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Recovery from stress – a cell cycle perspective

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ABSTRACT

We develop a Boolean model to explore the dynamical behaviour of budding yeast in response to osmotic and pheromone stress. Our model predicts that osmotic stress halts the cell cycle progression in either of four possible arrest points. The state of the cell at the onset of the stress dictates which arrest point is finally reached. According to our study and consistent with biological data, these cells can return to the cell cycle after removal of the stress. Moreover, the Boolean model illustrates how osmotic stress alters the state transitions of the cell. Furthermore, we investigate the influence of a particular pheromone based method for the synchronisation of the cell cycles in a population of cells. We show this technique is not a suitable method to study one of the arrest points under osmotic stress. Finally, we discuss how an osmotic stress can cause some of the so called *frozen* cells to divide. In this case the stress can move these cells to the cell cycle trajectory, such that they will replicate again.

Keywords: Boolean network, state transition, cell cycle, stress response, osmotic stress, alpha factor.

1 INTRODUCTION

The cell cycle is the most fundamental chemical clock underlying all forms of life. It produces a sequences of timed biochemical signals that trigger biological events such as DNA replication, chromosome segregation and cell division. This (re-)production process must be achieved reliably in a broad range of external conditions to which the cell has to respond flexibly. Therefore, the reaction to environmental fluctuations, or stresses, has to be coordinated with the cell cycle.

In this paper we substantially extend a recently developed Boolean model of *S. cerevisiae*'s cell cycle [1] to incorporate the cell cycle stress response, particularly to osmotic stress and application of a pheromone called alpha factor. Our model predicts that the states of the cell cycle trajectory are attracted to either of four fixed points under osmotic stress, i.e. the cell cycle will be arrested in one of four distinct states. It also describes the sequence of states towards the fixed point, and the activity of all cell cycle components at this point. Also, it shows that the state of the cell at the onset of stress dictates the arrest state of the cell.

Likewise, our model reveals that all four fixed points are drawn to the cell cycle trajectory after removal of the osmotic stress (0.4 M-1 M NaCl). Therefore, the cell can recover from these osmotic stresses. Furthermore, our model illustrates the state transitions during the recovery from the stress, which allows us to analyse the stress response in great detail.

We also note that the cell cycle trajectory lies in the largest basin of the state transition network, i.e. many states are converging towards it. We will hypothesise that this is the result of an evolutionary process. Our calculations also show that one of the four osmotic stress fixed points has a relatively small basin. This basin, however, can be reached by suitable experimental conditions and it will play a major role in the stress response. Hence, rather than the mere size of the basin, it is crucial to consider whether typical environmental/experimental fluctuations can take the cell to the states in that basin.

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One key result of our model regards a standard experimental procedure. The reaction to stresses is often studied in populations of cells, whose cell cycles have been synchronised. One of the most frequently used methods for cell cycle synchronisation is via the alpha factor, which arrests the cells in the G1 phase. After release from the alpha factor the cells behave synchronously for two or three cycles [2]. To describe the synchronisation as part of different experiments, we expand our Boolean model by adding the corresponding links and nodes. Our model demonstrates that one of the osmotic stress arrest points cannot be studied via alpha factor synchronisation.

Our model also predicts that osmotic stress can *connect* certain nodes of the state transition network, which are disconnected in unstressed conditions, to the cell cycle basin. We call these disconnected nodes *frozen* states. They describe cells in particular states, which are not able to divide in a *normal* environment, i.e. even if the stress is removed. These cells cannot form colonies and are often considered to be *dead*. The application of an osmotic stress reconnects these states to the cell cycle trajectory. These cells can form colonies again and are perfectly healthy. Hence, if we accept the definition that cells that cannot form colonies in unstressed conditions are dead, this leads to the curious consequence that osmotic stress can *revive* cells.

The organisation of this paper is as follows: we first explain the architecture of *S. cerevisiae*'s cell cycle regulatory networks and its interaction to osmotic stress and the alpha factor. We then introduce our Boolean model and construct its corresponding state transition graphs in different conditions. In the results section, we analyse the model and discuss some of its predictions. Finally, we summarise the main results and discuss their biological implications.

2 THE BUDDING YEAST CELL CYCLE NETWORK UNDER OSMOTIC STRESS

The cell cycle is controlled by a complex molecular network, which is responsible for the self-sustained oscillatory expression of many genes. The cell cycle of eukaryotes consists of four distinct phases, denoted by G1 (Gap 1), S (DNA Synthesis), G2 (Gap 2) and M (Mitosis). In the G1 phase cells substantially increase in size and prepare for the S phase, during which DNA replication occurs. The G2 phase provides cells with additional time to grow and to activate regulatory mechanisms. Finally, during the M phase, chromosome segregation and nuclear division take place and the cell divides into two identical cells.

In baker's yeast (*S. cerevisiae*), the cell cycle oscillation is governed by the binding of Cyclin-Dependent Kinases (CDK) with different cyclins sequentially. Basically, the START of the cell cycle is marked by an increased activity of Cln3, which is an upstream activator of two different transcription factors, SBF and MBF. These, in turn, increase the levels of G1 phase cyclins (represented by Cln2 in Fig. 1A) and S phase cyclins (illustrated by Clb5 in Fig. 1A). The presence of Sic1, which is the Cyclin Kinase Inhibitor (CKI) in the G1 phase, maintains Clb5 in an inactive form during most of G1 phase. To enable the G1-S transition Cln2 inactivates Sic1. Therefore, the absence of active Sic1 triggers the activity of Clb5, whose activity then initiates DNA replication and thereby transfers the cell to the S phase. The next cell cycle transition, G2-M, is mainly governed by the activity of G2 phase cyclins (labled by Clb2 in Fig. 1A) [3]. G2 phase cyclins are regulated by several mechanisms: on the one hand, the protein kinase Swe1, controls the Clb2 activations [4]. On the other hand, Clb2 activates its own transcription factor (Mcm1) [5]. Hence, active Clb2 transfers the cell to the M phase, during which the replicated chromosomes are segregated. Then, exit from mitosis is achieved mainly by the inactivation of Clb2 [6]. This is mediated by the degradation of Clb2, due to Cdc20 and Cdh1 [7, 8]. Finally, the cell is prepared to return to the G1 phase mediated by the phosphatase Cdc14, which promotes Swi5, and also dephosphorylates Cdh1 and Sic1 (Fig. 1A).

This cycle of biochemical events is altered in the presence of different stresses, such as osmotic stress. The cell's reaction to osmotic stress is a homeostatic process. Osmoregulation is highly conserved across species, which regulates the internal turgor pressure, as well as the water content and the volume of the cell. Osmotic stress causes the activation of different signaling pathways [9], among which the so called High-osmolarity glycerol (Hog) MAPK signaling plays a key role [9, 10]. Downstream of the Hog MAPK network is doubly phosphorylated Hog1, which is the main player linking the osmotic stress response to the cell cycle networks. In baker's yeast, if osmotic stress is applied during the G1 phase, Hog1 delays the progression towards the S phase via two different mechanisms: (i) downregulation of the transcription of the G1 cyclins [11, 12], (ii) and also direct phosphorylation and stabilisation of Sic1 [12, 13]. The S and G2/M phases are also delayed by osmotic stress, due to three main Hog1 dependent mechanisms: (i) direct downregulation of S phase cyclin [14], (ii) accumulation of Swe1 via phosphorylation of Hsl1 [15] and (iii) also downregulation of G2 phase cyclin (Fig. 1A).

