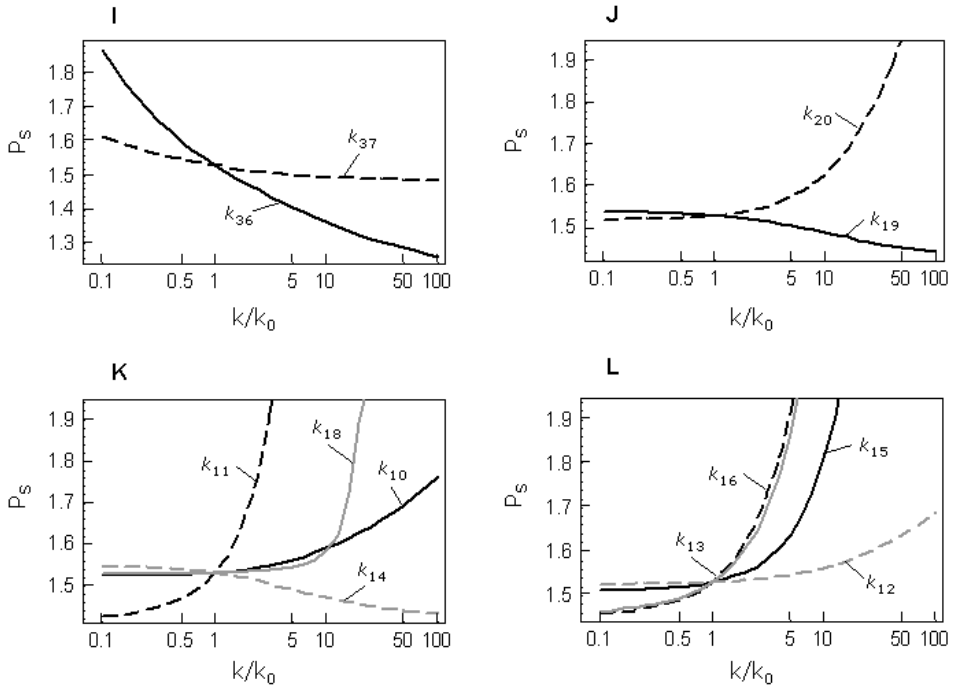

Fig. 5 (I-L). Sensitivity analysis of the P_S value. The kinetic parameters have been varied from 0.1-fold to 100-fold. See Table 1 and [1] for the details of the reactions.

The analysis of the DNA replication initiation focused especially on the critical steps of the G_1/S transition. The involvement of the Cki Sic1 in the control of the replication events represents a good example. The nucleo/cytoplasmic localization of Sic1 differs in glucose- and ethanol-grown cells. It is known that in G_1 cells growing in glucose-supplemented media Sic1 is mostly nuclear, while in G_1 cells grown in ethanol a large amount of Sic1 remains cytoplasmic [11]. This means that in glucose, where Sic1 is almost entirely nuclear, high levels of the nuclear Cdk1-Clb5 complex accumulate, albeit in the inactive form of a ternary complex with Sic1 itself. Such a pre-accumulation allows semi-synchronous liberation of the active Cdk1-Clb5,6 complex after Sic1 degradation in the nucleus, resulting in quite sharp firing of DNA origins and a short S phase (Figs. 2A and B). In ethanol-growing cells, only a minor fraction of Sic1 enter the nucleus, so that the nuclear Cdk1-Clb5,6 complex accumulates slowly and steadily, resulting in a less synchronous firing of DNA replication origins and a longer S phase than observed for glucose-grown cells (compare Figs. 2B and D). A similar situation is observed in *sic1Δ* strains growing on glucose (Fig. 3B). These simulation results allow to give a satisfactory interpretation to a phenotype not explained by a purely inhibitory role for Sic1, but which essential function is also to bind to the cytoplasmic Cdk1-Clb5,6 complex and to promote its nuclear import (as experimentally observed, [11]).

