

A logical circuit for the regulation of fission yeast growth modes

Jürg Bähler^a, Saša Svetina^{b,c,*}

^aThe Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK

^bInstitute of Biophysics, Faculty of Medicine, University of Ljubljana, Lipičeva 2, 1000 Ljubljana, Slovenia

^cJožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

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Abstract

Growth of fission yeast at the ends of its cylindrical cells switches from a monopolar to a bipolar mode, before it ceases during mitosis and cell division. Here we assume that these growth modes correspond to three stable states of an underlying regulatory circuit, which is a relatively simple and to a large degree autonomous subsystem of an otherwise complex cellular control system. We develop a switch-like logical circuit based on three elements defined as binary variables. Effects of circuit variables on each other are expressed in terms of logical operations. We analyse this circuit for its behavior (“phenotypes”) after removing single or multiple operations (“mutants”). Known fission yeast polarity mutants such as those defective in the switch to bipolar growth can be classified based on these predicted ‘phenotypes’. Differences in growth patterns between daughter cells in different bipolar growth mutants are also predicted by the circuit model. The model presented here should provide a useful framework to guide future experiments into mechanisms of cellular polarity. This paper illustrates the usefulness of simple logical circuits to describe and dissect features of complex regulatory processes such as the fission yeast growth patterns in both wild type and mutant cells.

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1. Introduction

Cells often operate by switching from one stable steady state to another to exert various biological functions. It is usually difficult to unravel all details of the underlying regulatory mechanisms, because they are confounded by complex interactions between many different proteins. Models that emphasize essential features of biological control processes can therefore provide a valuable basis to identify important information and to decide on critical follow-up experiments to test and refine the models. To develop a model, it helps to choose a system with a sufficient amount of

accumulated data and to concentrate on a cellular process that is relatively isolated from other processes. The regulation of fission yeast growth modes satisfies these criteria.

The fission yeast *Schizosaccharomyces pombe* is a relatively simple unicellular model organism, which grows at the ends of its cylindrically shaped cells. Newly born cells generated by cell division initiate growth in a monopolar fashion, growing at the cell end that was already present in the mother cell. After DNA replication, growth is induced also at the new cell end created by cell division, leading to a bipolar growth mode (Mitchison and Nurse, 1985). During mitosis, cells cease growth at their ends, and the growth machinery engages in the formation of the division septum at the cell center (Gould and Simanis, 1997). Thus, fission yeast cells exhibit three well-defined growth modes during the cell cycle, switching from one to the next in a defined order: (1) monopolar growth, (2) bipolar

*Corresponding author. Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Lipičeva 2, 1000 Ljubljana, Slovenia. Tel.: +386 1 5437600; fax: +386 1 4315127.

E-mail addresses: jurg@sanger.ac.uk (J. Bähler), sasa.svetina@biofiz.mf.uni-lj.si (S. Svetina).

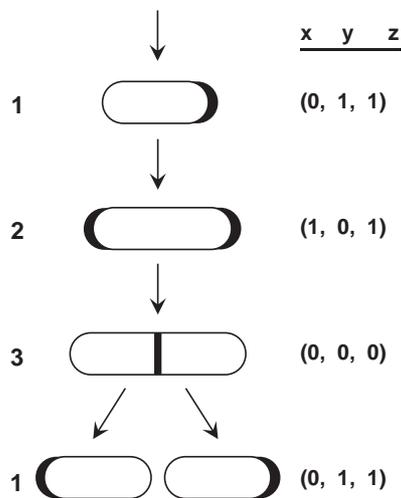


Fig. 1. Schematic representation of the different fission yeast growth modes. Growing regions are indicated by black areas. The values of the binary variables x , y , and z for the three circuit elements that define the corresponding logical circuit are shown for each growth mode. See text for details.

growth, (3) no growth at cell ends, (4) monopolar growth, etc. (Fig. 1).

Polarized growth correlates with the localization of actin patches to the growth sites at cell ends (Marks and Hyams, 1985). Polarized growth may result from an interplay between cell end markers and a self-organizing, microtubule-mediated delivery of growth determinants to the cell ends (reviewed by Snell and Nurse, 1993; Bähler and Peter, 2000; Hayles and Nurse, 2001; Chang and Peter, 2003; Chang and Verde, 2004). Several mutants show modified growth behaviors such as defects in the switch to bipolar growth. These mutants differ in various characteristics, including severity of defects and specific growth patterns. The growth behavior and mutant phenotypes of fission yeast indicate that cell polarity is under control of a regulatory circuit. The viability of many growth mutants further suggests that this circuit is a relatively simple subsystem of a more complex cellular control system. We hypothesize that the different growth modes of fission yeast represent different stable steady states of the underlying regulatory network. Due to the multitude of stable states, such a network must involve positive feedback loops (Thomas, 1998; Soulé, 2003).