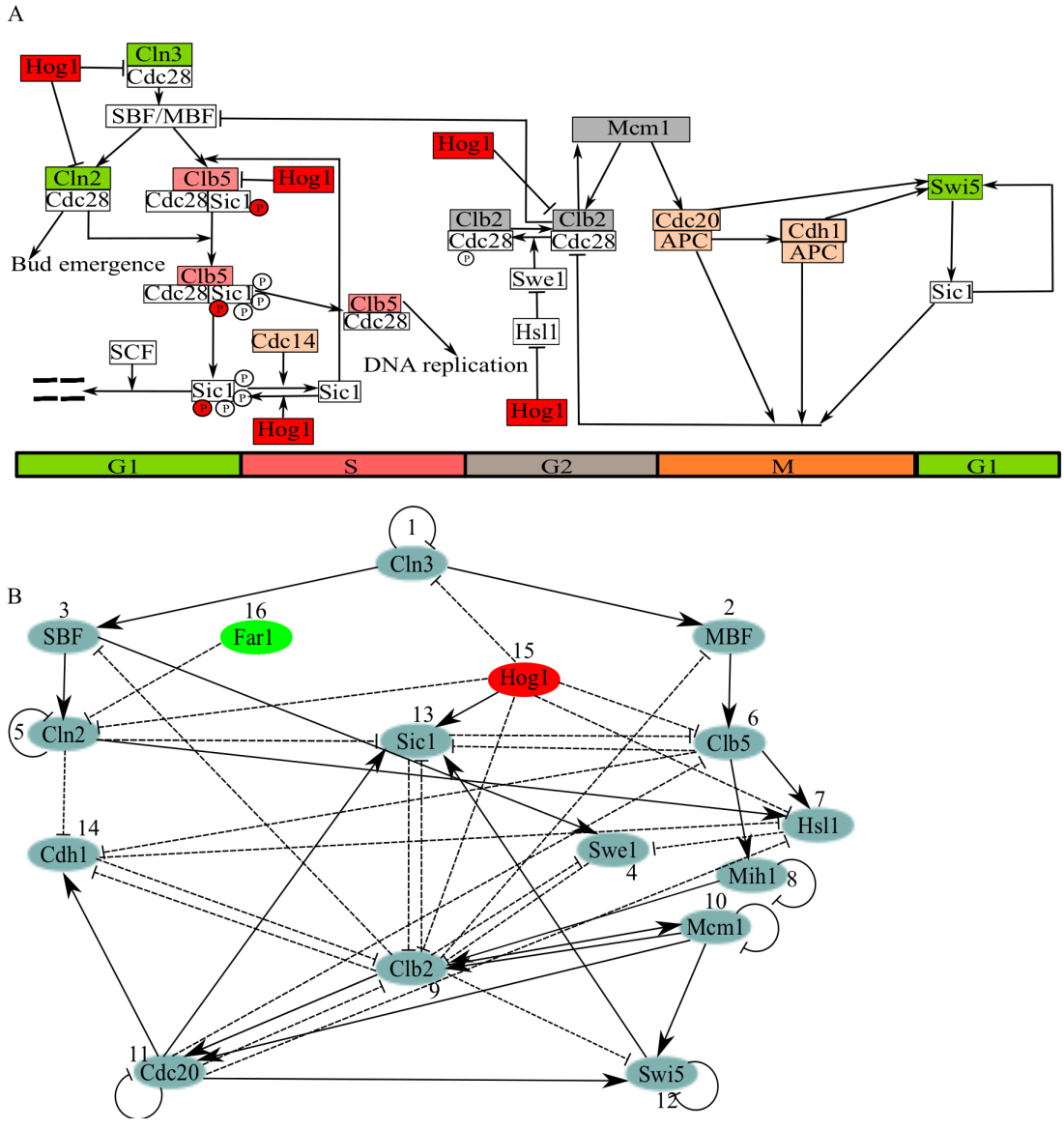

Figure 1 – **A)** Molecular regulatory network of the cell cycle of baker's yeast from START to cell division. Activity of Cln3 triggers the cell cycle. A bud emerges upon activation of Cln2. DNA replication is controlled by Clb5, and Clb2 regulates the G2 and M phases. Osmotic stress activates Hog1, which inhibits the cyclins of the different phases. Moreover, Hog1 causes Sic1 and Swe1 to accumulate. As a result of the interactions of Hog1 with different components, the cell arrests in different phases (see text for details). **B)** Boolean model of the regulatory network of the cell cycle under two different stresses; (i) osmotic stress and (ii) alpha factor. The Boolean network consists of 16 nodes, 14 of which are cell cycle regulated. The other two nodes, Hog1 and Far1, do not have any input from the rest of the network. Solid lines represent positive regulations and dashed lines represent deactivation (inhibition, repression, or degradation).

The cell cycles of population of baker's yeast are synchronised by application of a pheromone called alpha factor. Alpha factor induces the transcription of Far1. Far1 is a Cyclin Kinase Inhibitor (CKI) of the G1 cyclin. Hence, activity of G1 cyclin (Cln2) is inhibited upon presence of the alpha factor [16]. After release from the alpha factor the cells will be synchronised for two or three cycles [2].

3 BOOLEAN MODEL OF THE CELL CYCLE REACTION TO OSMOTIC STRESS AND THE ALPHA FACTOR

In this section we describe our Boolean model of the cell cycle reaction to osmotic stress and the alpha factor. We start from the recently developed Boolean model of the cell cycle by Li et al. [1], which contains 11 core components. In first step, we then add

three further cell cycle proteins (Swe1, Mih1 and Hsl1), which will later allow us to include the stress response into our cell cycle model. To this network of 14 components we add another node, Hog1, which is part of the cell's osmotic stress response and interacts with Cln2, Cln3, Sic1, Clb2, Clb5 and Hsl1, all components of the cell cycle (Fig. 1B). Finally we introduce Far1 and its link to Cln2. This allows us to model the cell's response to the alpha factor (Fig. 1B).

To unveil the dynamical properties of the cell cycle and the stress response, we build a network in which each vertex, l, represents one of the components with a binary value $s_l(t) \in \{0, 1\}$ that depends on its activity: "on" or "off". Each edge stands for an interaction between the corresponding vertices. Figure 1B illustrates the resulting Boolean network. The incidence matrix of the combined cell-cycle-osmotic-stress-pheromone model is a 16×16 matrix, $B = [b_{lk}]_{16\times16}$, $l, k \in \{1, 2, \ldots, 16\}$. The elements b_{lk} are called weight factors, and are defined such as, $b_{lk} = 1$ if protein k activates protein l, $b_{lk} = -1$ if protein k inhibits protein l, and finally $b_{lk} = 0$ if there is no link from the protein k to l. The incidence matrix and the connectivity graph (Fig. 1B) both provide the same information.

