CODING BIOLOGICAL SYSTEMS IN A STOCHASTIC FRAMEWORK

The Case Study of Budding Yeast Cell Cycle

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Abstract: In biology, modelling is mainly grounded in mathematics, and specifically on ordinary differential equations (ODEs). Using programming languages originally thought to describe networks of computers that exchange information is a complementary and emergent approach to analyze the dynamics of biological networks. In this work, we focus on the process algebra language called BlenX and we show that it is possible to easily reuse ODE models within this framework. In particular we focus on a well characterized biological network: the cell cycle of the budding yeast. This system has been studied in great details in the deterministic framework and data about a lot of mutants are available for the chosen model. It is interesting to note that the experimental phenotypic characterization of some mutants cannot be explained by the deterministic solution of the model, so in this work we propose a translation of the model in the stochastic framework in order to be able to verify if the inconsistencies are due to the noise that is affecting the system.

1 INTRODUCTION

In the last decade, the classical approach to model biological systems using mathematical formalisms in which continuous variables evolve deterministically was proved to be not enough for explaining the behavior of some molecular mechanisms in which the population of elements is so small that their stochastic variation in time can lead the system to behave differently from the average (McAdams and Arkin, 1997).

Moreover, as a recent paper on Nature (Nurse, 2008) points out, not only the kind of the results of a model are important but also the way in which it is formalized should be taken carefully into account: according to him, indeed, life science field requires the development of new and more appropriate languages to describe biological systems. After the work of Regev et al. (Regev and Shapiro, 2002), a promising trend in this direction is to use technologies based on programming language theories in order to capture the intrinsic concurrency, causality and probabilistic nature into algorithmic descriptions that can be executed, analyzed and simulated by computers. Algorithmic approaches require modelers to think about the mechanisms governing the behavior of the system under question (Priami, 2009): this strategy diverges from classical mathematical modeling because it is executable and not just simply solvable (Fisher and Henzinger, 2007). Mathematical equations describe the variation of variables (i.e. concentrations of species) from one state to another, while executable models highlight the causality relation among the events that constitute the history of the dynamics of the model. As a consequence, a number of process calculi have been adapted or newly developed for building biological models and performing stochastic simulations (i.e. stochastic pi-calculus (Priami et al., 2001), BioAmbients (Regev et al., 2004), Brane Calculi (Cardelli, 2005), CCS-R (Danos and Krivine, 2004), k-calculus (Danos and Laneve, 2004), Bio-PEPA (Ciocchetta and Hillston, 2007)). In this work, we will use a new programming language, BlenX, (Dematté et al., 2008), that is evolved from stochastic pi-calculus and Beta Binders (Priami and Quaglia, 2005). This language follows the main paradigms of process calculi and defines some new primitives that allow an easy encoding of deterministic models presented in the literature in a stochastic framework.

The biological system on which we want to focus our study is the well known molecular network driv-

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ing the cell cycle of *Saccharomyces cerevisiae*. In the literature (see (Csikasz-Nagy, 2009) for a state of the art review), beside models written in terms of ordinary differential equations (ODE) (Nasmyth, 1996), we find stochastic cell cycle models built with Langevine type stochastic DE (Steuer, 2004), with the Gillespie method (Sabouri-Ghomi et al., 2007; Kar et al., 2009) and with stochasticity on transitions (Lecca and Priami, 2007; Mura and Csikasz-Nagy, 2008a). However the most complete model of this system (Chen et al., 2004a) has only been studied in the deterministic framework and, as recent studies on reduced models suggest (Kar et al., 2009), the noise can have an important impact on the behaviour of the system.

The main contribution of this paper is, therefore, a codification in the BlenX language of the model described in (Chen et al., 2004a) and some interesting analysis that can be performed on the simulation runs of the BlenX model.

