Chapter 6

Budding yeast cell cycle

6.1 Introduction

Yeast, and most particularly budding yeast, has for long been a reference model system for the study of the cell cycle control mechanism. *Saccharomyces cerevisiae* is a simple, unicellular organism, used from time immemorial for brewing and bakery - hence its common name of brewer’s or baker’s yeast. In contrast with the regulatory network controlling the cell cycle in mammals, which is poorly known in regard to its complexity, the budding yeast cell cycle network has been extensively studied. During the 1970s, large mutation screens have been carried on, facilitated by the possibility to grow haploid cells, thereby avoiding the pitfall of recessive mutations. Many key regulators of cell division have been discovered in this context (Simchen, 1978). Consequently, yeast cell cycle also constitutes a system of choice to develop or assess computational methods dedicated to the dynamical modelling of biological regulatory networks.

Several models published by the groups of Béla Novák and John Tyson deal with this organism. Most particularly, a model published by Chen *et al.* (2004) represents one of the most comprehensive attempts to model the budding yeast cell cycle regulatory network (see Figure 3.4 in this manuscript). Other articles focusing on specific control modules, such as the morphogenetic checkpoint (Ciliberto *et al.*, 2003, see Figure 6.1) and the FEAR and MEN pathways (Queralt *et al.*, 2006, see Figure 6.2) have been published by the same groups. The possibility of grafting the morphogenesis checkpoint module to the core model was already discussed in (Chen *et al.*, 2004); however, the authors warned that it would not be an easy step, and indeed no coupled model has been published so far – although in a recent paper, Randhawa *et al.* (2008) announced that the coupling of the two modules had been successfully carried on and was about to be published. However, when we started the project, the perspective of a coupled model encompassing the core model, the MCP module and the FEAR and MEN network still appeared quite remote.

The development of modelling frameworks enabling the definition and analysis of large and complex regulatory networks is a challenging problem. Although the appropriateness of a modular strategy is recognised (Hartwell *et al.*, 1999; Snoep *et al.*, 2006), modelling frameworks supporting systematic composition still need to be properly defined. One promising strategy to tackle this issue relies on a modular approach to regulatory networks, which allows the identification of functional modules that can
be separately modelled and analysed. Then, to obtain comprehensive networks, one needs to compose submodels. In this respect, in Randhawa et al. (2008), the authors present a tool to guide the modeller in the course of model composition which they apply to retrieving the generic model published by Tyson and Novak (2001). Within the logical framework, such a modular approach has been used to build multicellular models from the serialisation of non-overlapping, cellular network modules (Schaub et al., 2007; Sánchez et al., 2008).

In this context our aim was double: first, use the Chen model as a benchmark for our modelling approach; indeed, with its 26 variables/species and more than 130 documented mutants, the Chen model represented a real challenge. Second, use this model as a blueprint to set the basis of a more comprehensive model. Our aim was to create a more comprehensive and realistic model by plugging additional control modules, and updating parts of the model after new evidence had arisen.

Our logical version of this model, presented in Fauré et al. (2009), successfully accounts, in qualitative terms, for the behaviour of these many mutants. To this core model, I have coupled the morphogenesis checkpoint module adapted from Ciliberto et al. (2003), and updated the subnetwork with the exit module adapted from Queralt et al. (2006). The resulting model encompasses the main features of its constituents, including the simulation of more than 150 different perturbations (see Part 9.1 for further discussion).
6.1. INTRODUCTION

Figure 6.2: Wiring diagram of the exit from mitosis module published by Queralt et al. (2006). Release of Cdc14, that triggers Clb2 degradation and thus mitotic exit by activating Cdh1, is indirectly coupled to sister chromatids separation via the activation of Separase. Compare with Figure 3 in Fauré et al. (2009).
The coupling method proposed in this article is facilitated by the use of compact logical expressions instead of logical parameters whenever useful. This feature will be implemented in the next public versions of our software GINsim. In the near future, we also plan to formalise the systematic method for composition and to implement it into GINsim.
Modular Logical Modelling of the Budding Yeast Cell Cycle

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Abstract

Systems biologists are facing the difficult challenge of modelling and analysing regulatory networks encompassing numerous and diverse components and interactions. Furthermore, available data sets are often qualitative, which complicates the definition of truly quantitative models. In order to build comprehensive and predictive models, there is clearly a need for incremental strategies, enabling the progression from relatively small to large scale models. Leaning on former models, we have defined a logical model for three regulatory modules involved in the control of the mitotic cell cycle in budding yeast, namely the core cell cycle module, the morphogenetic checkpoint, and a module controlling the exit from mitosis. Consistency with available data has been assessed through a systematic analysis of model behaviours for various genetic backgrounds and other perturbations. Next, we take advantage of compositional facilities of the logical formalism to combine these three models in order to generate a single comprehensive model involving over thirty regulatory components. The resulting logical model preserves all relevant characteristics of the original modules, while enabling the simulation of more sophisticated experiments.

1 Introduction

The development of modelling frameworks enabling the definition and analysis of large and complex regulatory networks is a challenging problem. One promising strategy to tackle this issue relies on modularity of regulatory networks, which allows the identification of functional modules to be separately modelled and analysed. Such models have then to be composed to obtain comprehensive networks. Within the logical framework, such a modular approach has been used to build multicomponent models from non-overlapping, cellular network modules (Schaub et al., 2007; Sánchez et al., 2008). Here we explore the coupling of partly overlapping modules involved in cell cycle control.