In this paper, we develop and discuss a possible regulatory circuit that is consistent with available data on the fission yeast growth patterns. First, we introduce a logical circuit model together with a description of the requirements for the logical analysis approach. We then compare ‘mutants’ of the model with real fission yeast mutants, before discussing the benefits of the logical circuit analysis for gaining deeper insight into possible regulatory mechanisms of cellular growth patterns.

2. Theoretical background: A switch-like logical circuit model for fission yeast growth modes

Logical circuits (boolean networks) provide a convenient method to address the dynamics of modules regulating biological systems (Thomas and d’Ari, 1990; Huang, 1999). A logical circuit description of a given cellular system can also be exploited to predict its behavior under a variety of perturbing conditions. In this section, we first give a short account on key aspects of the logical circuits approach, then derive a suitable irreducible logical circuit, and finally perform its ‘mutational’ analysis.

2.1. A brief introduction into logical circuits

The relevant fission yeast growth modes are (1) monopolar growth, (2) bipolar growth and (3) no-growth at the cell ends (Fig. 1). We propose a model in which these growth modes correspond to three stable steady states of an underlying regulatory circuit. This circuit is approximated by a logical circuit that involves three circuit variables. We assume that different growth modes can be represented by differently activated circuit variables. The first tasks then are to define the relevant binary representation for these variables and to assign their values (0 or 1) for each growth mode. Growth at one or both cell ends is represented in the circuit by the binary variable (z) that takes the value 1 when end growth is “on” and the value 0 when it is “off”. The other two variables reflect growth regulatory mechanisms leading to either monopolar or bipolar growth modes. They are represented by the binary variables y and x , respectively, which are defined to be 1 when the corresponding growth mode is turned “on” and to be 0 when it is turned “off”. Since these two growth modes exclude each other, it follows that when variable y is 1, variable x is 0, and when variable x is 1, variable y is 0 (Fig. 1).

The states of a logical circuit based on the three circuit variables x , y and z are conveniently described by $2^3 = 8$ vectors whose components are the binary values of these variables (Table 1, column Vectors). According to the above assignments, the three stable states of the logical circuit that correspond to the three growth modes are $(x, y, z) = (0, 1, 1)$ for monopolar growth, $(1, 0, 1)$ for bipolar growth, and $(0, 0, 0)$ for no-growth at the cell ends (Fig. 1). In general, the stable states of a regulatory circuit are the result of the operations exerted by system variables on each other. In logical circuits these operations define logical functions X , Y and Z that attain the values 0 or 1, dependent on the current values of the circuit variables x , y and z . The state is stable when the logical functions attain values of the vector that defines this state. The state is not stable when at least one logical function attains a different value. We

Table 1
State tables of different logical circuits*

Vectors x y z	Circuit A X Y Z	Circuit B X Y Z	Circuit C X Y Z	Circuit D X Y Z	Circuit E X Y Z	Circuit F X Y Z
0 0 0	0 0 0					
0 0 1	1 1 0	1 1 0	1 1 0	1 1 0	1 1 0	0 1 0
0 1 0	0 0 1	0 0 1	0 0 1	0 0 0	0 0 1	0 0 1
0 1 1	0 1 1	0 1 1	0 1 1	0 1 0	0 1 1	0 1 1
1 0 0	0 0 1	0 0 1	0 0 0	0 0 1	0 0 0	1 0 1
1 0 1	1 0 1	1 1 1	1 0 0	1 0 1	1 1 0	1 1 1
1 1 0	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	1 0 1
1 1 1	0 0 1	0 1 1	0 0 1	0 0 1	0 1 1	1 1 1

*Stable states are represented by bold numbers. The first column (Vectors) shows the $2^3 = 8$ states of the logical circuit with three variables (x, y, z). Circuit A shows the vectors that are obtained by application of the logical functions defined by Eqs. (7)–(9) on the vectors in the Vectors column (see text for details). The next four columns show the corresponding vectors for the ‘mutants’ of circuit A defined by Eqs. (10)–(12) (B), Eqs. (13)–(15) (C), Eqs. (16)–(18) (D), and Eqs. (19)–(21) (E). Circuit F represents vectors obtained by logical functions $X = x$, $Y = z$, and $Z = x + y$.

want to identify a logical circuit defined by a set of logical functions that give rise to the three chosen stable states while the remaining five states are unstable. Table 1 shows an example of such a logical circuit (circuit A), which will be defined and discussed below. Table 1 also shows other circuits (B to F) that will be discussed and exhibit different sets of stable states. In general, a combination of the vector column together with any of the circuit columns in Table 1 forms the state table representation of the respective logical circuit, revealing the stable states (Thomas and d’Ari, 1990; Thellier et al., 2004).