The time evolution of the cell cycle components $l \in \{1, \ldots, 14\}$ is described by an iterative process in which the subsequent state of each node is determined by the following Boolean rule:

$$s_l(t+1) = \begin{cases} 1 & \text{if } \sum_k b_{lk} s_k(t) > 0 \\ 0 & \text{if } \sum_k b_{lk} s_k(t) < 0 \\ s_l(t) & \text{if } \sum_k a_{lk} s_k(t) = 0. \end{cases} \quad (1)$$

The sums calculate the weighted average of all activating and inhibiting inputs to a node. If the overall input is positive the node becomes active, otherwise it becomes inactive. The state of a cell cycle node at time $t + 1$ is hence given by the weighted average of the input nodes at time t. This definition is the same as the one used by Li et al. [1].

Note that Hog1 and Far1 (nodes 15 and 16 in Fig. 1B) do not have any incoming links from the cell cycle. To our knowledge no cell cycle regulation mechanisms for these two components have been reported. Hence, in our model they are independent variables that they become active at the onset of the corresponding stresses, and then remain active. Hence, the Boolean function for Hog1 and Far1 is independent of the other components, i.e. $s_l(t+1) = s_l(t)$ if $l \in \{15, 16\}$.

In every iteration the states of all other nodes of the Boolean network are updated synchronously due to intrinsic biological properties of the cell cycle [1]. Note that the set of fixed points, which is central to our subsequent arguments, is independent of the update rule, i.e. synchronous update, asynchronous update and a combination of both all lead to the same set of fixed points (see Appendix, Corollary 7.2) [17].

Next, we will explain how to construct the state transition matrix of our model, i.e. the matrix that describes how the overall state of the Boolean network transits to a subsequent state. Then, by employing the state transition matrix, we will discuss the cell cycle's dynamical reaction to osmotic stresses in different experimental setups.

4 STATE TRANSITION SPACE OF CELL CYCLE

In this section we introduce the state transition matrix of our Boolean model. It describes transitions between the global states of the Boolean network. This transition matrix can be considered as the adjacency matrix of the state transition graph. Note that this network is much larger than the Boolean one. In fact, every node of the transition network corresponds to the state of the entire Boolean network of N nodes, i.e. each of the 2^N states is represented by a node in the transition network. Hence, the state transition matrix ($T = [t_{ij}]$) is a $2^{16} \times 2^{16}$ square matrix, where $N = 16$ is the number of nodes in the corresponding Boolean network.

We can reduce the size of the transition matrix of our Boolean model by considering the fact that Hog1 and Far1 are not cell cycle regulated. As a consequence, instead of using a single $2^{16} \times 2^{16}$ square matrix, we can split it into four $2^{14} \times 2^{14}$ square matrices. Each of these describes one of the four stress combinations, where two stress nodes can be either active or inactive. The first matrix corresponds to the case when both Hog1 and Far1 are inactive; we call this matrix U (unstressed). When Hog1 is active and Far1 is inactive we denote the corresponding matrix by O (osmotic). The third matrix A (alpha factor) represents when Hog1 is inactive and Far1 is active. Finally, the fourth matrix C (combinatorial) describes the situation when both Hog1 and Far1 are active. So each of the U, O, A and C matrices models the state transition of the cell cycle in different environmental conditions. Consequently, the state transition matrix T is of the form:

$$T = \begin{bmatrix} U & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & O & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & A & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & C \end{bmatrix}_{2^{16}\times2^{16}} . \quad (2)$$

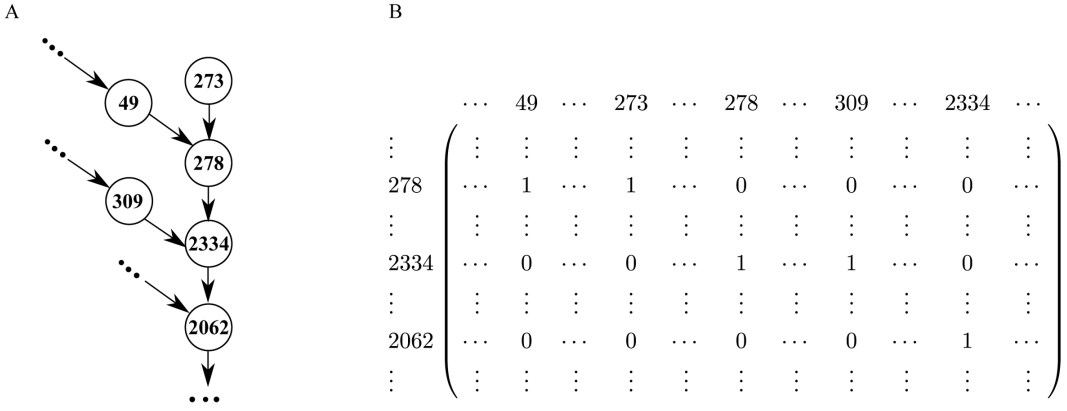

Figure 2 – **A)** An example of a state transition network. Numbers inside each circle are the decimal representation of the corresponding states of Boolean network nodes. **B)** State transition matrix of the graph shown in part A. See text for details of the calculation.

Next, we need to derive the different state transition matrices (U, O, A and C), which will enable us to study the cell cycle reaction in different environmental conditions. The following discussion is exemplified for the state transition matrix U (unstressed), but it is valid for the other blocks as well.

If a transition from one of the states j to another i is possible by iteration with the Boolean function Eqn. (1), the element u_{ij} of U is 1; otherwise it is 0. Hence, the transition matrix describes the time evolution of the Boolean network.

It is convenient to map the binary representation of each state of the Boolean network $(s_1\ s_2\ \ldots\ s_{14})$ to the respective decimal representation, i.e.

$$\left(s_1\ s_2\ \ldots\ s_{14}\right)_2 = (i)_{10}, \tag{3}$$

where $i \in \{1, \ldots, 2^{14}\}$ and $s_k,\ k \in \{1, \ldots, 14\}$. We use this decimal representation to label the respective states in the state transition network. Figure 2A shows part of a state transition graph and Figure 2B depicts the corresponding state transition matrix. For example, according to Figure 2A state 273 and 49 transit to state 278 as time evolves, therefore, $u_{278,49} = 1$ and $u_{278,273} = 1$.

The matrix U (unstressed) with elements $u_{i,j}\ i, j \in \{1, 2, \ldots, 2^{14}\}$ is the adjacency matrix of the state transition graph. Our model shows that the state transition graph of the cell cycle is, in fact, a directed tree (Fig. 3A). We will discuss the reasons behind this structure later. Since every state transfers to a unique state $d_j^{out} = \sum_i u_{ij} = 1$. The number of the states being mapped to state i after one time application of Boolean function is $d_i^{in} = \sum_j u_{ij}$. Having obtained the state transition matrix we are able to study the dynamics of transitions among all states. The set of all possible states $\mathbf{X}$ is finite. Hence, starting from any element of $\mathbf{X}$ we will, in principle, reach either a fixed point or a limit cycle after sufficient number of iterations with U (see Appendix 7.1) [17]. As our state transition graph has the structure of a directed tree, it cannot, by definition, contain any cycles.