2 THE BLENX LANGUAGE

A detailed description of the BlenX language is out of the scope of this paper. We just summarize the subpart of BlenX needed for understanding the code presented below. We refer the reader to (Dematté et al., 2008; Dematté et al., 2008) for the formalization of the language and its modeling approach, and for a description of the tools that support this language.

The basic metaphor of BlenX is that a *biological entity* (i.e. a component that is able to interact with other components to accomplish some biological functions) is represented by a computational device called *box* that has a set of *interfaces* and an *internal program*. Interfaces have associated types and they are used by an entity to interact with other entities; the internal program, instead, codifies for the set of actions that a box can perform after a specific interaction with another entity in the system. For example, if a box is modeling a protein, its interfaces may represent sensing and effecting domains. Through sensing domains the protein receives signals that are then propagated through the internal actions that activate/inactivate a specific effecting domain. The exchange of signals can happen between boxes whose interfaces have a certain degree of affinity, which codes the strength of their interaction. Each action/event in BlenX is associated to a rate function used to run stochastic simulation of the coded system using a variant of the Gillespie algorithm. The rate functions can be declared as simple real numbers if the reaction that they are modeling is a simple mass action law, or they can be arbitrary functions (e.g. Michaelis-Menten, Hill response) if the reaction is an aggregated process whose elementary mechanism of interaction between entities is not known.

The primitives of the language used in Sect. 3 for the translation from ODEs to BlenX are graphically summarized in Fig. 1(b).

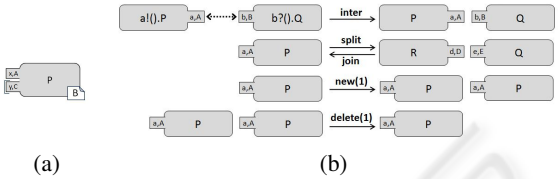


Figure 1: (a) Boxes as abstractions of biological entities. The small squares on the border are the interfaces; x and y are the interfaces subjects (omitted when not necessary); A and C are the interfaces types (omitted when not necessary); the line surrounding C indicates that the interface is hidden; P is the internal program and B is the name of the box; (b) Intuitive behaviour of some BlenX primitives. Each row represents one of the primitives used in our translation. The first codifies the interaction between two boxes, through the exchange of an input/output signal (input is in the form of b?() and the output is in the form a!()). The last three rows are the graphical representation of events of the form: "when(*conditions*) *verb*", where the action *verb* is triggered when *conditions* are satisfied. The *verb* can be one of split/join, new or delete, that model respectively the substitution, creation, and deletion of boxes in the system. Conditions are in the form of "*entity_name : : function*", whose meaning is that the action after the condition is triggered, at rate *function*, on the entity *entity_name*.

The last peculiar feature of the BlenX language that we will use later in the code are *templates*, that are a feature of many programming languages that allows to code parametric processes which can contain variable parts instantiated later by the compiler with respect to the base grammar. In BlenX template code is specialized and instantiated at compile time using interfaces identifiers, code or names that are passed as template arguments. As we will see in the next section, the usage of templates reduces the amount of code that need to be written and it makes the whole model more modular and easy to be modified.

3 REGULATORY NETWORK OF *SACCHAROMYCES CEREVISIAE*

The cell cycle is a coordinated set of steps by which a cell replicates all its internal components and divides them into two nearly identical daughter cells so that each of them contains the molecular machinery necessary to repeat the process. The cell cycle mechanism of the budding yeast is one of the most under-

