Dynamics of the cell-cycle network under genome-rewiring perturbations

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Abstract
The cell-cycle progression is regulated by a specific network enabling its ordered dynamics. Recent experiments supported by computational models have shown that a core of genes ensures this robust cycle dynamics. However, much less is known about the direct interaction of the cell-cycle regulators with genes outside of the cell-cycle network, in particular those of the metabolic system. Following our recent experimental work, we present here a model focusing on the dynamics of the cell-cycle core network under rewiring perturbations. Rewiring is achieved by placing an essential metabolic gene exclusively under the regulation of a cell-cycle’s promoter, forcing the cell-cycle network to function under a multitasking challenging condition; operating in parallel the cell-cycle progression and a metabolic essential gene. Our model relies on simple rate equations that capture the dynamics of the relevant protein–DNA and protein–protein interactions, while making a clear distinction between these two different types of processes. In particular, we treat the cell-cycle transcription factors as limited ‘resources’ and focus on the redistribution of resources in the network during its dynamics. This elucidates the sensitivity of its various nodes to rewiring interactions. The basic model produces the correct cycle dynamics for a wide range of parameters. The simplicity of the model enables us to study the interface between the cell-cycle regulation and other cellular processes. Rewiring a promoter of the network to regulate a foreign gene, forces a multitasking regulatory load. The higher the load on the promoter, the longer is the cell-cycle period. Moreover, in agreement with our experimental results, the model shows that different nodes of the network exhibit variable susceptibilities to the rewiring perturbations. Our model suggests that the topology of the cell-cycle core network ensures its plasticity and flexible interface with other cellular processes, without a need for an optimal setting of the kinetic parameters.

5 Online supplementary data available from stacks.iop.org/PhysBio/10/066001/mmedia

Introduction
The operational principles of the eukaryotic cell-cycle have been found to be universal across a wide range of organisms, from yeast to mammals [1–4]. The common picture emerging is of the cell-cycle progression driven by a robust core network [5–9]. However, its interface with other intracellular processes, in particular the metabolic system, remained elusive [10–12]. Clearly, the cell-cycle system itself is...
influenced by, and affects the intracellular metabolism. However, whether the major cell-cycle regulators directly interact with metabolic processes and what are the consequences of such interactions on the cell-cycle functionality are not known. Evidently, the cell-cycle network is not an autonomous isolated ‘oscillator’, but rather an integral part of the cellular complex web of interactions. It has been demonstrated that in the budding yeast hundreds of genes (~800) that do not directly participate in the cell-cycle process exhibit temporal dynamics similar to the genes that directly regulate the cell-cycle progression, and are synchronized with its dynamic phases [11, 13]. However, it is not entirely clear why these genes are expressed periodically with the cell-cycle core regulators. Moreover, the significant role of these dynamics remained unclear as well as the relations between the cell-cycle regulatory genes and other intracellular processes. In recent years, the major players in the cell-cycle network and their interactions have been identified. Accordingly, simplified models capturing its main features have been constructed and demonstrated the robust dynamics observed experimentally. These models however, focused on the autonomous dynamics of the cell-cycle network, largely neglecting the more delicate issue of its interface with processes external to it [14]. The question arises: What is the susceptibility of the dynamics to perturbations resulting from interactions of the cell-cycle core regulatory network with other cellular processes?