The cell cycle is the process by which a cell replicates its genetic material and divides itself into two daughter cells. This regulatory system relies on an intricate molecular network, that is highly conserved among eukaryotes. The core cycling engine is completed by a set of checkpoints placing cell division under external control and ensuring that every single step has been completed before the next one begins, such that, for example, sister chromatids are not separated until chromosomes are correctly aligned on the metaphase plate.

Most of these mechanisms have been well characterised in the budding yeast. In mammals (and multicellular organisms in general), an additional layer of controls coordinates the cell growth and division with the needs of the whole organism, and disruptions of these checkpoints constitute important steps towards cancer. The considerable amount of data available on molecular details and mutant phenotypes makes budding yeast an appealing system to develop a model of the eukaryotic cell cycle control.

Indeed, the cell cycle core engine has been modelled in great detail, most notably by the groups of Béla Novák and John Tyson, using a differential formalism. Several models focusing on different regulatory modules have been developed (Goldbeter, 1991; Tyson, 1991; Romond et al., 1999; Tyson et al., 2001; Pomerening et al., 2003; Ciliberto et al., 2005; Pomerening et al., 2005; Csikász-Nagy et al., 2006; Calzone et al., 2007; Novák et al., 2007, and references therein). In parallel, the lack of supporting quantitative data that hampers the development of differential models, as well as the numerical instabilities inherent to large non linear systems, motivated the development of simplified Boolean models of the core...
cycling engine (Li et al., 2004; Fauré et al., 2006; Davidich and Bornholdt, 2008a). However, Boolean modelling appears too crude to properly account for several subtle aspects of cell cycle control, such as the effect of cellular mass. The use of a multi-level logical formalism offers a good compromise between Boolean drastic simplification and daunting quantitative models. Moreover, the use of a logical formalism facilitates the development of more integrated models, through the articulation of control modules with the core cell cycle engine. Hereafter, we present three logical (multi-level) models for the core cycling engine (from now on, the core model), the morphogenetic checkpoint (MCP module), and a module rewiring the FEAR (Cdc Fourteen Early Anaphase Release) and MEN (Mitotic Exit Network) networks (exit module). These three models heavily rely on previous modelling studies by the groups of Novák and Tyson (Chen et al., 2004; Ciliberto et al., 2003; Queralt et al., 2006), which constitute a challenging benchmark for our modular approach. Once validated through a systematic analysis of their behaviours for wild-type and numerous mutant situations, these modules are then integrated into a comprehensive logical model (from now on, the coupled model) through a simple formal procedure. The resulting model preserves the main dynamical features of each of the three modules. Furthermore, this model allows the recovery of additional reported properties involving components belonging to different modules.

2 Results

2.1 Logical modelling of the core cycling engine.

In a first step, we have defined a logical version of the model for the core cell cycle engine of the budding yeast published by the groups of Novák and Tyson (Chen et al., 2004). This first step was also a benchmark for our modelling approach to the cell cycle, as building a qualitatively valid logical adaptation of such a complex differential model is not a trivial task (see materials and methods). Presented in Figure 1, the resulting logical model is thus 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.

Following the principles described in Section 4 and using well defined logical rules for the updating of each considered regulatory node (cf. examples in Tables 3 and 4, and the supplementary material for a complete listing of these rules), the simulation of this model qualitatively recapitulates the wild-type sequence of events the cell has to go through to be considered viable: firing of the origins of replication (ORI goes up), spindle alignment (SPN goes up), separase activation (Esp1 goes up), division (CYTOKINESIS goes up to level 2) after the formation of a bud (BUD must have reached level 1, although it may go down afterwards before cell division), and origin relicensing (ORI goes down). The different phases of the cycle are defined in terms of the levels of activity of key components, as follows: low Clb5 and Clb2 activity (either =0 or sequestered by Sic1 / Cdc6) define a G0/G1 phase; high Clb5 activity (i.e. not sequestered by the CKI) and low Clb2 activity define S/G2 phase; high Clb2 activity defines M phase, which can be further divided into prophase/metaphase (low Esp1 activity) and anaphase/telophase (high Esp1).

Many different mutants (gene knock-outs, partial loss-of-functions, temperature-sensitive mutations, ectopic and over-expressions) or perturbations (e.g. culture in presence of drugs such as nocodazole), have been analysed experimentally and reported in the literature. Over 130 of these mutants have been considered by Chen et al. In the present article, we set out to reproduce their results with our logical version of the model. Mutant dynamical behaviours have been systematically computed on the basis of the wild-type model, using a functionality of our software GINsim enabling the definition and recording of multiple mutants in terms of logical rules. Mutant simulations are evaluated qualitatively regarding viability (according to the above sequence of events), or arrest in a particular phase of the cell cycle. As shown in the supplementary material, dynamical analysis of most of the individual or multiple perturbations leads to results qualitatively consistent with reported phenotypes, as well as with the results published by Chen et al. (2004).