Considering the structure of the network we built as a platform of the essential events that happen in the G_1/S transition, the results obtained now permit us to link this model to the late events of the cell cycle. In particular, we demonstrated that it is crucial to consider the proper network construction - the spatial localization of the regulatory key players - to simulate correctly the onset of DNA replication.

Acknowledgments
This work was supported by the European Commission (Project ENFIN, contract number LSHG-CT-2005-518254), and the German Research Foundation (DFG; the International Research Training Group "Genomics and Systems Biology of Molecular Networks").

References

[1] Barberis, M., Klipp, E., Vanoni, M., and Alberghina, L., Cell size at S phase initiation: an emergent property of the G1/S network, *PloS Computational Biology*, 3:e64.doi:10.1371/journal.pcbi.0030064.
[2] Chen, K.C., Csikasz-Nagy, A., Gyorffy, B., Val, J., Novak, B., and Tyson, J.J., Kinetic analysis of a molecular model of the budding yeast cell cycle, *Mol. Biol. Cell*, 11(1):369-391, 2000.
[3] Chen, K.C., Calzone, L., Csikasz-Nagy, A., Cross, F.R., Novak, B., and Tyson, J.J., Integrative analysis of cell cycle control in budding yeast, *Mol. Biol. Cell*, 15(8):3841-3862, 2004.
[4] Csikasz-Nagy, A., Battogtokh, D., Chen, K.C., Novak, B., and Tyson, J.J., Analysis of a generic model of eukaryotic cell-cycle regulation, *Biophys. J.*, 90(12):4361-4379, 2006.
[5] Obeyesekere, M.N., Tucker, S.L., and Zimmerman, S.O., A model for regulation of the cell cycle incorporating cyclin A, cyclin B and their complexes, *Cell Prolif.*, 27(2):105-113, 1994.
[6] Obeyesekere, M.N., Herbert, J.R., and Zimmerman, S.O., A model of the G1 phase of the cell cycle incorporating cyclin E/cdk2 complex and retinoblastoma protein, *Oncogene*, 11(6):1199-1205, 1995.
[7] Kohn, K.W., Functional capabilities of molecular network components controlling the mammalian G1/S cell cycle phase transition, *Oncogene*, 16(8):1065-1075, 1998.
[8] Aguda, B.D. and Tang, Y., The kinetic origins of the restriction point in the mammalian cell cycle, *Cell Prolif.*, 32(5):321-335, 1999.
[9] Qu, Z., Weiss, J.N., and MacLellan, W.R., Regulation of the mammalian cell cycle: a model of the G1-to-S transition, *Am. J. Physiol. Cell Physiol.*, 284(2):C349-C364, 2003.
[10] Alberghina, L., Rossi, R.L., Querin, L., Wanke, V., and Vanoni, M., A cell sizer network involving Cln3 and Far1 controls entrance into S phase in the mitotic cycle of budding yeast, *J. Cell Biol.*, 167(3):433-443, 2004.