To deal with this issue, one has to develop novel experimental and modeling approaches. To this end, we utilized a genome-rewiring methodology [15, 16] elucidating these dynamical aspects of the cell-cycle, in particular the flexibility of its interface with the metabolic system. In a recent experimental work, we introduced a direct regulatory perturbation by rewiring the genome; placing an essential metabolic gene exclusively under a promoter of the cell-cycle in the budding yeast (figure 1) [17]. Harnessing cell-cycle regulators to directly control an essential metabolic process increases the load on its regulatory network at a specific phase of the cycle and demands re-distribution of its resources. Such a perturbation, introduces a complex challenge to the cells by requiring the cell-cycle regulators to operate outside of their natural context and in concert with arbitrarily chosen metabolic demands. We have shown that yeast cell populations with rewired cell-cycle promoters could rapidly adapt to grow at normal rates despite an increased inhibition of the metabolic rewired gene. Furthermore, our experiments demonstrated that the cell-cycle system forms a flexible and adaptive interface with the cellular metabolic processes. This interface can support the multitasking utility of the cell-cycle promoters. It enables the concurrent regulation of their native function while controlling a foreign essential metabolic gene under inhibiting conditions. Nevertheless, rewiring different essential nodes of the cell-cycle network showed that they had variable susceptibilities to the rewiring perturbation and that the observed response was specific to the rewired cell-cycle promoter [17]. The aim of this paper is to develop a framework for modeling the perturbations caused by rewiring of the cell-cycle promoters. We show that this framework enables the study of the interface of the cell-cycle regulatory genes with other cellular processes as well as the variability in susceptibilities to such perturbations among the different cell-cycle promoters. Moreover, the modeling methodology presented here can be generalized to other cellular networks, allowing the study of their interface with other processes beyond their core interactions. It extends our capability to model the commonly found multitasking properties of regulatory genes.

Models of the cell-cycle may be generally grouped into two categories. The first employs a simplified Boolean approach (first proposed by Kauffman [18, 19] and applied to the cell-cycle by Li et al [8]). The second utilizes differential equations, usually with a large number of unknown parameters [5]. In the Boolean approach, the nodes in a directed graph represent the regulatory genes and their interactions are the directed edges. A gene can be in one of two states, on (1) or off (0), according to a Boolean function taking into account the state of the other genes connected to it. Given its simplicity, it is somewhat surprising that the emerging dynamics of these Boolean networks still reflect the essential features of the biological gene regulatory network [8]. Although there are many advantages to this methodology, it has some major drawbacks. It employs a two way switch that may be too simplistic to model a complex and delicate process such as the expression of genes in a realistic manner. The dynamics of a Boolean network is usually further simplified by parallel synchronous update of the entire network. Yet, a deterministic model with a synchronous update of the entire network may strongly affect its dynamics and fails to describe the stochastic behavior characterizing a natural gene expression process [20]. Moreover, attractors which are stable under the synchronous update simplification may be unstable if stochasticity is imposed on the transmission times [21]. By contrast, the models based on differential equations can represent more faithfully the biological kinetic processes. However, those models usually have many equations and parameters, most of which are unknown, making them hard to comprehend and to work with. Furthermore, these models can also be easily compromised by over-fitting of the parameters, and there is no consensus about their robustness [22–25].

Modeling the rewiring of the cell-cycle to a metabolic process requires a different approach. The cell-cycle core network comprises two basic types of processes: protein–DNA interactions carried out by transcription factors (TFs), and protein–protein interactions. The TFs regulate the expression of genes related to the cell-cycle at different phases. They also govern a periodic regulation of their own expression and determine the inner clock of the cell-cycle [26]. There are also important check-points involving other mechanisms at various phases of the cycle [27], and an essential feedback to ensure coherent entry into the cycle [28]. Here, we propose a framework that combines the two levels of interaction, protein–DNA and protein–protein, in differential equations that emphasize the fundamental differences between these two types of processes. The concentration of a given protein is affected by the activity of the respective TFs as well...
Figure 1. Rewiring the cell-cycle network. A schematic diagram of the cell-cycle transcription regulation [26]. In the rewired model of the cell-cycle, an essential metabolic gene is exclusively regulated by a duplicate of the cell-cycle promoter that regulates SWI4, NDD1, or SWI5. The metabolic gene is marked HIS3, the gene used in our experiments [17], but in principle represents any arbitrarily chosen gene that is foreign to the cell-cycle core network. Note that the native genes of the cell-cycle core network and their regulatory links remained intact in this rewiring process.