As mentioned above, the state transition matrix U (unstressed) captures the time evolution of its corresponding Boolean network. The state $\mathbf{x}$ is represented by a 2^{14} dimensional vector with exactly one non-vanishing component, which is equal to one:

$$\mathbf{x} = \begin{bmatrix} 0 \\ \vdots \\ 1_j \\ \vdots \\ 0 \end{bmatrix} \quad j \in \{1, \ldots, 2^{14}\}. \tag{4}$$

In fact, the state of the Boolean network after the k^{th} iterations starting from any initial condition $\mathbf{x^0}$ can be evaluated by $\mathbf{x^k} = U^k\mathbf{x^0}$. Hence, for the fixed point ($\mathbf{fp}$), independently of the value of k, we always have $\mathbf{fp^k} = \mathbf{fp^0} = U^k\mathbf{fp^0}$. Note, that this fixed point equation is an eigenvalue equation to a unit eigenvalue. Therefore, the number of fixed points (n_{fp}) of a Boolean network equals the number of unit eigenvalues of the state transition matrix U (unstressed).

The fixed points allow us to partition the space of all possible states $\mathbf{X}$ to *equivalence* classes which are defined as follows:

Definition 4.1. Let $\mathbf{X}$ be the set of all states. If $\mathbf{x}$ and $\mathbf{y}$ are in $\mathbf{X}$, $\mathbf{y}$ is a *descendant* of $\mathbf{x}$ if there exists an integer $m \geq 0$ such that $\mathbf{y} = U^m\mathbf{x}$. Two states $\mathbf{w}$ and $\mathbf{z}$ in $\mathbf{X}$ are said to be *equivalent* if they have a common *descendant*.

A fixed point is *descendant* of all states which are being attracted towards it.

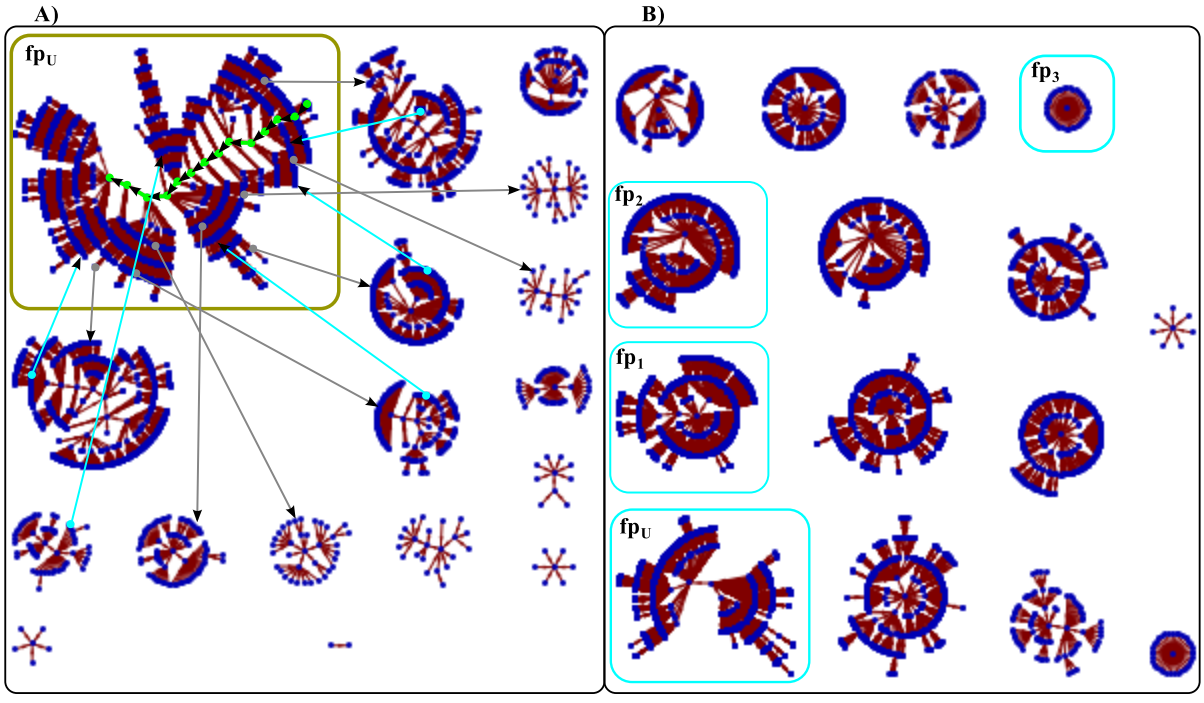

Figure 3 – **A)** Directed state transition tree of the global set of initial conditions. The cell cycle trajectory is in the largest basin of the attraction (**U**-basin). The nodes of this trajectory are shown in green and the edges in black. We call the states in the other basins frozen states. Presence of the stress can bring some of the frozen states back to the **U**-basin. Examples of these states are shown in light blue. Moreover, osmotic stress can kick some states of the **U**-basin out to the other basins. Hence, they are arrested forever after removal of the stress. Some of these states are shown in grey. Some of these states are artificial; they are obtained by mathematically generating all possible combinations of "on"/"off" states of all constituents. **B)** Directed state transition tree of the global set of initial conditions, when the Hog1 node is active. The states of the cell cycle trajectory converge to either of the basins which are highlighted by a light blue box.

Definition 4.2. The *equivalence class* of each fixed point is called *basin* of that fixed point or *basin of attraction*.

Moreover, to partition $\mathbf{X}$ into its basins it is convenient to first determine the minimal k such that $U^{k+1} = U^k$. For an arbitrary initial state, k is greater or equal to the number of iterations after which it reaches its *descendant* fixed point. Note that k is the largest diameter of all basins. Hence, since the state transition graph of U (unstressed) is a directed tree the matrix U^k has n_{fp} nonzero rows. The nonzero elements of each of these n_{fp} nonzero rows constitute their corresponding basin.

Following the same argument, we can find the other state transition matrices (O (osmotic), A (alpha factor) and C (combinatorial)) and their corresponding networks. The dynamical behaviour of C is beyond the scope of this paper as we do not describe combinatorial stresses. The corresponding state transition graphs of the matrices U and O are shown respectively in Figures 3A and 3B.

The state transition graph of our Boolean cell cycle model is a directed tree. In Figure 4 we have labelled the consecutive states of the cell cycle from *CC1* to *CC14*. If the initial condition is *CC1*, time evolution of our Boolean network brings that state to the end of the cell cycle trajectory and the cell cycle is completed. Note that the Hamming distance, i.e. the number of different entries in the binary state vectors, between the first and the last states in the Figure 5 is one. If the cell is in the final state, *CC14*, stationary G1, the onset of the cell division signal will return the cell back to the initial state. This signal activates the Cln3 node, and changes its Boolean value from 0 to 1. Cln3 is the cyclin that triggers cell cycle progression [18]. The exact mechanisms that activate Cln3 are still unknown. Since we are rather interested in the state transitions in one cycle, the cell division signal is not considered explicitly in our model.