Only four cases led to major discrepancies: the mutant cln1Δcln2Δcln3Δcdh1Δ, which should be arrested in telophase, appears to be viable in our simulations; so do the pds1Δcdc20Δ and CLB5-dbΔpds1Δcde20Δ mutants. Finally, in our simulations, the mutant CLB2-dbΔclb5Δ on galactose appears to be arrested in telophase, as when grown in glucose, whereas it should be viable in the slow-growth rate medium. The problems encountered here are linked to the importance of synthesis rates in the phenotypes of these mutants. This is clearly exemplified by the case of the CLB2-dbΔclb5Δ mutant, whose viability depends on the growth medium. Such characteristics are hard to account for in the logical formalism. Other minor discrepancies relate to timing issues for some mutants, especially over the issue of BUD formation, which in Chen et al. model heavily re-
Figure 1: Logical regulatory graph for the core engine controlling cell cycle in the budding yeast derived from Chen et al. (2004). In this model, Cln2 represents both Cln1 and Cln2, Clb5 both Clb5 and Clb6, and Clb2 both Clb1 and Clb2; Cdc28, the kinase partner of the cyclins, is implicit. A newborn daughter cell is stuck in a G1 state characterised by an absence of cyclin activity, which is kept low by the CDK inhibitors (CKI) Sic1 and Cdc6, and by the APC activator Cdh1. The cell must grow up to a certain size (MASS) in order to produce enough Cln3 and Bck2 and turn the MBF and SBF transcription factors on. Their cyclin targets Cln2 and Clb5 then begin to accumulate. Finally, Cln2 activates the synthesis of the bud (BUD), but Clb5 activity is impaired by the CKI Sic1. Once this inhibition is relieved by the action of Cln3, Bck2 and Cln2, Clb5 will help Cln2 and Cln3 to inhibit Cdh1. In the meantime, Clb5 initiates the replication (ORI). Once Cdh1 is off, Clb2 is activated in a positive feedback loop with its transcription factor Mcm1. Clb2 shuts the MBF and SBF off, reinforces the inhibition of the CKI and Cdh1, and plays a role in the assembly and maintenance of the mitotic spindle. Once the spindle is complete, the checkpoint is relieved, and activated Cdc20 initiates mitosis. Cdc20 degrades Pds1, while free Esp1 separates sister chromatids; Cdc20 also degrades Clb5 and initiates the degradation of Clb2, an important step for Cdh1 and the CKI reactivation. Cdc20 would also promote the degradation of a (hypothetical) phosphatase PPX that plays a role in Net1 activation. As the spindle checkpoint is relieved, Net1 is also inhibited by Cdc15. In the absence of its competitive inhibitor, Cdc14 can re-activate Cdh1 and CKI, which resets the cell to its initial G1 state by inhibiting the cyclins. Graphical conventions: Boolean and multi-level nodes are denoted by circles and rectangles, respectively; activatory versus inhibitory interactions are denoted by green normal versus red T-headed arrows; black, circle-headed arrows denote context-depending signs; thick and thin arcs as in Figure 5.
lies on bud synthesis rate; more elaborate priority rules might help solve these problems in a logical context (see the supplementary material for a detailed presentation of the mutants).

2.2 Logical modelling of the morphogenetic checkpoint module

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). Presented in Figure 2, this model accounts for the fact that the cell cycle is temporarily blocked in G2 phase in case of budding defect. This G2 blocking can be bypassed in the presence of high Clb2 activity level, which, according to Ciliberto et al., correlates with the growth of the cell. Consequently, nuclear division occurs without cell division, thereby giving rise to dimucleate 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 (Ciliberto et al., 2003, and references therein). Our logical model of the MCP recapitulates the wild-type and knockout phenotypes considered by Ciliberto et al. (2003), as well as three additional knockout mutants described by 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 of the possible values of MASS, when BUD formation is allowed or impaired (six different situations in total, all described in the corresponding section of the supplementary material). In a wild-type context, Clb2 can be fully activated (\( \text{Clb2} = 2 \)) for \( \text{MASS} = 1 \), whereas when budding is impaired (\( \text{BUD} = 0 \)), \( \text{MASS} \) must cross its second threshold and reach level 2 to force the activation of Clb2. In this latest situation, we do obtain a second stable state with \( \text{Clb2} = 2 \) and \( \text{MASS} = 1 \), which can be considered an artefact of the isolation of the MCP module. In fact, all paths leading to this stable state involve an activation of Clb2 before the activations of MBF and Swe1, which does not happen in a normal cycle. As we shall see, this artefactual stable state can be eliminated through a proper coupling of the MCP module with the core engine model. We have then coupled the MCP module to the core model, following the procedure detailed in the material and methods section. The coupled model preserves all the properties mentioned above for the wild-type and loss-of-function backgrounds of the core and MCP modules. As hinted above, the artefactual activation of Clb2 before BUD formation for \( \text{MASS} = 1 \) observed in the isolated MCP module disappears under the updating assumption considered for the coupled model. Furthermore, the behaviour of the MCP module now constraints that of the core model, in particular regarding the timing of bud formation. Such behaviour is difficult to assess with a logical model; nonetheless, in the coupled model, as long as \( \text{MASS} \) is kept in the lowest priority class, the activation of Clb2, and thus the G2/M transition, is conditioned to bud formation, which was not the case in the core model, as hinted by Chen et al. (2004) (see also Table 2).