as through protein–protein interactions. The contribution of a TF, participating in the activation of a specific gene, is linear in its concentration. The protein–protein contribution on the other hand, is proportional to the multiplication of the concentrations of all the participating proteins, the affecting proteins and their target, as implied by the law of mass action. Our model reproduces the robust cycle dynamics of the network similar to previous modeling approaches. It differs however, from previous models by treating the TFs as limited ‘resources’. Thus, loading a TF by duplicating its binding promoter to control a foreign metabolic gene causes redistribution of this limiting resource and can potentially affect the network dynamics. Another fundamental difference between our approach and previous models is the treatment of check-points. We treat the check-points as essential processes that naturally integrate with the core cell-cycle regulatory system [27]. By contrast, in previous models check-points are fixed-points of the dynamics, requiring special external stimuli to proceed in the cycle [8]. Thus, check-points in our model could be regarded as part of the re-distribution of resources in the network; a perturbation that can halt the cycle but do not require a special signal for its continuous dynamics.

Methods

The model algorithm was computed using the following steps: 1. Selecting a gene randomly; 2. Updating the protein concentration of the selected gene according to the updating rules (elaborated in the model description in Results); 3. Computing the concentrations of all proteins and 4. Advancing time by one unit and repeating the series of computations.

We checked the simulation on a simple network with two nodes x and y, with the following dynamics:

\[
\frac{dx}{dt} = 0.2x - xy - 0.05x
\]

\[
\frac{dy}{dt} = 0.8x - 0.05y.
\]

This system can be solved analytically and has two fixed-points: an unstable one at \((x, y) = (0, 0)\) and a stable one at \((x = 0.009375, y = 0.15)\). The simulation converged from a variety of start vectors to the stable fixed point. However, when initiated from the point \((0,0)\) it remained there.

The simulations of the cell-cycle were performed with arbitrary initial concentration vectors. The parameters were also set arbitrarily, starting by dividing the outgoing weights of each gene evenly. Figure S1 (available from stacks.iop.org/PhysBio/10/066001/mmedia) shows the regulatory network of the yeast cell-cycle used in the simulation (as in figure 2) with the weights indicated above the edges serving as a baseline for the statistical computations. For the genes CDH1 and MCM1 we added another vertex, labeled Const, that interacted positively with both genes and showed no degradation (see the model description in Results). For all other genes, the degradation coefficient was set arbitrarily to be the same constant, \(D_x = 0.8\), chosen to be within a reasonable range of the outgoing weights. The initial concentrations of the genes were also chosen arbitrarily: a value of 0.5 was assigned to genes that peak during the M and G1 phases. A value of 0.05 was assigned to the remaining genes, since experimental data have shown an order of magnitude reduction for most of the regulatory genes during the cell-cycle [8]. The
Figure 2. The model cell-cycle core network. The interactions between genes are either positive (green arrows) or negative (red arrows). Light green and light red arrows are the connections used in the model of [8]. The regulatory network was modified (i) by adding the gene MCM1, additional edges (dark green—positive, dark red—negative) and (ii) by using two levels of interactions; protein–protein (PP) and TF. These are the minimal modifications required to enable proper cycle dynamics. The SBF complex contains SWI4 and SWI6, the MBF complex also contains SWI6, and the SFF complex contains NDD1.

main C++ code for the simulation was computed within a Matlab framework, thus enabling us to compute an ensemble of models with randomly varying values around these baseline weights. We checked that the simulation is insensitive to the initial choice of parameters. The simulation was computed for 1000 random weight matrices. Each weight was chosen randomly from a window 0.5–1.5 around the initial value w, under the network’s rules. Almost half of the runs ended in a limit cycle matching the cell-cycle’s expression of genes (see figure 5). Estimation of the cycle’s periodicity was conducted by examining the power-spectrum of the protein concentration time series. In practice, the power-spectrum was assessed by applying FFT in Matlab to the time series of the SBF concentration produced by the simulation. Then, the cycle frequency was identified with the peak of the absolute power-spectrum value. Simulation runs that did not produce a cycle were easily recognized by their power-spectrum not showing such a peak.

Since the kinetic parameters are unknown, they were treated as random variables and we studied the statistical behavior for an ensemble of models with arbitrary parameters. The model was measured in two main aspects; the number of runs ending in a cycle and the mean cycle frequency in each ensemble (table 1 statistics).