5 RESULTS

5.1 Osmotic stress drives the cell into one of the four fixed points

The state transition graph of baker's yeast's Boolean network is shown in Figure 3A. The state transition matrices, described in the last section, contain all information about the dynamics of the system. Their fixed points, i.e. eigenvectors to a unit eigenvalue,

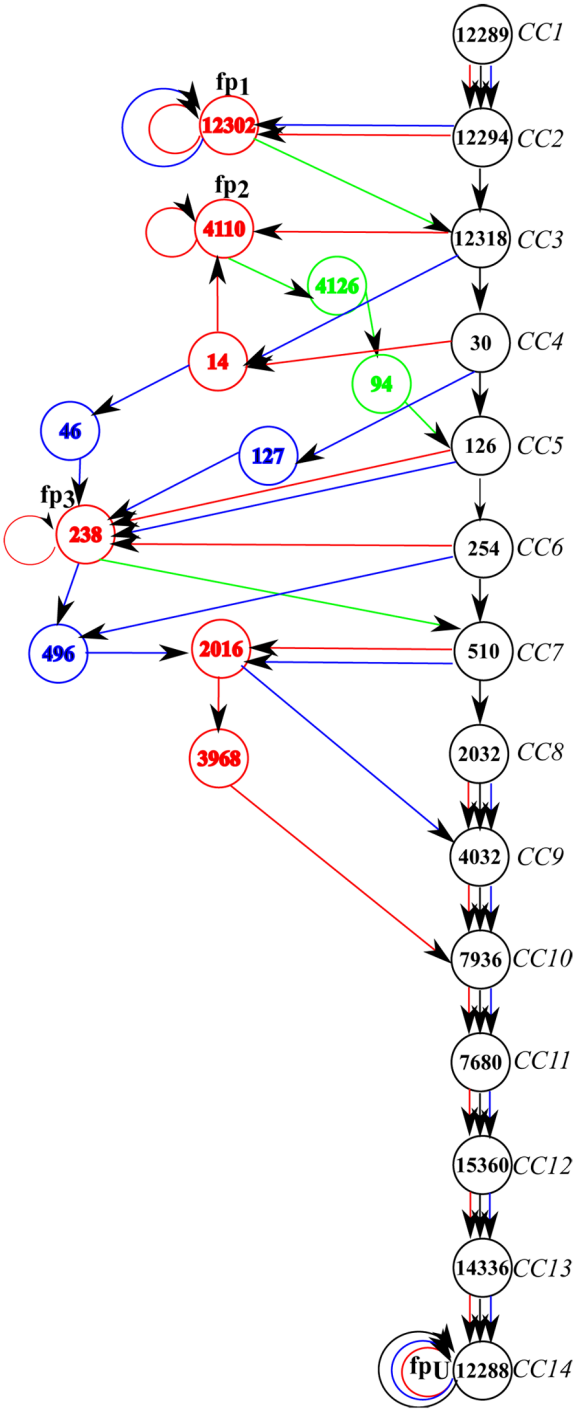

Figure 4 – Cell cycle trajectory and its response to osmotic perturbation. The black edges show the *normal* state transitions. The red edges illustrate the perturbation from the cell cycle trajectory by Hog1. Moreover, the green edges represent the state transition from the arrest points of osmotic stress to the unperturbed cell cycle trajectory. Osmotic stress cannot take the cell out of its basin, and all perturbations from the limit cycle of the cell cycle can be compensated after the removal of the osmotic stress. Blue edges illustrate the perturbation from the cell cycle trajectory which is caused by the activity of the alpha factor. The alpha factor arrests the cell in $\mathbf{fp}_1$, which is also a fixed point of the cell in osmotic stress condition. The cell which is released from the alpha factor will converge to the state *CC3*. Hence, the alpha factor is not a proper population synchronisation method to study $\mathbf{fp}_1$. Numbers inside each circle are the decimal representation of the corresponding states of cell cycle components.

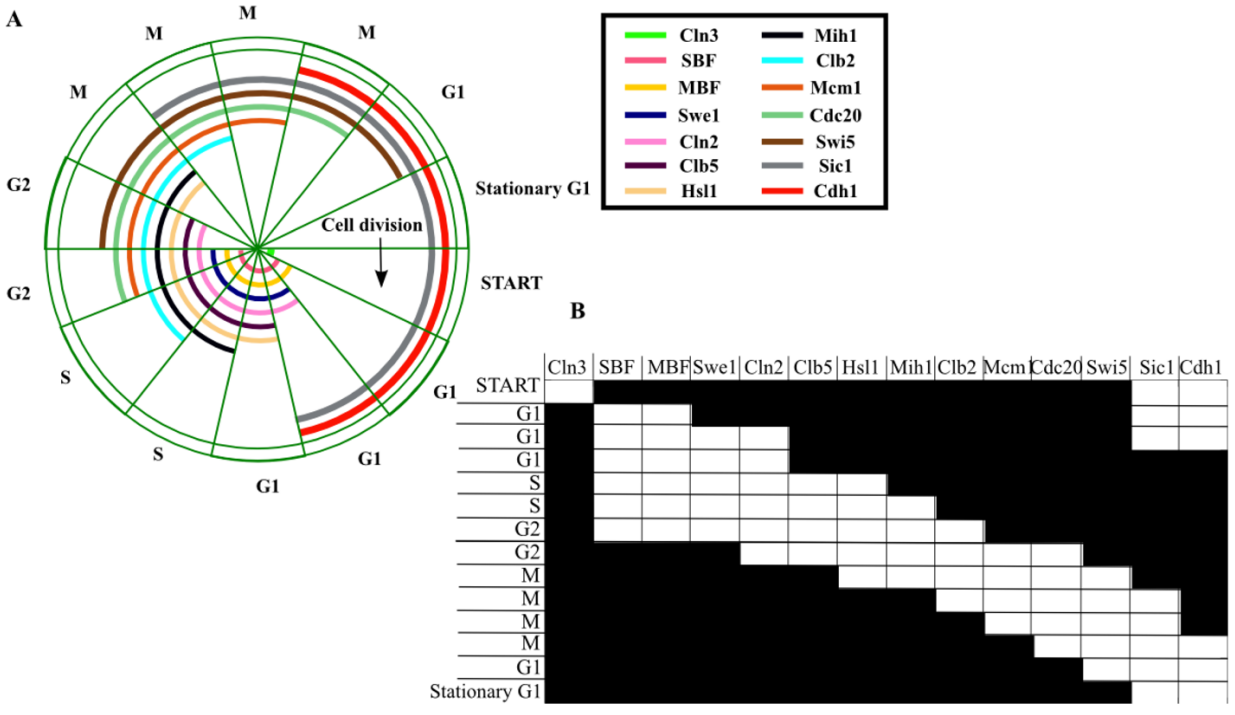

Figure 5 – **A)** Sequential activity of the cell cycle components during the state transition from START to $\mathbf{fp_U}$ (stationary G1). The components are shown with different colours and they are active during their corresponding sectors. **B)** Boolean activity profile of the cell cycle component. Note that Cln3 activity is different in the stationary G1 and START states. Cln3 triggers a cascade of state transitions from START to $\mathbf{fp_U}$. We did not consider the signal which reverses stationary G1 to START. Hence, we consider one period of the cell cycle. The cascade of events ends in the fixed point $\mathbf{fp_U}$. Activity of Cln3 returns the cell from that fixed points to START. An active component is shown by white, whereas black block depicts an inactive component.