[11] Rossi, R.L., Zinzalla, V., Mastriani, A., Vanoni, M., and Alberghina, L., Subcellular localization of the cyclin dependent kinase inhibitor Sic1 is modulated by the carbon source in budding yeast, *Cell Cycle*, 4(12):1798-1807, 2005.
[12] Bell, S.P. and Dutta, A, DNA replication in eukaryotic cells. *Annu. Rev. Biochem.*, 71:333-374, 2002.
[13] Takeda, D.Y. and Dutta, A, DNA replication and progression through S phase, *Oncogene*, 24(17):2827-2843, 2005.
[14] Wells, W.A., Does size matter?, *J. Cell Biol.*, 158(7):1156-1159, 2002.
[15] Rupes, I., Checking cell size in yeast, *Trends Genet.*, 18(9):479-485, 2002.
[16] Raghuraman, M.K., Winzeler, E.A., Collingwood, D., Hunt, S., Wodicka, L., Conway, A., Lockhart, D.J., Davis, R.W., Brewer, B.J., and Fangman, W.L., Replication dynamics of the yeast genome, *Science*, 294(5540):115-121, 2001.
[17] Wyrick, J.J., Aparicio, J.G., Chen, T., Barnett, J.D., Jennings, E.G., Young, R.A., Bell, S.P., and Aparicio, O.M., Genome-wide distribution of ORC and MCM proteins in *S. cerevisiae*: high-resolution mapping of replication origins, *Science*, 294(5550):2357-2360, 2001.
[18] Newlon, C.S., Petes, T.D., Hereford, L.M., and Fangman, W.L., Replication of yeast chromosomal DNA, *Nature*, 247(5435):32-35, 1974.
[19] Petes, T.D. and Williamson, D.H., Fiber autoradiography of replicating yeast DNA, *Exp. Cell Res.*, 95(1):103-110, 1975.
[20] Lord, P.G. and Wheals, A.E., Variability in individual cell cycles of *Saccharomyces cerevisiae*. *J. Cell Sci.*, 50:361-376, 1981.
[21] Vanoni, M., Vai, M., Popolo, L., and Alberghina, L., Structural heterogeneity in populations of the budding yeast *Saccharomyces cerevisiae*. *J. Bacteriol.*, 156(3):1282-1291, 1983.
[22] Vanoni, M., Rossi, R.L., Querin, L., Zinzalla, V., and Alberghina, L., Glucose modulation of cell size in yeast. *Biochem. Soc. Trans.*, 33(Pt 1):294-296, 2005.
[23] Johnston, G.C., Singer, R.A., Sharrow, S.O., and Slater, M.L., Cell division in the yeast *Saccharomyces cerevisiae* growing at different rates, *J. Gen. Microbiol.*, 118:479-484, 1980.
[24] Rivin, C.J. and Fangman, W.L., Cell cycle phase expansion in nitrogen-limited cultures of *Saccharomyces cerevisiae*, *J. Cell Biol.*, 85(1):96-107, 1980.
[25] Rivin, C.J. and Fangman, W.L. Replication fork rate and origin activation during the S phase of *Saccharomyces cerevisiae*, *J. Cell Biol.*, 85(1):108-115, 1980.
[26] Cipollina, C., Alberghina, L., Porro, D., and Vai, M., SFP1 is involved in cell size modulation in respiro-fermentative growth conditions, *Yeast*, 22(5):385-399, 2005.
[27] Mendenhall, M.D., An inhibitor of p34CDC28 protein kinase activity from *Saccharomyces cerevisiae*, *Science*, 259(5092):216-219, 1993.
[28] Schwob, E., Bohm, T., Mendenhall, M.D., and Nasmyth, K., The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in *S. cerevisiae*, *Cell*, 79(2):233-244, 1994.
[29] Lengronne, A. and Schwob, E., The yeast CDK inhibitor Sic1 prevents genomic instability by promoting replication origin licensing in late G1**,** *Mol. Cell*, 9(5):1067-1078, 2002.

[30] Dirick, L., Bohm, T., and Nasmyth, K., Roles and regulation of Cln-Cdc28 kinases at the start of the cell cycle of *Saccharomyces cerevisiae*, *EMBO J.*, 14(19):4803-4813, 1995.

[31] Tyers, M., Tokiwa, G., Nash, R., and Futcher, B., The Cln3-Cdc28 kinase complex of *S. cerevisiae* is regulated by proteolysis and phosphorylation, *EMBO J.*, 11(5):1773-1784, 1992.

[32] Costanzo, M., Nishikawa, J.L., Tang, X., Millman, J.S., Schub, O., Breitkreuz, K., Dewar, D., Rupes, I., Andrews, B., and Tyers, M., CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast, *Cell*, 117(7):899-913, 2004.

[33] Porro, D., Brambilla, L., and Alberghina, L., Glucose metabolism and cell size in continuous cultures of *Saccharomyces cerevisiae*, *FEMS Microbiol. Lett.*, 229(2):165-171, 2003.

[34] Alberghina L., Porro D., Cazzador L., Towards a blueprint of the cell cycle, *Oncogene*, 20(9):1128-1134, 2001.