Results

Model description

The cell-cycle core network of interactions includes TFs and protein–protein interactions. Protein–protein interactions provide a higher level of cell-cycle regulation and can be further divided into different components including cyclins, inhibitors, degraders and competitors. In the yeast Saccharomyces cerevisiae there is a single cyclin-dependent kinase (CDC28) which controls many cell-cycle processes by phosphorylation and its activation depends on the formation of complexes with the activated cyclins. There are nine cyclins transcribed in four distinct waves during the cell-cycle. The inhibitors, degraders and competitors interact with the cyclin/CDC28 complexes (e.g. anaphase promoting complex). It was shown that those two levels of control work in tandem to regulate the periodic process of the cell-cycle [29]. To model the cell-cycle network we adopted the connectivity graph used in [8] with modifications as shown in figure 2. We emphasize that this is a core network and not an exhaustive description of the molecular interactions involved in the regulation of the cell-cycle. In biological terms, the positive interactions represent transcription at the TFs level and protein stabilization at the protein–protein interaction level, resulting in the extension of the protein life half time. The negative interactions represent, mainly, the action of inhibitors and degraders, resulting in shortening of the proteins half-life.

| Rewired promoter/load | Mean | Statistics |
|-----------------------|------|------------|
| SWI4/low              | 0.0045 | 0.722 |
| SWI4/medium           | 0.0038 | 0.573 |
| SWI4/high             | 0.0037 | 0.632 |
| NDD1/low              | 0.0047 | 0.574 |
| NDD1/medium           | 0.004  | 0.963 |
| NDD1/high             | 0.0037 | 0.973 |
| SW15/low              | 0.0046 | 0.533 |
| SW15/medium           | 0.0044 | 0.595 |
| SW15/high             | 0.0044 | 0.597 |
| No rewiring           | 0.0045 | 0.482 |
time. We expanded the connectivity graph used previously [8], by adding more interactions according to published experimental data (figure 2; dark green—positive interactions, dark red—negative interactions). Special emphasis was paid to the different role of MCM1 in the context of the TFs’ complexes in which it participates [26, 30–32]. We found that these modifications enable to obtain cycle dynamics. Thus, in a sense, the graph presented in figure 2 may be regarded as a minimal model that can faithfully reproduce the essential cell-cycle dynamics. Future work should examine the effects of additional possible interactions, not included in our model, on the dynamics.

In our differential approach we propose a realization of the kinetics in the following way. The change in the concentration of a protein \( C_i \) is determined by three main processes: (i) An increase that is linear with the concentration of the TFs that regulate it, (ii) A change due to protein–protein interactions which, according to the law of mass action, is proportional to the multiplication of the relevant concentrations and (iii) A decrease in concentration due to protein degradation. This will yield the following differential equation for every gene of the core network:

\[
\frac{dC_i}{dt} = \sum_j W_{ij}^{TF} C_j + \left( \sum_k W_{ik}^P C_k \right) C_i - D_i C_i.
\]

The first term represents the sum over the concentrations of the entire set of TFs that affect the transcription of gene \( i \), weighted by the relevant kinetic constant \( W_{ij}^{TF} \) (proportional to the weight on the directed edge from the TF \( j \) to gene \( i \)). The second term is the sum over the concentrations of proteins that affect protein \( i \) via protein–protein interaction, weighted by the relevant kinetic rate \( W_{ik}^P \) (where \( W_{ik}^P \) is proportional to the weight on the directed edge from the protein \( k \) to protein \( i \)). We impose the constraint: \( \sum_k W_{ik}^P C_k \leq 0 \) since only TFs can activate the transcription of a new protein. Finally, \( D_i \) is the degradation rate of protein \( i \). We checked our methodology on a simple network with two nodes and three edges that can be solved analytically (see Methods). The simulation of this simple process indeed converged to the analytically solution.