are of particular interest, as they correspond to an *arrested* state, i.e. a state that the cell cannot leave without an external signal. We calculate the eigenvectors to the 17 unit eigenvalues of the matrix U (unstressed) of our Boolean network. These fixed points partition the state space into 17 different basins, i.e. equivalence classes. We denote the basin which contains the cell cycle trajectory **U**-basin to emphasise that it is the basin of the unstressed case. The fixed point of the **U**-basin is denoted by $\mathbf{fp_U}$. It is worth noting that because of the biological properties of the cell cycle we choose to update the states synchronously [1], but it can be shown that the asynchronous update will not alter the existence and number of the fixed points (see Appendix, Corollary 7.2) [17].

Osmotic stress causes activation of the MAPK Hog network, which as a result, activates the Hog1 node. The activity of the Hog1 node in turn changes the state transition matrix from U (unstressed) to O (osmotic). The matrix O has 15 unit eigenvalues, and consequently 15 basins (Fig. 3B). A short calculation shows that states from the unstressed cell cycle trajectory (Fig. 5) are now elements of four different basins of O (out of 15). It is noteworthy that one of these fixed points is $\mathbf{fp_U}$. Osmotic stress halts cells which are stressed upstream of the state *CC7* in Figure 4. Accordingly, these cells are arrested in either of the three fixed points ($\mathbf{fp_1}$, $\mathbf{fp_2}$, $\mathbf{fp_3}$ in Fig. 4). Whereas the cells which are after the state *CC7*, will go through the same state transitions as unstressed cells, until they reach $\mathbf{fp_U}$. Hence, the state of the cells at the onset of the stress dictates their development and ultimately which fixed point they are attracted to.

Moreover, our model also predicts the state transitions from the four fixed points of O (osmotic) to the cell cycle trajectory when the stress is removed. According to our model, a perturbation from the cell cycle trajectory by the osmotic stress can be compensated when the stress node is off[1](Fig. 4). The cell cycle trajectory and its response to the osmotic perturbation are depicted on Figure 4. The black edges show the state transitions for the unperturbed case, whereas the red edges illustrate the network's response to the activity of Hog1. Finally, the green edges

[1]Note that our Boolean model includes the mechanism for the stress dose between 0.4 M-1 M NaCl. Higher dose of stress can cause cell death, which would require us to take other regulatory mechanisms into account.

represent the state transitions from the fixed points of O to the cell cycle trajectory, when osmotic stress is removed. Remarkably, the presence of osmotic stress cannot take the cell out of the its basin, and all of these perturbations from the limit cycle of the cell cycle can be compensated after the removal of the osmotic stress.

5.2 Biological importance of the size of basins

In the previous section we have discussed the fixed points of the state transition matrices and their relevance for the interpretation of the cells' stress response. In this section we will study the importance of the respective basins and particularly their size.

We start our discussion with the observation that one of the basins of U (unstressed) is much larger than the others. This is consistent with what has been observed previously in the Boolean model of the cell cycle [1]. Like before, the cell cycle trajectory (Fig. 5) lies in this largest basin (**U**-basin) [1].

To interpret this result, one should keep in mind that many of the states in the **U**-basin are to some extent *artificial*; they are obtained by mathematically generating all possible combinations of states of all constituents. How many of them are viable or can be reached by careful experimentation or environmental fluctuation is a challenging question. The special relevance of the **U**-basin is that the cell cycle trajectory lies in this basin, rather than its mere size. It can be speculated however, that those states that can be reached by typical environmental fluctuations should be preferentially linked to the **U**-basin on evolutionary time scales, to allow the cells to return to a normal cell cycle and to reproduce.

The size of one of the four cell cycle basins of O (osmotic) (node $\mathbf{fp_3}$ in Fig. 4) is very small compared to the others. But since we know how to reach that particular basin experimentally, it is a crucial basin for our following discussion.

So rather than the mere size of basin it is important to know how to reach the states in that basin and whether they correspond to viable cells. We therefore suggest to consider only those states which are *observable*, i.e. can be reached by environmental or experimental perturbations.

5.3 Influence of the alpha sector synchronisation on the cell cycle dynamics

In this section we will discuss an experimental procedure that is frequently used to synchronise populations of cells. In a culture of cells the individuals will be at different stages of their cell cycle and this adds an additional complication to many experiments. Synchronising these cells in terms of their cell cycles allows to look at more specific settings and still preserves the advantage of large ensembles of cells, which are required for many experimental techniques.

This is why many traditional techniques for studying the cell reaction to environmental fluctuations at a particular phase is to synchronise the population of cells. Yet, it is important to understand the influence of the chosen synchronisation technique on the cell cycle dynamics and the state transitions. Ignoring the influence of the synchronisation method on the dynamics, may mislead the interpretation of the results.

One of the standard methods of synchronisation of the cell cycle of a culture of baker's yeast is via the alpha factor. This activates a cascade of events which at the end inhibits Cln2 and arrests the cells in the G1 phase.

We first add Far1 and its corresponding edge to our Boolean model of the cell cycle (Fig. 1B). We then investigate the state transitions of the cell cycle when the alpha factor is applied by deriving matrix A. States of the cell cycle trajectory evolve into either of two fixed points which we denote by $\mathbf{fp_1}$ and $\mathbf{fp_U}$ respectively (Fig. 4). Cells in states which are attracted to $\mathbf{fp_U}$ will divide exactly once upon presence of the cell cycle division signal despite the presence of the alpha factor. But those which are in $\mathbf{fp_1}$'s basin will get arrested at that fixed point when Far1 is active. The state transitions of the cell during and after release from the alpha factor is depicted on Figure 4.

Interestingly, these two fixed points are also osmotic stress fixed points (Fig. 4). Considering the fact that the cell will get arrested in $\mathbf{fp_1}$ when the alpha factor is used, we see that synchronisation with the alpha factor will limit us to study those cell cycle states which are downstream of *CC3* in Figure 4. This indicates that using the alpha factor for population synchronisation will allow us to study all osmotic stress fixed points apart from $\mathbf{fp_1}$ and its basin. To study $\mathbf{fp_1}$ other methods of population synchronisation must be used.

5.4 Osmotic stress can retrieve some frozen states to the cell cycle trajectory

The stresses we have considered in the paper so far, are all *reversible*, in the sense that, once the stress is released, the cells will resume their normal cell cycle. That means that the system stays within the **U**-basin (cell cycle basin). As discussed before, there are many other basins which are disconnected from the **U**-basin (Fig. 3A). If a stress moves the system into one of those basins, the cells will not recover after the removal of the stress. They will not resume their normal cell cycles and they will not

form colonies. Following a common definition in experimental biology these cells can be considered to be *dead* in spite of their remaining metabolic activity. Our model suggests that some of these frozen states, might be returned to the **U**-basin if an appropriate experimental procedure, e.g. stress, is applied.