2.3 Logical modelling of the mitotic exit module.

In their differential model of the core engine, Chen et al. introduced the hypothetical PPX phosphatase to account for the sequential activation of Cdc20 and Cdh1 (Chen et al., 2004). Recent evidence about the FEAR pathway was mentioned in the article, but had come out too late to be integrated in their model. Recently, Queralt et al. proposed a model accounting for the role of separase in the initiation of mitotic exit (Queralt et al., 2006). We rely on their model to propose a logical model of mitotic exit and replace the PPX mechanism in our logical model of the core engine with this more detailed version.

Briefly, when securin is degraded by Cdc20 at anaphase onset, free separase cleaves the cohesin that maintains sister chromatids together and downregulates PP2A\(^{Cdc55}\), a phosphatase that opposes Net1 phosphorylation by Clb2. Downregulation of PP2A\(^{Cdc55}\) 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.
Figure 3: Logical regulatory graph for the module controlling the exit from mitosis. At anaphase onset, Cdc20 is activated. It degrades the securin (Pds1), setting the separase (Esp1) free, and initiates the degradation of Clb2. Free separase then inactivates the PP2A phosphatase, initiating the phosphorylation of Net1, a competitive inhibitor of the Cdc14 phosphatase. This partial release of Cdc14 allows the activation of Cdc15, which together with Tem1 fulfills the inhibition of Net1, achieving the complete release of Cdc14. Free Cdc14 then activates Cdh1, which completes the degradation of Clb2, thus achieving mitotic exit. Graphical conventions as in Figure 1.

Our model (Figure 3) qualitatively recapitulates the behaviours of the wild-type situation and of nine reported mutants (cf. supplementary material). In each case, we have tested mitotic exit, i.e. we have checked whether a stable state corresponding to G1 (with active Cdh1 and low Clb2) can be reached from the state corresponding to metaphase (high Clb2, no Cdh1).

The procedure to couple the exit module to the core engine is described in the material and methods section. The coupled model (Figure 4) preserves the behaviour observed for the wild-type core module, as well as the properties of numerous mutations, such as those implied in the G1/S transition and in the MCP (the Table 1 displays a simulation of the wild-type cell cycle). The coupled model now further fits the data that were used to build the exit module. In particular, inactivation of separase (the esp1ts mutant mentioned by Chen et al. (2004)) now provokes an arrest in telophase, which is consistent with the results reported by Queralt et al. (2006). Selected mutant simulations are listed and commented in Table 2, while a summary of the results of all mutant simulations can be found in the supplementary material.

3 Discussion

Although the appropriateness of a modular strategy is widely advocated, modelling frameworks supporting systematic composition are still lacking. A first step in this direction can be found in Randhawa et al. (2008), where the authors present a tool to ease model composition, with an application to the generic model published by (Tyson et al., 2001b). Leaning on previous dynamical modelling efforts by the groups of Novák and Tyson, the present article focuses on the development of a modular modelling approach and its application to the integration of three multi-level logical models for regulatory modules involved in the control of yeast cell cycle.

The first of these models is the most comprehensive and already implements regulatory mechanisms involved in the spindle checkpoint, the G1/S transition, as well as a preliminary version of the exit control module. The morphogenesis checkpoint module constrains activation of Clb2, and thus entry into mitosis, with respect to bud formation. Lastly, the exit module integrates recent data on the role of separase and securin in the release of Cdc14, thereby driving mitotic exit. Although these three differential models have been developed by the same groups, they were not yet explicitly integrated into a unique model. Indeed, such an integration appears really challenging in the differential framework, as numerical instabilities arise when the number of variables and the number of nonlinearities increase.

When applying the composition method described in subsection 4.3, the resulting comprehensive model preserves the essential properties of the three modules, while enabling the simulation of perturbations involving components that belong to several modules. Provided that these modules have been modelled with the prospect of their integration in mind, a systematic integration method can be defined. Our logical framework is thus suited for incremental modelling strategies and eases the concerted refinement of the different modules composed to build a comprehensive model. Module integration is further facilitated through the development of novel GIINsim functionalities enabling the copying and pasting of (sub)networks (along with thresholds and logical rules) from one file to another, or yet the automatic computation of stable states for all pre-defined perturbations.

For each module, logical rules have been derived on a case-by-case basis, using differential equations as a template to determine positive and negative regulatory influences and infer distinct qualitative levels. Based on this experience, it would be particularly interesting to delineate generic rules to ease the translation of differential models into logical ones. A first step in this direction can be found in (Davidsich and Bornholdt, 2008b), where the authors introduce a systematic Boolean transposition of an ODE model, which recapitulates its most salient qualitative dynamical properties.
Figure 4: Logical regulatory graph for the coupled model, encompassing the core engine (Figure 1), the MCP module (Figure 2) and the exit module (Figure 3). Compare with Figure 1. Graphical conventions as in Figure 1.
Table 1: Simulation of the coupled model in the wild-type condition. The model is simulated using a set of synchronous priority classes 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 (Cf. supplementary material and model file for further details).