A finite copy-number of TFs, produced over a relatively short time window at the relevant cell-cycle phase [13] and activating simultaneously a foreign metabolic gene presents a multitasking challenge to the system. We regard the copy-number of the TFs as limited ‘resources’ and present the challenge by constraining the sum over all weights of the outgoing edges of a particular TF to a constant (which is set arbitrarily to one). The addition of a new edge by rewiring a TF to regulate a foreign metabolic gene, results in the redistribution of the outgoing weights and thus applying pressure on the network dynamics. One way to think of the TFs as limited resources is to imagine a particular TF as a fixed number of particles flowing through a set of ‘channels’ (the outgoing edges of a network’s node). Thus, opening more channels to the node inevitably reduces the number of particles directed into any of the other channels. In our experiments, the pressure on the system caused by genome rewiring was controlled by utilizing different concentrations of a drug (3AT) that specifically inhibits the activity of the metabolic protein (HIS3p), the product of a gene rewired to a specific promoter of the cell-cycle [17]. Such an applied pressure is realized in the model, by increasing the weight of the rewired connection relative to all other outgoing weights of a node in the network (keeping the total sum of weights to one).

Since the kinetic parameters are unknown, we treat them as random variables and study the statistical behavior of the model under a spectrum of such arbitrary parameters. The results of the model were found to be robust against perturbations of the chosen parameters (see below). Figure S1 (available from stacks.iop.org/PhysBio/10/066001/mmedia) depicts the weights used as baseline to the simulations. Simulating the expression of the genes CDH1 and MCM1 posed a particular challenge, since they are expressed constitutively during the cell-cycle and there is no relevant experimental data about their regulation. To address this problem, we added an artificial external vertex to these nodes that interacts positively with both genes and has no degradation (see figure S1, available from stacks.iop.org/PhysBio/10/066001/mmedia). For all other genes, the degradation coefficient was set arbitrarily to the same constant and the initial concentrations of the genes were also chosen arbitrarily (see Methods).

Results of the cell-cycle model

We first test the basic model without recruitment of a metabolic gene to a cell-cycle promoter. Figure 3(A) shows that under such conditions the network exhibits intrinsic oscillatory dynamics for the various genes of the cell-cycle. The model oscillatory dynamics are similar to the expression patterns measured experimentally for a synchronized population of cells, following a temporary arrest of the cell-cycle (figures 3(B), (C) [13]. This is a remarkable result, demonstrating that the core network in our model reproduces a limit cycle, with no intermediate traps at fixed-points. Thus, it is consistent with the interpretation of the check-points as ‘emergency’ halting mechanisms of the cycle, rather than fixed-points of the network dynamics.

Next, following the experiments in [17], we introduce a rewiring perturbation to the model by recruiting an essential metabolic gene (realized in the experiment by HIS3 from the histidine biosynthesis pathway), to three different promoters of the cell-cycle regulating SW4, SW15 and NDD1 (figure 1). We realize the application of a variety of pressure loads (realized in the experiment by adding the HIS3p inhibiting drug, 3AT), by forcing different redistributions of their outgoing weights. For each rewiring event, we set an increasing weight to the ingoing edge of the metabolic gene and redistribute the rest of the weights according to the model’s rules. This simulated an increased pressure set by the recruitment of the foreign gene on the cell-cycle network. The model was then computed for different loads for a large number of random weight matrices, with a uniform distribution around the basic values presented in figure S1 (available from stacks.iop.org/PhysBio/10/066001/mmedia). The ingoing weights to the recruited metabolic gene were kept constant throughout these simulations in order to preserve the...
Figure 3. The dynamics of regulatory genes of the cell-cycle. (A) Concentrations of the regulatory genes, as computed by the simulation without rewiring. (B) The heat map of experimental transcript levels of regulatory genes in yeast cells released from α-factor synchronization. The genes expression is from the analysis of microarray measurements taken from public data of [13]. (C) The heat map of concentration levels of regulatory genes as computed by the simulation of the network without rewiring. The concentration levels are expressed as the log 2 change from the average of each gene. (D) The concentrations of selected genes, computed by the simulation of the network. The essential metabolic gene is rewired to the NDD1 promoter with low (continuous curves; weight 0.25) or high (dotted curves; weight 0.7) pressure loads. The different colors represent the dynamics of different genes: red—MCM1, green—SBF, blue—SFF and cyan—SWI5. (E) and (F) The heat maps of concentration levels of regulatory genes as computed by the simulation with rewiring as in (D) with low and high pressure loads, respectively.