stood control system between eukaryotes (Csikasz-Nagy, 2009) and its function is constrained by the phenotypic properties of more than 100 genetically engineered strains. In (Chen et al., 2004a), the authors show a mathematical model built on this control system that is successfully explaining the phenotypes of a lot of mutants characterized so far but some inconsistencies between the model and experiments indicate aspects of the mechanism that require more careful studies. Moreover given the small size of a yeast cell, the number mRNA molecules for most of the regulatory proteins is very low (possibly less than 10) and the total number of molecules of each regulatory protein in a cell is quite low (Kar et al., 2009): the intrinsic molecular fluctuations are then probably the main responsible for the fluctuation in cell cycle properties like the size at division. (Kar et al., 2009) also point out that although comprehensive deterministic models of the control system are available for budding yeast, stochastic simulations have not been carried out because the full reaction network needs to be expressed in terms of elementary reaction steps in order to perform exact stochastic simulations that takes precisely into account the effect of the intrinsic noise. Although this consideration is generally true, we want to make a remark, already discussed by several authors (Arkin and Rao, 2003; Ciocchetta and Hillston, 2007; Mura, 2008), about the usage of the Gillespie SSA algorithm (Gillespie, 1977) when high-level mathematical representation of non-elementary reactions are incorporated in the form of rate-dependent functions. The rate dependent functions are defining the reaction propensities of the stochastic model. Following the approach of (Mura and Csikasz-Nagy, 2008b) who model a smaller version of the cell cycle mechanism studied in this work, we assume that the fundamental hypothesis of Gillespie holds, i.e. each reaction time is a random variable following a negative exponential distribution with rate equal to the value of the propensity function. If this hypothesis is valid, a stochastic characterization of the reaction times as negatively distributed random variables is an accurate modeling choice, as proved by Gillespie (Gillespie, 1977). In our case, because of the presence of many non-elementary reactions, a careful validation of the stochastic model has been performed: here just few examples are shown for the sake of conciseness and because the main focus of the paper is the modeling procedure. Detailed studies on all the experimentally characterized mutants described at (Chen et al., 2004b) and (Panning et al., 2008) are a work in progress and some preliminary results are presented in section 4. Moreover an "unpacking" of all the non-elementary reaction mechanisms into their elementary steps is part of our future studies: we will use the model described here as the starting component of an iterative modeling cycle that starts from the model with abstracted complex mechanisms and refines it until it contains only elementary reactions. This is a complementary approach w.r.t. the one of (Kar et al., 2009) who are studying simplified version of the mechanism and they increase the general complexity of the model at each iterative modeling step.

Before explaining the modeling procedure that has been followed to code the cell cycle network in BlenX, we briefly summarize the biological background of the chosen system, referring the reader to (Chen et al., 2004a) for all the details about the mathematical model, its parameters and more detailed studies in the deterministic framework.

Newborn daughter cells must reach a critical size to have enough Cln3 and Bck2 protein to activate the transcription factors which drive synthesis of Cln2 and Clb5. Cln2 is primarily responsible for bud emergence and Clb5 for initiating DNA synthesis. In G1 phase Clb5 is not active because inhibited by CKIs. After the CKIs are phosphorylated by Cln2, they are rapidly degraded allowing Clb5 to do its job. Other cyclins like Clb2 are not present in G1-phase because their transcription factor is inactive, their degradation pathway Cdh1/APC is active, and their stoichiometric inhibitors CKI are abundant. Cln2- and Clb5-dependent kinases remove CKI and inactivate Cdh1, allowing Clb2 to accumulate and it activates its own transcription factor. Clb2 turns off the transcription factors of Cln2 and Clb5. As Clb2 drives the cell into mitosis, it also stimulates the synthesis of Cdc20 that, when the replicated chromosomes are attached, will initiates mitotic exit, degrading Pds1 and releasing a protease involved in sister chromatid separation. It also degrades Clb5 and partially Clb2, lowering their potency on Cdh1 inactivation. As the attached chromosomes are properly aligned on the metaphase spindle, Tem1 is activated, which in turn activates Cdc15, which in turn phosphorylates Net1 and this releases its hold on Cdc14. Cdc14 then does battle against the Cdks: activating Cdh1, stabilizing CKIs, and activating their transcription factor. In this manner, Cdc14 returns the cell to G1 phase (no cyclins, abundant CKIs, and active Cdh1). The mathematical model of this system contains 36 equations that are accounting for regulatory proteins, like the ones described above, and some dummy variables tracking specific events in the cell cycle progress. For example, BUD is accounting for the activity of Cln2 (when BUD = 1, a new bud is initiated); ORI (related to Clb5) represents the state of *origins of replication* (when ORI = 1, DNA synthesis is initiated); at cell division, when the concentra-

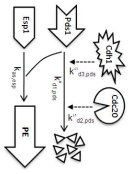
tion of Clb2+Clb5 drops below a threshold level, ORI is reset to zero as origin relicenses. Finally, SPN represents the alignment of replicated chromosomes on the mitotic spindle and it is driven by Clb2 activity.