Within the framework of our model, the Boolean state of the stress node moves some frozen states, that are originally outside of the **U**-basin, into the **U**-basin. This means that time evolution with the matrix O (osmotic) brings these states to the **U**-basin. Therefore these states can reach the cell cycle trajectory and allow the cell to divide. Hence, a stress can "thaw" frozen states (blue nodes in Fig. 3A).

A direct description of stresses that move states out of the **U**-basin is beyond the scope of this paper. However, some experimental evidence, reported by Reiser et al. [19], suggests that this prediction might be biological fact. The experiment regards the behaviour of certain mutated cells in the presence of osmotic stress. This particular mutation affects one key component of the so-called Mitotic Exit Network (MEN). In the absence of osmotic stress these knock-out mutant cells are arrested and cannot exit mitosis, i.e. the mutation modifies the transition network (it deletes edges) so that the **U**-basin splits into disconnected components. This is different from the situation discussed above, but dynamically the situation is similar. The cell is in a state that cannot reach the fixed point of the **U**-basin in unstressed conditions; the cells cannot divide and do not form colonies. Remarkably, and consistent with our Boolean model, it was found that these cells do exit mitosis when an osmotic stress is applied. This suggests that a similar observation might be made when the cells are removed from the **U**-basin by stresses. But in this case the stress could bring frozen cells back to the cell cycle trajectory, so that even after removal of the stress the cells would keep dividing.

6 CONCLUSION

We present a Boolean model that describes how environmental changes influence the cell cycle dynamics through the entire cell cycle. The model integrates recent experimental findings about the interaction of the osmotic stress response and cell cycle networks across the G1-S-G2-M phases. The finite number of states in our discrete model enables us to study the cell cycle reaction in all different states.

Osmotic stress kicks the cell away from its cell cycle trajectory. Our model predicts that Hog1 can take the cell into the basin of either four different fixed points. The state of the cell at the onset of the stress determines the arrest point. Furthermore, our model describes the state transitions under osmotic stress as well as how the cell recovers from osmotic arrest after the removal of the stress. According to our model, all of the four arrest points can reach the cell cycle trajectory when the stress is removed.

We discuss that many of the states are to some extent artificial; they are obtained by mathematically generating all possible combinations of states of all constituents. We show that the special relevance of the **U**-basin (cell cycle basin) is that the cell cycle trajectory lies within this basin, rather than its size. Also, we demonstrate that the size of one of the four cell cycle basins under osmotic stress, is very small compared to the others. We argue that despite its small size, since we know the experiments to reach that particular basin, it is a crucial basin in some experimental settings. We therefore suggest to consider only those states which are *observable*, i.e. can be reached by environmental or experimental perturbations. This suggests a method for reducing the size of state space.

Our Boolean model also enable us to study the dynamical changes of cells which are exposed to the alpha factor. This is highly relevant for a common experimental setting: the alpha factor is one of the standard methods for population synchronisation of yeast. We have shown that with this particular method of synchronisation it is not possible to study one of the arrest points of osmotic stress. Hence, other methods of population synchronisation have to be used to study this fixed point and its basin.

Remarkably, our model demonstrates that osmotic stress can move some of the states which are outside of the **U**-basin back into this basin. This prediction of our model explains the reaction of MEN mutant cells to osmotic stress. Cells with the MEN mutation cannot go through cell division and are arrested. However, consistent with our Boolean model, these cells divide in the presence of osmotic stress [19]. Hence, osmotic stress takes the MEN mutant cells, the state of which are outside the **U**-basin, back into this basin. In general, some cells that are incapable of forming a colony can be simulated to divide again by osmotic stress. In this case the stress can return them to the cell cycle trajectory, such that they will remain in the **U**-basin even after the osmotic stress is removed.

7 APPENDIX

7.1 The synchronous or asynchronous update of states does not affect the existence of fixed points

In this section we prove that the method of update does not alter the existence and number of fixed points. The Definitions and Lemma 7.1 are taken from the monograph by Robert [17].

The finite but large set of states of the Boolean network is denoted by $\mathbf{X}$, and F is a Boolean map from $\mathbf{X}$ to itself. We are interested in studying the dynamical behaviour of successively applying F to the elements of $\mathbf{X}$. Given an arbitrary initial state $\mathbf{x^0} \in \mathbf{X}$, the sequence of states, i.e. the time evolution is given by

$$\mathbf{x^{p+1}} = F(\mathbf{x^p}) \quad (p = 0, 1, 2, \dots). \tag{5}$$

Since $\mathbf{X}$ is a finite set, the sequence of state transitions can have two possible types of dynamical behaviour: (i) either the sequence converges to a stationary state $\mathbf{y}$ after a finite number of iterations; at this fixed point of F whereas $F(\mathbf{y}) = \mathbf{y}$, (ii) the sequence repeates after a certain number of steps. The states of that recurrent sequence form a cycle $\{\mathbf{y}_1, \mathbf{y}_2, \dots, \mathbf{y}_k\}$, which is defined by $\{\mathbf{y}_1 = F(\mathbf{y}_k), \mathbf{y}_2 = F(\mathbf{y}_1), \dots, \mathbf{y}_k = F(\mathbf{y}_{k-1})\}$ [17].

Definition 7.1. The iteration given by Eqn. (5) is a *synchronous update* or *parallel mode of operation*. At each step all nodes are updated simultaneously.

Definition 7.2. A *serial mode of operation* or *asynchronous update* is an iteration in which not all of the elements are updated at the same time. Starting from an arbitrary condition $\mathbf{x^p}$, one element is updated first, then the second, considering the effects of the changes to the first element, and so on. This leads to the system

$$\begin{aligned} x_1^{p+1} &= f_1\left(x_1^p, x_2^p, \dots, x_n^p\right) \\ x_2^{p+1} &= f_2\left(x_1^{p+1}, x_2^p, \dots, x_n^p\right) \\ &\vdots \\ x_n^{p+1} &= f_n\left(x_1^{p+1}, x_2^{p+1}, \dots, x_{n-1}^{p+1}, x_n^p\right), \end{aligned} \tag{6}$$

where $x = (x_1, x_2, \dots, x_n)$ and $p \in \{0, 1, 2, \dots\}$.