Table 2: Example of mutant simulations where the coupled model shows improved consistency with biological data.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>GAL-CLB2</td>
<td>In the core model, there is no constraint on BUD formation, and these mutants can divide despite the absence of bud. In the coupled model, the morphogenesis checkpoint delays entry into mitosis long enough for the cell to grow a bud.</td>
</tr>
<tr>
<td>GAL-CLB2 sic1Δ</td>
<td></td>
</tr>
<tr>
<td>GAL-CLN2 cln1Δ cln2Δ cdh1Δ</td>
<td>In the core model, the esp1ts mutant displayed sustained oscillations, consistent with the simulations reported in Chen et al. (2004). In the coupled model, this mutant arrests in telophase, consistent with the results reported by Queralt et al. (2006)</td>
</tr>
<tr>
<td>Separse inactivation</td>
<td>In the core model, this mutant could exit mitosis with sister chromatids still attached, consistent with the simulations presented by Chen et al. (2004). In the coupled model, due to the constraints on Cdc55 introduced by the exit module, activation of separase is required for mitotic exit.</td>
</tr>
<tr>
<td>CLB1 clb2Δelta</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Example of mutant simulations where the coupled model shows improved consistency with biological data.
The results presented here confirm that the qualitative knowledge of the regulatory network of the system is sufficient to explain most of its dynamical properties, thus pointing once more to cell cycle network robustness (Chen et al., 2004; Li et al., 2004). However, detailed analysis of the mutants reveals that, in some cases, incorporation of refined information about expression or activity levels, or yet synthesis or growth rates may be necessary to fully reproduce some properties of the system. Mainly relying on qualitative information, the logical formalism used here offers a flexible mean to integrate this information into discrete dynamical models, including the possibility to use multi-level components whenever this is functionally justified. Wether the definition of subtler updating rules (priorities, delays) could enable the modelling of finer kinetic behaviours remain to be assessed.

Finally, although logical models can be used independently to gain understanding of the articulation of the different modules in comprehensive models and predict the concerted behaviour of components belonging to different modules, they can serve in turn as scaffolds to develop more quantitative (differential or stochastic) models. In this respect, GINsim enables the conversion of logical models into Petri nets, thereby providing an access to complementary analysis methods (e.g. model checking tools), or yet to hybrid or stochastic extensions (Cl. Mura and Csikász-Nagy, 2008) for an application of Stochastic Petri nets to a simplified model of budding yeast cell cycle).

4 Materials and methods

4.1 The logical modelling framework

The main features of the logical approach used are defined in Thomas and D’Ari (1990); Thomas et al. (1995); Chaouiya et al. (2003). Briefly, a logical model is defined by a regulatory graph, where the nodes and arcs represent the regulatory components and interactions, respectively. To each node is associated an integer interval (from 0 to max) defining discrete levels of activity, and each arc is accordingly associated to an interval defining the set of values for which its source may influence its target. The dynamical behaviour of each node is then defined by logical functions (also represented in terms of logical parameters), which associate a target value for this node with each combination of regulators. The dynamics of the system is represented in terms of a state transition graph, where the nodes denote states of the system (i.e., vectors giving the levels of activity of all the variables), and the arcs denote state transitions (i.e., changes in the value of one or several variables, depending on the values of the relevant logical functions). When several transitions are enabled, the resulting dynamical pathway depends on the updating assumption selected: synchronous, asynchronous, or user-defined through the setting of priority classes (Fauré et al., 2006). Time is thus implicitly defined by the sequence of transitions. For several years, our group has been developing a software suite, GINsim, to facilitate the definition and the dynamical analysis of such logical models (González et al., 2006). In particular, this software enables the definition and storage of different initial states and alternative genetic backgrounds (i.e., mutants). For more details, see the GINsim web site at the url http://gin.univ-mrs.fr/GINsim.

4.2 Logical modelling of cell cycle control mechanisms

Direct activations or inhibitions (post/transcriptional regulations, including protein de/phosphorylations or degradations) are simply represented by regulatory arcs from the regulator to the target gene or protein, together with the definition of consistent logical rules for the regulated target. Although the logical formalism is particularly well suited to represent regulatory interactions (activations, inhibitions), it is less adapted to the representation of mass flow, and in particular of multiproteic complex formation. Consequently, we usually represent the complexes implicitly: a complex is considered present if all its components are present; all components, whether regulatory or enzymatic subunits, regulate the targets of the enzymatic member, with a logical AND rule – or AND NOT in the case of sequestration (cf. Figure 5). In the models presented here, this is the case for the sequestration of cdc14 by Net1, the inhibition of Net1 by Cdc15 and Tem1, the sequestration of Tem1 by Bub2-Bfa1, the sequestration of separase by securin in the exit module, and the sequestration of Cib5 and Cib2 by Cdc6 and Sic1. In the latter case, for example, we have represented inhibition of the Cibs by introducing arcs from the CKI towards the Cibs targets, so that inhibition of the Cibs is represented by an inhibition of the components they activate and by an activation of the components they inhibit. In addition, for the sake of simplicity, complex subunits that are not dynamically regulated are usually not represented (e.g., the different CDKs), unless we want to test mutations (as in the case of Cdc14). Regarding the number of thresholds considered, we started with Boolean models (Fauré et al., 2006), which were progressively refined through the consideration of a minimal number of additional levels of activity for some regulatory components (rectangular nodes in Figures 1-4) in order to solve discrepancies between some mutant behaviours and published data. Beyond the representation of specific molecular actors (regulatory proteins and complexes), the models considered encompass a series of phenomenological variables: MASS, CYTOKINESIS, BUD, ORI, SPN, denoting cel-
Figure 5: Complex formation. **Left:** wiring diagram representing the sequestration of a molecule A by a molecule B. Free A activates the synthesis of C and the degradation of B; in complex with B, A is inactive. **Right:** the corresponding regulatory graph. A directly activates C and inhibits B (thick arrows); B sequesters A, which is represented by thin arrows of the opposite sign directed to A’s targets.

| Logical growth, cell division, the formation of the bud, the firing of the origins of replication and the formation of the spindle, respectively. Here, different activity levels are associated with qualitatively different effects. Although the evolution of these components is still incremental (**i.e.* one can not go directly from level 0 to level 2), they do not directly represent quantitative information.