The ODEs that formalize the biochemical network described above can be easily translated in BlenX following the codification procedure explained in (Palmisano et al., 2009): here, we show the translation of few reactions (see Fig. 2), referring the reader to the work mentioned above for further details.

$$\frac{d[Esp1]}{dt} = \ldots - k_{as,esp}[Esp1][Pds1]$$

$$\frac{d[Pds1]}{dt} = \ldots - k_{as,esp}[Esp1][Pds1] - k'_{d1,pds}[Pds1] + - k''_{d2,pds}[Cdc20][Pds1] - k''_{d3,pds}[Cdh1][Pds1]$$

$$\frac{d[PE]}{dt} = \ldots + k_{as,esp}[Esp1][Pds1]$$




```
//.prog
when(PDS1, ESP1 : : Assoc_to_form_PE) join(PE);
when(PDS1 : : degradation_1_PDS1) delete(1);
when(PDS1 : : degradation_2_PDS1) delete(1);
when(PDS1 : : degradation_3_PDS1) delete(1);
//.func
let Assoc_to_form_PE:function = kasesp*alpha*|PDS1|*|ESP1|;
let degr_1_PDS1:function = kd1pds_p*|PDS1|;
let degr_2_PDS1:function = kd2pds_p_p*alpha*|CDC20|*|PDS1|;
let degr_3_PDS1:function = kd3pds_p_p*alpha*|CDH1|*|PDS1|;
```

Figure 2: Partial network of reaction involving Pds1 (ODE on top left, cartoon representation on top right, BlenX codification on the bottom). The formation of the complex PE from the single species Pds1 and Esp1 is modelled in the ODEs with the three terms that contain $k_{as,esp}$ rate (written in the ODEs associated to each involved species); in BlenX this complex formation is coded with just a single join event (with stochastic rate defined by the `Assoc_to_form_PE` function). The three degradation mechanisms of Pds1 (i.e. spontaneous at rate $k'_{d1,pds}$, catalyzed by Cdc20 at rate $k''_{d2,pds}$ and catalyzed by Cdh1 at rate $k''_{d3,pds}$) are coded in BlenX with three different delete event with the appropriate stochastic rate.

The whole model can be translated following the rules in (Palmisano et al., 2009), but because of the size of the model, this can lead to a pretty complex model, difficult to be maintained and integrated in other models. In order to have a more effective codification, we can improve the structure of the entire model without changing the underlying mathematical interpretation. A way for doing that is to recognize that there are some mechanisms that are "structurally equal" even if applied on different proteins: for example there are a lot of species that are degraded because of the interaction of another species with a mass action kinetic law. In BlenX this can be coded in an easy way through the usage of the templates. Let's consider the degradation of Clb2 and Clb5 proteins: in the first version of the code, the spontaneous degradation mechanism and the catalyzed one has been translated for both proteins with a delete event whose rate is depending on $kdb2\_p$, $kdb5\_p$, $kdb2p$, $kdb5\_p\_p$ respectively. We can drop those four events and code the mechanisms with the code in Fig. 3 (details about the interpretation of the code are given in the caption of the figure).

```
//.prog
template receiving_degr_sign : pproc<<name channel>>
                                    = channel?().die;
template spont_degradation : pproc<<rate kin_rate>>
                                = die(rate(kin_rate));
let CLB2 : bproc = #(clb2_ch, CLB2_ty)
   [ receiving_degr_sign<<clb2_ch>> |
     spont_degradation<<kdb2_p>> | ... ];
let CLB5 : bproc = #(clb5_ch, CLB5_ty)
    [ receiving_degr_sign<<clb5_ch>> |
      spont_degradation<<kdb5_p>> ];
let CDC20 : bproc = ..., #(cdc20_out, CDC20_out_ty),...
    [ ... | rep cdc20_out!().nil ];
//.types
(CLB2_ty, CDC20_out_ty, rate(Vdb2_2)),
(CLB5_ty, CDC20_out_ty, rate(Vdb5_1))
//.func
let Vdb5_1 : const = kdb5_p_p*alpha;
let Vdb2_2 : const = kdb2p*alpha;
```