We utilized the frequency distribution as a measure of the susceptibility of the various nodes of the networks for the rewiring perturbation (Methods). Figure 4 depicts for each rewired network, the histograms of the cell-cycle frequency distributions for three representative pressure loads. The frequencies are presented in arbitrary units, as computed from the power-spectrum of the protein concentration (Methods). The frequency distributions depict a significant shift of the mean under increasing pressure load. Interestingly, the results of the model demonstrate that one of the cell-cycle nodes is more robust than the other two. For the two rewired networks, linking a foreign metabolic gene to the SWI4 and NDD1 nodes, increased pressure results in a longer cycle (lower frequencies; figures 4(A) and (B)). For the third case of SWI5, the cycle was not significantly changed, indicating that this node is more robust to perturbation than the other ones tested (figure 4(C)). These results of the model are in agreement with our experimental results showing that the promoter of SWI5 is indeed more robust to the rewiring perturbation than SWI4 or NDD1 [17]. In our experiments, the strain with SWI5 rewiring (figure 1) adapted faster and was capable to adapt to higher stress loads than those with SWI4 or NDD1 rewiring.

The model further provides an opportunity to study the role of specific core genes in maintaining the cell-cycle regulation. By randomly perturbing the initial weights of each node, we could measure the robustness of the model to these perturbations. This was done by measuring the

differences in the pressure loads and for obtaining a faithful statistical representation of the dynamics. The redistribution of resources resulted in the elongation of the overall cycle duration as well as changes in the relative durations of the phases, while preserving their ordered dynamics (figures 3(D)–(F)). Note that the effect on the cycle’s elongation depends on the load imposed by the foreign metabolic gene on the cell-cycle’s promoter. Eventually, for a sufficiently large pressure the oscillatory dynamics of the cycle is lost. Note that in figure 3(D) the amplitudes are much higher under high pressure (dotted curves) than the ones under low pressure (solid curves). While the model does not allow quantitative measures of the protein concentrations, it may indicate an exit at the G1 phase under high pressure load due to the required higher concentrations of the relevant TFs.
fraction of failures to maintain oscillatory dynamics, for a large number of simulations with different random weight matrices. For each gene and for each simulation, the outgoing weights were randomly chosen from a uniform distribution around the basic weight matrix of figure S1 (available from stacks.iop.org/PhysBio/10/066001/mmedia). The results show that \( MCM1 \), in its two states (alone and in a complex with the SFF complex of TFs) is the most significant gene, ensuring a limit cycle (figure 5). Indeed, it has been suggested that \( MCM1 \) has a central role in the cell-cycle regulation and in its stabilization. Moreover, \( MCM1 \) plays a different role regarding the complexes it forms with other TFs, and it was proposed that its role at the TF level is similar to the role of cyclines at the protein–protein interaction level [33, 34]. Rewired networks

\[ \text{Figure 4. Distributions of cell-cycle model frequencies. The distributions of frequencies of limit cycles with random initial weights for an essential metabolic gene, rewired to the (A) NDD1, (B) SWI4 and (C) SWI5 promoters. Each curve was computed for a different stress load (blue—low, green—intermediate, red—high and grey—no rewiring as a reference). Values of the weights: low load-0.25; high load-0.7 for SWI4-NDD1 or 0.925 for SWI5. For each stress load, the simulation was computed for 1000 random weight matrices, with a uniform distribution around the initial weight matrix. Each weight was chosen randomly from a window of } 0.5w–1.5w \text{ around the initial value } w, \text{ under the network’s rules. The histograms are normalized to unit area.} \]
Figure 5. Breakdown of the limit cycle dynamics. Histograms depicting the number of simulation runs not resulting in a limit cycle. For each gene, the simulation was computed for 1000 random weight matrices. The outgoing weights of the selected gene were chosen, each time, randomly from a uniform distribution around the initial weight matrix $w \sim (0.3w - 1.7w)$. The genes not showing a bar never failed in our simulations.