In Chen et al. (2004), the mass of the cell is hypothesised to drive the cycle by amplifying synthesis, thus increasing the concentration of the cyclins, that belong to the nuclear compartment, whose volume is constant. In our models, MASS is represented by a multi-level node. A first threshold represents the minimal level above which a normal cell can go through the G0/G1 transition (which involves the inhibition of the CKI, depending on the activation of Cln3 and Bck2). A second threshold is considered in the context of the implementation of the morphogenetic checkpoint that controls the G2/M transition, to represent the requirement of higher cellular mass to bypass the checkpoint when budding is impaired.

Following Chen et al., we consider that mitosis is triggered when Cln2 level decreases below a specific threshold (Chen et al., 2004). In our model, a high level (levels 2 or 3) of Cln2 thus enables the node CYTOKINESIS to reach the value 1. The CYTOKINESIS node will reach its highest level 2 only after Cln2 inactivation. The MASS, BUD and SPN nodes are then reset to zero, before the CYTOKINESIS node also reaches its bottom level.

A similar mechanism is implemented for the control of the relicensing of replication origins (ORI).

As our focus in this paper lies in the delineation and validation of an efficient module composition method, we have kept the treatment of concurrent molecular processes as simple as possible, while preserving a qualitative matching between model simulation and the order of events documented in the literature or explicitated in previous models. Consequently, all transitions have been treated synchronously, excepting the special cases of CYTOKINESIS and MASS updateings. Indeed, a correct sequential in/activation of these two phenomenological nodes requires the definition of specific priority classes. The resulting model thus implements four priority classes: the first priority class (highest priority) contains the CYTOKINESIS up-regulation as unique member; the third class contains CYTOKINESIS down-regulation; the fourth class (lowest priority) contains MASS up-regulation; finally, all the other transitions are grouped into the second class and are treated synchronously.

For each model, mutants are defined as sets of alternative logical rules for particular components of the system. These alternative rules can then be used in replacement of the default (**i.e.* wild-type) rules of the model. This allows us to analyse the behaviour of the system for various alternative genetic backgrounds, encompassing gene knock-outs, over-expressions, ectopic expressions, or yet non-degradable forms of one or several components. The logical rules for wild-type and all mutants for each model are described in the supplementary material.

### 4.3 Coupling procedure

#### 4.3.1 Principles

Leaning on the properties of the logical formalism, we have delineated a systematic method to merge logical modules (or submodels) into comprehensive models. The first step consists in the definition of the comprehensive regulatory graph. In the case of the coupling of a completely novel module (**e.g.* the MCP module), this regulatory graph simply encompasses all the components and interactions from the original modules (MCP module and core engine). In the case of the replacement of part of model with a more detailed, or updated version (**e.g.* the exit module), the corresponding nodes and interactions of the original model (**e.g.* the core engine) are replaced by the more detailed graph.

Next, the logical rules associated with the nodes of the new graph are defined as follows. The rules of the nodes that are present in only one model are left unchanged, as their activities are already consistently defined. In contrast, the rules associated to nodes that are present in two modules must be modified to integrate the effects of all regulators. In the models presented, several components of the core, MCP and exit modules amount to simplifications of their counterparts in another module (for example, MBF and BUD in the MCP module, Cdc15 in the core model, or Cln2, Cdc20 and Cdh1 in the exit module). In these cases, it is the most detailed rule that is conserved in the coupled model. Moreover, when
a component receives input from regulators that belong to different modules, as in the case of Clb2 present in both the MCP module and the core engine, or in the case of Net1 and Bub2-Bfa1 present in both the exit module and the core engine, novel logical formulae are systematically defined through proper combinations of the original ones. We proceed in two steps: (i) the thresholds are first interpolated; (ii) the logical formulae associated with each logical value are then combined (logical AND). Finally, accordingly with our modelling of complex formation (cf. Materials and methods), we have to take into account both partners of a complex in the regulation of their targets, as in the case with the sequestration of Clb2 by Sic1 and Cdc6.

4.3.2 Coupling of the MCP module to the core engine

In order to define a comprehensive model encompassing the two logical modules already defined, we proceed in the following way. First, all the components considered in only one module are simply conserved as such, i.e. with identical logical rules, level numbers and interactions, with the exception of Clb2 targets. In this case, we have to take into account the sequestration of Clb2 by the CKIs, which were not present in our MCP module. This means that, in the new logical formulae for Swe1 and Mih1, Clb2 (presence of Clb2) has to be replaced by Clb2 \( \land \{ \text{Sic1 OR Cdc6} \} \) (i.e., “Clb2 AND NOT (Sic1 OR Cdc6)”), meaning that Clb2 has an enzymatic action on its targets only when not sequestered by Sic1 or Cdc6. Four components are involved in both modules: MASS, MBF (MBF_SBF in the core model), BUD and Clb2. BUD and MBF are Boolean components in both models, and their regulation in the MCP model is a simplification of their regulation in the core model; accordingly, we retain the more detailed definitions of the core engine in the coupled model.