Figure 3: Templates for degradation mechanisms. CLB2 and CLB5 boxes contain the parallel composition of the two different mechanisms (i.e. the spontaneous degradation, and the degradation induced by the CDC20 signal). Both are coded using the templates defined at the beginning of the .prog file that, in a general way, define the sequence of actions that each one has to perform (an input on a *channel* and a die action for the degradation induced by the external signal; and a die with a specific rate for the spontaneous degradation of the box). The internal program of CDC20 contains an infinite source of output signal: this output is linked to the input channels of both CLB2 and CLB5 through the rate defined in the .types file defining the interaction stochastic rate between the pairs of types. Finally, the internal program of CLB2 and CLB5 contains the spontaneous degradation of the box at the appropriate rate.

We want to substitute the delete event with the code in Fig. 3 because events are global rules that drive the deletion of a box from the system, but the mechanism that we want to model is the "local suicide" of a box that decides, from its internal code, to disappear from the system after the interaction of another specific species (in this case the one involved in the rate of the delete event): in this way we can reduce the amount of code that has to be written because even if a protein is degraded by different species, its internal code can be the same and the sources of the signal can be different (with possibly different rates). Moreover, using the templates, we can write the code that is

accounting for this behaviour just once, and then we can instantiate it in different boxes just specifying the local information that differentiate each species (in our case the rate of the self degradation, or the channel on which the communication is happening). The templates used in this model are accounting only for the simple mass action kinetics because we want to keep the same level of abstraction of the original model. However, as a general modeling principle, writing different kind of mechanisms (i.e. enzymatic reactions) in a parametric way will help the user to generate a model that is maintainable and reusable w.r.t. a model written only with global events and rules.

In the next section we show some validation results on stochastic simulations of the cell cycle model. The complete BlenX code of the model can be downloaded at http://www.cosbi.eu/downloads/attachment/CellCycleModel.zip. The three textual files can be used to reproduce the results presented in this paper using the BetaWorkbench: we refer the reader to (Dematté et al., 2008) for a description of the usage of the different tools in the BetaWorkbench framework.

4 VALIDATION OF THE STOCHASTIC MODEL

In this section we show that our model successfully reproduces the behavior of wild type and mutant cells quantitatively. At (Chen et al., 2004b), full details about the deterministic model of the different mutants can be found. On this website, the authors summarize the basic experimental results on which the original wiring diagram in (Chen et al., 2004a) is based. They also present a set of 131 mutants built using the same equations and parameter values of the wild type except for those parameter changes that are dictated by the nature of the mutation.

We performed Multiple stochastic simulative Replications in Parallel (MRIP) of the wild type model and of some of the mutants available. We used MRIP approaches to speed up simulations by working out independent replications of the same stochastic trajectory on multiple computers. Therefore, to guarantee the trustworthiness and the statistical accuracy of the analysis, we ran a batch of 300 simulations for each parameter set (wild type or a specific mutant) and each simulation reproduces about 30 complete cell cycles. In Fig. 4(a), we show the output of a simulation run for the wild type model.