Definition 7.3. The operator (Boolean function) F_i is defined by

$$F_i(x) = \begin{bmatrix} x_1 \\ \vdots \\ f_i(x_1 \cdots x_n) \\ \vdots \\ x_n \end{bmatrix}. \tag{7}$$

Lemma 7.1. (Theorem 2 in [17]). *Every asynchronous update is a method of successive approximations of a Boolean function* $G : \mathbf{X} \to \mathbf{X}$*, called associated asynchronous operator for* $F : \mathbf{X} \to \mathbf{X}$.

$$G = F_n \circ \cdots \circ F_2 \circ F_1. \tag{8}$$

Proof. We will successively apply the asynchronous update to all $\mathbf{x} = (x_1 \cdots x_n) \in \mathbf{X}$:

$$\begin{aligned} g_1(x_1 \ \dots \ x_n) &= f_1(x_1 \ \dots \ x_n) \\ g_2(x_1 \ \dots \ x_n) &= f_2(g_1(x) \ \dots \ x_n) \\ &\vdots \\ g_i(x_1 \ \dots \ x_n) &= f_i(g_1(x) \ \dots \ g_{i-1}(x) \ x_i \ \dots \ x_n) \\ &\vdots \\ g_n(x_1 \ \dots \ x_n) &= f_n(g_1(x) \ \dots \ g_{n-1}(x) \ x_n). \end{aligned} \tag{9}$$

Hence, the final state, after successive application of the asynchronous update p times, can be obtained by directly applying the corresponding Boolean function G

$$\mathbf{x}^{p+1} = G(\mathbf{x}^p) \quad (p = 0, 1, 2, \dots). \tag{10}$$

□

Corollary 7.2. *The sets of fixed points for the synchronous and asynchronous updates are identical.*

Proof. This corollary is a direct consequence of Lemma 7.1. For every asynchronous map we can find a Boolean function G which is a sequential composition of F_i Eqn. (8). Hence, if $\mathbf{y}$ is a fixed point of F, i.e. $F(\mathbf{y}) = \mathbf{y}$, this implies that $\mathbf{y}$ is also a fixed point of

$$G(\mathbf{y}) = F_n \circ \cdots \circ F_2 \circ F_1(\mathbf{y}) = \mathbf{y}.$$

Moreover, if $\mathbf{y}$ is a fixed point of G, then $G(\mathbf{y}) = \mathbf{y}$. According to Eqn.(9), we have

$$\begin{aligned} g_1(y_1 \ \dots \ y_n) &= f_1(y_1 \ \dots \ y_n) = y_1 \\ g_2(y_1 \ \dots \ y_n) &= f_2(g_1(y) \ \dots \ y_n) = y_2 \\ &\vdots \\ g_i(y_1 \ \dots \ y_n) &= f_i(g_1(y) \ \dots \ g_{i-1}(y) \ y_i \ \dots \ y_n) = y_i \\ &\vdots \\ g_n(y_1 \ \dots \ y_n) &= f_n(g_1(y) \ \dots \ g_{n-1}(y) \ y_n) = y_n. \end{aligned} \tag{12}$$

Hence, $g_1(y_1, \dots, y_n) = f_1(y_1 \dots y_n) = y_1$. Therefore y_1 is a fixed point of F_1. With the same argument we can show that y_2 is the fixed point of F_2 and argue iteratively for all F_n. As a result, $\mathbf{y} = (y_1 \dots y_n)$ is a fixed point of F. Thus, F and G have the same set of fixed points. The synchronous or asynchronous application of the Boolean function does not change the existence and the set of fixed points. □

Furthermore, the set of fixed points does not change if instead of updating the states purely synchronously or asynchronously we use a combination of both. The argument is analogous to the one above.

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REFERENCES

[1] LI F, LONG T, LU Y, OUYANG Q & TANG C. 2004. The yeast cell-cycle network is robustly designed. PNAS, 101(14): 4781–4786.

[2] BREEDEN LL. 1997. Alpha-factor synchronisation of budding yeast. Methods in Enzymology, 283: 332–341.

[3] SURANA U, ROBITSCH H, PRICE C, SCHUSTER T, FITCH I, FUTCHER B & NASMYTH K. 1991. The role of CDC28 and cyclins during mitosis in the budding yeast *S. cerevisiae*. Cell, 65(1): 145–161.

[4] SIA R, HERALD H & LEW DJ. 1996. Cdc28 tyrosine phosphorylation and the morphogenesis checkpoint in budding yeast. Molecular Biology of the Cell, 7(11): 1657–1666.

[5] AMON A, TYERS M, FUTCHER B & NASMYTH K. 1993. Mechanisms that help the yeast cell cycle clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. Cell, 74(6): 993–1007.

[6] STEGMEIER F & AMON A. 2004. Closing mitosis: The functions of the Cdc14 phosphatase and its regulation. Annual Review of Genetics, 38: 203–232.

[7] VISINTIN R, PRINZ S & AMON A. 1997. CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. Science, 278(5337): 460–463.

[8] BÄUMER M, BRAUS GH & IRNIGER S. 2000. Two different modes of cyclin clb2 proteolysis during mitosis in *Saccharomyces cerevisiae*. FEBS Letters, 468(2-3): 142–148.

[9] DE NADAL E, ALEPUZ PM & POSAS F. 2002. Dealing with osmostress through MAP kinase activation. EMBO reports, 3(8): 735–740.

[10] BREWSTER JL, DE VALOIR T, DWYER ND, WINTER E & GUSTIN MC. 1993. An osmosensing signal transduction pathway in yeast. Science, 259(5102): 1760–1763.

[11] BELLÍ G, GARÍ E, ALDEA M & HERRERO E. 2001. Osmotic stress causes a G1 cell cycle delay and downregulation of Cln3/Cdc28 activity in *Saccharomyces cerevisiae*. Molecular Microbiology, 39(4): 1022–1035.

[12] ESCOTÉ X, ZAPATER M, CLOTET J & POSAS F. 2004. Hog1 mediates cell-cycle arrest in G1 phase by the dual targeting of Sic1. Nature Cell Biology, 6(10): 997–1002.

[13] ZAPATER M, CLOTET J, ESCOTÉ X & POSAS F. 2005. Control of Cell Cycle Progression by the Stress-Activated Hog1 MAPK. Cell Cycle: 6–7.

[14] YAAKOV G, DUCH A, GARCÍA-RUBIO M, CLOTET J, JIMENEZ J, AGUILERA A & POSAS F. 2009. The stress-activated protein kinase Hog1 mediates S phase delay in response to osmostress. Molecular Biology of the Cell, 20(15): 3572–3582.

[15] CLOTET J, ESCOTÉ X, ADROVER M, YAAKOV G, GARÍ E, ALDEA M, DE NADAL E & POSAS F. 2006. Phosphorylation of Hsl1 by Hog1 leads to a G2 arrest essential for cell survival at high osmolarity. The EMBO Journal, 25(11): 2338–2346.

[16] MENDENHALL MD & HODGE AE. 1998. Regulation of Cdc28 cyclin-dependent protein kinase activity during the cell cycle of the yeast *Saccharomyces cerevisiae*. Microbiology and Molecular Biology Reviews, 62(4): 1191–1243.

[17] ROBERT F. 1986. Discrete Iterations: A Metric Study. Springer Series in Computational Mathematics.

[18] TYERS M, TOKIWA G & FUTCHER B. 1993. Comparison of the *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. The EMBO Journal, 12(5): 1955–1968.

[19] REISER V, D'AQUINO KE, LY-SHA E & AMON A. 2006. The stress-activated mitogen-activated protein kinase signaling cascade promotes exit from mitosis. Molecular Biology of the Cell, 17(7): 3136–3146.