The case of MASS is slightly more complex, as it is Boolean in the core engine model, while it has an additional level in the MCP model. Consequently, MASS is also endowed with two significant levels of activity in the coupled model. This means that MASS can still regulate Clb2 differentially at levels 1 and 2, as it does in the MCP module, and will have the same effect on its other targets all along the \([1,2]\) interval. In the MCP model, MASS is only self-regulated. As in the core model, MASS in the coupled model is regulated by the CYTOKINESIS component, and takes its maximum value (1 in the original core model, 2 in the coupled model) when CYTOKINESIS is below its threshold. In addition, as we put MASS growth in lowest priority class, it will increase only if growth is necessary for cell cycle progression.

The case of Clb2 is more complex. In the core model, it receives input from MASS, Cdh1, Cdc20, and Mcm1, and can take three non-zero values. In the MCP model, Clb2 receives input from MASS, Swe1 and Mih1, and can take two non-zero values. First, we have to establish a correspondence between Clb2 levels of activity in the core and in the MCP models. We use the inhibition of MBF, which is common to both models, as a reference to set the threshold of activity of Clb2 on Swe1 in the coupled model at level 2. Consequently, the threshold of activity of Clb2 on Mih1 is set at level 1. In the coupled model, Clb2 thus has three levels of activity, as in the core model. The logical rules for Clb2 are then determined, for levels 2 and 3, by a logical AND between the rules for levels 2 and 3 of the core and level 2 of the MCP models (see Table 3, rules (e) and (g)).

Definition of the rule for level 1 was more delicate, as it was not defined in the core model; based on the role of the inhibitors Cdc20 and Cdh1, we defined the rule for level 1 of Clb2 as a logical AND between the rule for level 1 in the MCP model and the union (logical OR) of the rules for levels 2 and 3 in the core (see Table 3, rule (b)).

4.3.3 Integration of the exit module within the core model

The integration of the mitotic exit module within the core model essentially can be achieved through a re-wiring of a specific part of the core model. The hypothetical phosphatase PPX has to be replaced by the mechanism proposed in the exit module. All the other nodes from the core model can be conserved, and left unchanged, as well as the regulation of PP2A and Cdc5Polo, which are defined only in the exit module.

The regulation of several components of the exit module is a simplification of their regulation in the core model. This is the case for Cdc20, Cdh1, Clb2, Pds1 and Tem1, and we have thus kept the original, detailed rule of the core model for the regulation of these components. In the case of Clb2, which has different threshold configurations in the two modules, a mapping of these thresholds can be determined similarly to what has been done for the coupling of the MCP module. Note that the regulatory rules associated with Pds1 and Tem1 in the core model already encompass the rules defined in the exit module. Furthermore, the Cdc14 node is not affected by the coupling process, as the associated logical rules are identical in both models.

In the coupled model Cdc15 becomes regulated by Cdc14 (and thus Cdc14 competitive inhibitor Net1) and Clb2, according to the rules defined in the exit module. The regulation of Net1 is similarly redefined.

The sequestration of separase by securin, which was treated as a direct inhibition in the core model (since in this model Esp1 did not have any target), is now represented by an opposing effect of securin over the securase target, PP2ACdc55.

In the core model, the Bub2-Bfa1 complex is activated by the spindle assembly checkpoint: i.e., it takes the
Core engine | MCP module | Coupled model
--- | --- | ---

| Incoming interactions | Cdh1, Mcm1, Cdc20[^2][^3], MASS | Cdh1, Mcm1, Cdc20[^2][^3], MASS[^1][^2], Mih1[^1][^2], Swe1[^1][^2] |

Logical rules for the value 1

(a) \( (\text{MASS}[^1] \land \text{Swe1} \land \text{Mih1}[^2]) \lor (\text{MASS}[^2] \land \text{Swe1}[^2] \land \text{Mih1}) \) \( \lor \) \( (\text{MASS}[^1] \land \text{Swe1} \land \text{Mih1}[^2]) \lor (\text{MASS}[^2] \land \text{Swe1}[^2] \land \text{Mih1}) \land (\text{Cdh1} \land \text{Cdc20}[^1] \land (\text{Cdc20}[^2] \lor \text{Mcm1})) \)

Logical rules for the value 2

(c) \( \text{MASS} \land \text{Cdh1} \lor ((\text{Cdc20} \land \text{Mcm1}) \lor (\text{Cdc20}[^2] \lor \text{Mcm1})) \)

(d) \( (\text{MASS}[^1] \land (\text{Swe1} \lor \text{Mih1}[^2])) \lor (\text{MASS}[^2] \land (\text{Swe1}[^2] \lor \text{Mih1})) \)

(e) \( (\text{MASS}[^1] \land (\text{Swe1} \lor \text{Mih1}[^2])) \lor (\text{MASS}[^2] \land (\text{Swe1}[^2] \lor \text{Mih1})) \land (\text{Cdh1} \land ((\text{Cdc20}[^2] \land \text{Mcm1}) \lor (\text{Cdc20} \land \text{Mcm1}))) \)

Logical rules for the value 3

(f) \( \text{MASS} \land \text{Cdh1} \land \text{Cdc20} \land \text{Mcm1} \)