Just looking the time series of the different proteins involved, we see that the simulations are reproducing the solutions of the original ODE model, a part from the stochastic noise. However, in order to be

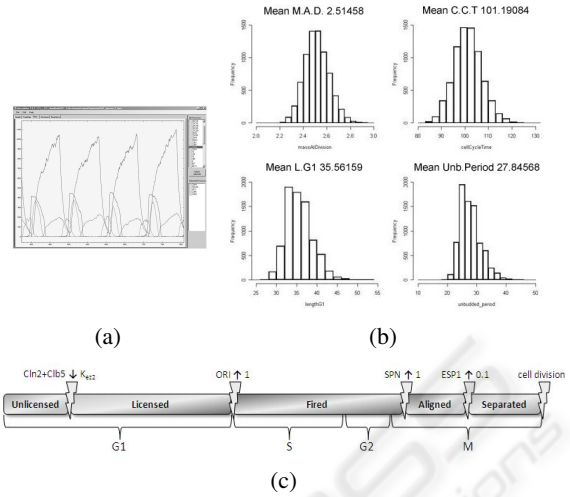


Figure 4: (a) Results of a stochastic simulation of the wild type cell cycle model. The units are minutes and number of molecules for the x-axis and y-axis respectively. The expected oscillatory behaviour is visualized using BetaPlotter between time instants 400 and 800. Oscillations are consistent with the the deterministic solution with variance in the peaks of some proteins caused by the stochastic noise. (b) Statistics of some properties of the wild type cell cycle model. Histograms title contains the mean value of the specific property. The values of the deterministic model are: Mass at division = 2.62, Cycle time = 101.2 min, Length of G1 phase = 36 min, Unbudded period = 33 min. (Chen et al., 2004b). (c) The five stages of the cell cycle, separated by the events described as rules of viability in (Panning et al., 2008). The up/down arrow in the condition of the triggers (lightning) identifies a rise/drop of the indicated quantity above/below the indicated threshold level.

able to compare with a statistical accuracy the computed behavior with the observed phenotype of the cells, we perform some analysis of the simulation output in order to measure some properties of the model like the cell cycle time, the mass at division and the length of the different phases. For each of the properties we compute the mean value and we generate an histogram of the distribution (Fig.4(b)): we verified that those values are in accordance with the experimental ones listed in (Chen et al., 2004b).

When comparing the model with the experimental data it is important to realize that much of the data from experiments is qualitative, usually in the form of “the cell is viable but considerably larger than wild type cells” or “the cell arrests in G1 phase”. So in order to compare the simulation results with experimental data, it is necessary to characterize cell cycle properties from the changing in time of the regulatory proteins of the model (that are the quantitative information generated by simulation runs). We analyzed the simulation output w.r.t. the rules for viability and

timing of phases defined in (Panning et al., 2008) and summarized in Fig. 4(c). Setting the thresholds in this way we were able to reproduce the statistics depicted in Fig. 5 that, for different mutants, are in accordance with the published phenotypic characteristics.