We believe that this lack of sensitivity to kinetic parameters is a common and important feature of biological regulatory networks ensuring their functionality [37]. Yet, some of the regulatory genes contribute more than others to the network’s stable dynamics. In particular, the model shows that $MCM1$ is central to this dynamics. However, reaching a true limit cycle in models of the cell-cycle is uncommon. Previous models have regarded the check-points in the cell-cycle system as strong fixed-points that dominate the ensemble of possible solutions. Thus, such check-points suspend the system’s oscillations unless a particular stimulus allows their bypass. By contrast, check-points in the present model served to enable the cycle to proceed continuously. A specific signal from a check-point serves to halt the oscillatory dynamics in case of a defected operation. We thus propose that check-points and other controls on the cell-cycle regulation act through periodic redirection of resources rather than being stable fixed-points. More experimental work however, is required in order to shed more light on the precise integration of the check-points in the cell-cycle regulatory network.

Our main motivation in developing this model was to study the direct interaction of the cell-cycle’s core regulatory genes with essential metabolic processes. More specifically, it follows our experimental work that utilized genome rewiring, linking an essential metabolic gene to a cell-cycle’s promoter [17]. We have shown in these experiments that such rewired cells adapted on short physiological time-scales even when the metabolic gene was strongly inhibited and required considerable resources from the cell-cycle network. Indeed, regarding the TFS of the network as limited resources and studying the effect of their redistribution due to an external load, elucidates the variable sensitivity of various nodes of the network to such perturbations.

Of the above features, i.e., the oscillatory dynamics without an external stimulus or the redistribution of resources due to load applied by a foreign gene on the cell-cycle regulatory system, none could be studied in the framework of previous models. In the Boolean models, the question whether
the cell-cycle is an autonomous oscillator or a sequence of transitions between stable attractors could not be studied, since the cycle is initiated by an artificial start signal [8]. On the other hand, in the continuous models the results are sensitive to noise that renders them dependent on the precise choice of parameters which are largely unknown experimentally [22–24]. While in most of these models the kinetic parameters are fixed, in our approach we study the statistical behavior of an ensemble of models with randomly varied parameters. This statistical approach with a distribution of kinetic parameters reflects, to our view, the intrinsic representation of a biological cell, rather than merely a maneuver to overcome a gap in our knowledge. Change of parameters leads to modifications in the cell-cycle duration. As depicted in figures 3(D)–(F), this change in the duration of the cycle occurs at specific phases (particular in G1). The results in [8] indicate the existence of a large attractor at the G1 phase. However, adding stochasticity to the deterministic Boolean model while leaving the regulatory network intact, results in oscillations rather than a stable attractor [38]. The phases of the cell-cycle were identified by the oscillations in concentrations of the various proteins, as described in [8]. The model however, is not capable of enforcing specific cell-cycle events (such as budding, origin relicensing and spindle alignment) at the right order.

Our model demonstrates the plasticity of the cell-cycle core regulatory network that can accommodate and maintain its limit cycle dynamics within a wide range of resource redistributions. Thus, the cell-cycle core regulatory network can support multitasking and a flexible interface with other cellular processes. This property is maintained in the model over a broad range of parameters, showing that fine-tuning to a working set-point is not essential. This result is compatible with our experimental findings [17]. The difference in stability due to rewiring of different promoters may shed light on these experimental results. Our experiments indeed demonstrated a shorter adaptation time for cells regulating the metabolic essential HIS3 gene by the promoter of SWI5 than the ones regulated by the promoters of SWI4 or NDD1. Moreover, the experiments showed that the rewired pSWI5-HIS3 cells could sustain much larger loads, applied by stronger inhibition of HIS3p by the drug 3AT.

In summary, the core of the presence study is in treating protein–DNA (TF) and protein–protein interactions separately in a continuous model. To overcome the lack of knowledge of parameters, we studied the statistics of a large number of models with random sets of parameters. Our model, constructed on the above principles, elucidates the interface of the cell-cycle with other cellular processes, and promotes the study of perturbations imposed by multitasking. Such an approach may well prove as an important tool in future studies of regulatory networks.

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