(g) \( (\text{MASS}[^1] \land (\text{Swe1} \lor \text{Mih1}[^2])) \lor (\text{MASS}[^2] \land (\text{Swe1}[^2] \lor \text{Mih1})) \land (\text{Cdh1} \land \text{Cdc20} \land \text{Mcm1}) \)

Table 3: Definition of the logical rules enabling the activation of the multi-level node Clb2 for the core engine (Figure 1), the MCP module (Figure 2) and the coupled model (Figure 4). Clb2 has two (non-zero) activation levels in the MCP module, and three (values 1 to 3) in the core engine and in the coupled model. Specific logical formulae are associated with values 1, 2 and 3 for the different models, thereby defining the conditions enabling the activation of Clb2 up to the corresponding levels. All other situations lead per default to the inactivation of Clb2 (value 0). Note that the value 1 is omitted for the core engine and the coupled model, meaning that this Clb2 level can only transiently occur for these models. In the logical formulae, atomic terms are denoted by the name of the component along with the corresponding interval of values (e.g. \( \text{Cdh1}[^1] \) denotes \( \text{Cdh1} = 1 \); bracketed values are omitted in the case of Boolean nodes, or when all non-zero values are considered). The symbols \( \neg \), \( \land \), and \( \lor \) denote the classical operators NOT, AND and OR, respectively. For example rule (c) states that the target value of Clb2 is 2 when \( \text{MASS} = 1 \) and \( \text{Cdh1} = 0 \) and \( \text{Cdc20} \) and \( \text{Mcm1} \) are both 0 or 1. For the coupled model, the rule (b) (target value 1) is defined as a conjunction of (a) (target value 1) and the disjunction of rules (c) (target value 2) and (f) (target value 3), while rules (e) and (g) are defined as the conjunction of (d) and (c) and the conjunction of (d) and (f), respectively.

value 1 as long as there are replicated chromosomes (\( \text{ORI} = 1 \)) that are not aligned on the metaphase plate (\( \text{SPN} = 0 \)) (see Table 4).

The checkpoint is lifted, and Bub2-Bfa1 is inhibited, when \( \text{SPN} = 1 \). In the exit module, Bub2-Bfa1 is the target of another checkpoint mechanism that monitors the separation of sister chromatids: as long as separase has not been released, Bub2-Bfa1 is kept active by the PP2A phosphatase, in spite of the Polo-like kinase Cdc5. To determine the rule for the coupled model, we have to take into account the fact that, in the system represented in the exit module, chromosomes are replicated and aligned on the metaphase plate: in other terms, the condition \( \text{ORI} = 1 \) and \( \text{SPN} = 1 \) is implicit in the rule for Bub2-Bfa1 activation. Thus, in the coupled model, as long as \( \text{ORI} = 1 \), presence of PP2A or absence of Cdc5Polo is sufficient to keep Bub2-Bfa1 active, whatever the level of SPN.

Finally, we have to take into account the sequestration of Clb2 by the CKI in the new logical formulae for Cdc5Polo, Cdc15 and Net1, similarly to what has been done for Swe1 and Mih1 in the previous section.

### 4.4 Availability

The software \textit{GINsim} implementing the logical formalism and the composition method is freely available to academic groups. Furthermore, we provide the XML files (GINML format) containing the four models (for the three modules plus their composition) on a dedicated webpage (http://gin.univ-mrs.fr/GINsim). This website further summarizes experimental data supporting the interactions considered. Finally, results of our dynamical analyses are provided for about a hundred of different conditions, along with corresponding experimental observations.
### Table 4: Definition of the logical rules for the coupled model (Figure 4) combining the core and exit modules (cf. Figures 1 and 3). Only the situations enabling the activation of the nodes Bub2-Bfa1 and Net1 are displayed. A complete listing of the rules defined for the three modules and for the coupled model is provided in the supplementary material. Notations of the logical formulae as in Table 3.

<table>
<thead>
<tr>
<th>Core engine</th>
<th>Exit module</th>
<th>Coupled model</th>
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<td><strong>Incoming interactions for Bub2-Bfa1</strong></td>
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<tr>
<td>ORI, SPN</td>
<td>Cdc5Polo, PP2A</td>
<td>ORI, SPN, Cdc5Polo, PP2A</td>
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<tr>
<td><strong>Logical rules for the activation of Bub2-Bfa1 (value 1)</strong></td>
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<tr>
<td>ORI ∧ SPN</td>
<td>PP2A ∨ Cdc5Polo</td>
<td>ORI ∧ (SPN ∨ PP2A ∨ Cdc5Polo)</td>
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<td><strong>Logical rules for the activation of Net1 at value 1</strong></td>
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<tr>
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<td>Net1, Cdc6, Bub2-Bfa1, Cdc15[2,3], PP2ACdc55[1,2], Cdc15, Cdc14, Sic1, Tem1</td>
</tr>
<tr>
<td>Bub2-Bfa1 ∨ PPX</td>
<td>(Cdc15 ∧ Tem1 ∨ Bub2-Bfa1) ∧ (Cdc14 ∧ Net1) ∧ PP2ACdc55[1,2]</td>
<td>(Cdc15 ∧ Tem1) ∨ Cdc15[2] ∨ Bub2-Bfa1 ∨ (Cdc14 ∧ Net1) ∨ PP2ACdc55[1,2]</td>
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### References