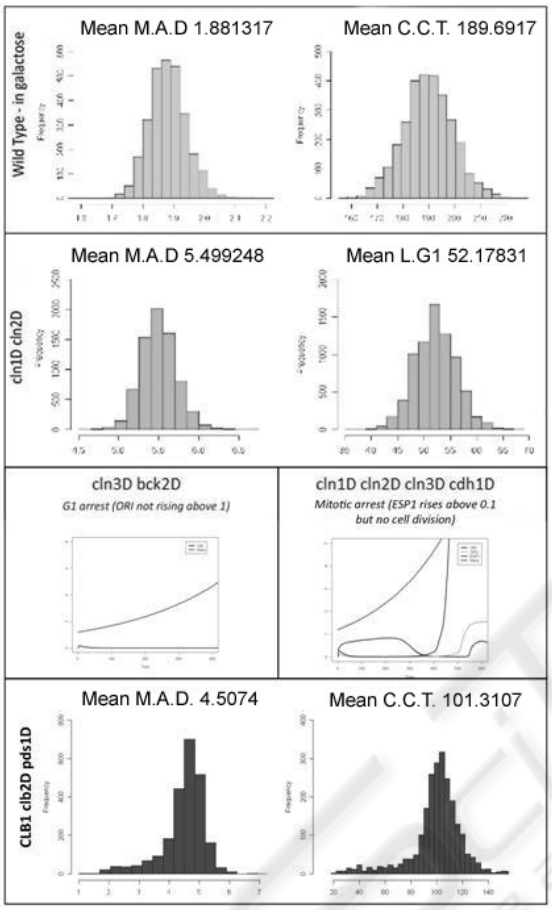


Figure 5: Statistics of different properties of some mutants of the cell cycle model. Mutant 1: wild type in galactose (Viable and, for the deterministic model, Mass at division = 1.92, Cycle time = 189.9 min). Mutant 2: cln1Δcln2Δ (Viable and, for the deterministic model, size is 2.4X WT). Mutant 3: cln3Δbck2Δ (Inviable, G1 arrest). Mutant 4: cln1Δcln2Δcln3Δcdh1Δ (Inviable, Telophase arrest). Mutant 5: CLB1clb2Δpds1Δ (Viable, but inconsistent with experimental results). The explaination of how to obtain the different mutants can be found in (Chen et al., 2004b). A detailed description of the biological meaning of those results is out of the scope of this paper, see the text for some comments about them.

Commenting on the plots in Figure 5, we want to point out that the third and fourth mutants are inviable and so all the simulations looks the same and no statistics can be performed. The other mutants are in accordance with the average behaviour of the deterministic model. In particular the last inconsistent mutant is viable also in the stochastic context but it has very instable cycles (the variance of the measured properties is very high), accounting in some way of the inviability found in the experimental outcome: those results suggest that this mutant is a good candidate for more careful studies on the effect of the noise on the viability of the cell cycle machinery.

The analysis on the complete set of 131 mutants has still to be completed, but the results on the mutants showed above are promising because, from one side, the average results are in accordance with the results on the deterministic models, but, from the other side, a careful analysis on the distribution of the stochastic results can explain some of the inconsistencies between the experimental result and the deterministic solution of the system.

5 CONCLUSIONS

In this paper we presented the codification of a budding yeast cell cycle model in a process-algebra language, called BlenX. This model can be stochastically simulated in order to answer questions that cannot be directly tackled by the deterministic approach, like checking the partial viability of mutants at the border of life and death, and whose mean behaviour is not enough for completely characterize the experimental observation of the system. The simple translation from the mathematical language is possible because of the expressive power of BlenX that allows the definition of general rate functions for reactions. However the usage of the SSA algorithm with simple and complex kinetic laws leaves to the user the responsibility to validate simulation results. The example proposed here uses different levels of abstraction and it has been validated against experimental results of wild type and some mutant strains.

It is our future goal to further analyze the mechanisms that are behind those complex kinetics in order to see more in details the effect of the stochastic noise on the system, because it can be the cause of some inconsistencies found between the experimental results and its deterministic evolution in time. Moreover unpacking the complex kinetics like the Michaelis-Menten or the Goldbeter-Koshland response can be very important both from the point of view of the structure of the model and for the biological insights that it can lead to. Indeed, regarding the former motivation, it can help the user to generate code that is maintainable, reusable and more easy to be integrated in bigger models. Regarding the latter motivation of interest, it can make evident the need of a refinement of the underlying network because, as showed

in (Ciliberto et al., 2007; Sabouri-Ghomi et al., 2007), the naive unpacking of enzymatic network can lead to network that loose their bistable behaviour unless they are structurally modified with the addition of background reactions: so using different template to unpack a specific mechanism can help the biologist to understand which is the basic structure needed for recovering the desired behaviour.

Finally, the complete study of all the mutants characterized in (Chen et al., 2004b) is under development: we will be able to provide to the user the stochastic counterpart of the knowledge about the budding yeast cell cycle discovered so far, which can help more comprehensive studies of that system.

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