Because of the multitude of possible unstable states, there are numerous possible logical circuits with three variables that give rise to the three selected stable states. Logical operations applied on a given vector can produce $7(2^3 - 1)$ vectors that are different, meaning that we can define 7 unstable states using different logical functions. Consequently, with 5 unstable vectors, there are $7^5 = 16\,807$ different sets of logical functions X , Y and Z (i.e. different logical circuits that exhibit the selected 3 stable states). Circuit A represents just one of them. These 7^5 circuits differ in the number of operations by which circuit variables exert effects on each other, and thus in the circuit complexity. Some of these circuits show redundancy, because certain operations can be removed without changing the circuit outcome. Through consecutive removal of redundant operations, different irreducible circuits can be reached, in which all remaining operations are essential.

2.2. An irreducible switch-like logical circuit

We want to find the logical circuit that gives rise to the chosen three stable states and that is consistent with experimental data. We aim at developing a logical circuit that is as simple as possible and then compare its predictions with available data. The next step would then be to introduce additional operations and variables

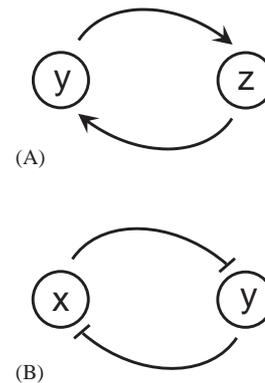


Fig. 2. Graphical representation of logical circuits. Linkages that end with an arrow denote positive (activating) effects, whereas linkages that end with a cross bar denote negative (repressing) effects. (A) Logical circuit described by Eqs. (1) and (2) (and if y is replaced by x, by Eqs. (3) and (4)). (B) Logical circuit described by Eqs. (5) and (6).

if required to remove discrepancies between predictions of the model and experimental data. The proposed approach is corroborated by the recent study of Azevedo et al. (2005), which indicates that evolution can drive regulatory networks towards the simplest solution.

We introduce a simple irreducible logical circuit that has the character of a switch, consistent with the chosen stable states. The switch-like behavior of the circuit is evident when considering stable states for pairs of the chosen variables with a constant value for the third variable. For instance, a switch-like behavior can be ascribed to the variables y and z when $x = 0$. In this reduced circuit, the switch from $(y, z) = (0, 0)$ to $(y, z) = (1, 1)$ represents the transition from no-growth to monopolar growth. The variables y and z in this switch are either both ‘on’ or both ‘off’, which means that they act on each other in a positive manner. The corresponding logical circuit can be represented by a graph in which the circuit operations are represented by linkages (Fig. 2A). The corresponding logical functions

can be formalized as

$$Y = z, \tag{1}$$

$$Z = y. \tag{2}$$

The logical function defined by Eq. (1) shows that the effect of variable z on the variable y is that y attains the current value of z . The logical function defined by Eq. (2) shows that z attains the current value of y . The effect of a variable on another variable can either be direct or via a chain of intermediate steps. To distinguish a variable from its operation on another variable, we write operations on right sides of logical functions in *italic* letters.

With $y = 0$, an analogous switch exhibiting mutual activating action between the variables x and z is expressed as

$$X = z, \tag{3}$$

$$Z = x. \tag{4}$$

A third standard switch is obtained with $z = 1$, where we have a switch from $(x, y) = (0, 1)$ to $(x, y) = (1, 0)$. The variables x and y act on each other in an inhibitory manner (Fig. 2B), which in terms of logical equations reads

$$X = \bar{y}, \tag{5}$$

$$Y = \bar{x}. \tag{6}$$

The overbar in \bar{y} and \bar{x} denotes the NOT logical operations, meaning in Eq. (5) that $X = 1$ if $y = 0$ and $X = 0$ if $y = 1$, and in Eq. (6) that $Y = 1$ if $x = 0$ and $Y = 0$ if $x = 1$.

The complete circuit for all three variables x, y and z , constructed as the simplest possible generalization of Eqs. (1)–(6), can be expressed by the following logical functions:

$$A: \quad X = z\bar{y}, \tag{7}$$

$$Y = \bar{x}z, \tag{8}$$

$$Z = x + y. \tag{9}$$

These logical functions take into account that for the monopolar or bipolar growth modes to be “on”, the growth variable z must be “on”, and at the same time the alternative growth mode must be “off”. This requirement is expressed by the logical SUMs represented by products of the respective operators (Eqs. (7) and (8)). Growth (z) is “on” if either the monopolar or bipolar growth mode is “on” as represented by the summation in Eq. (9) corresponding to the logical OR operation.

Note that logical operations given in Eqs. (7) and (8), which are described by logical SUMs, can be realized by different linkage connections. This is important, because in the cellular system each linkage may in molecular

terms represent a ‘signaling pathway’ composed of a complex chain or network of chemical reactions involving numerous proteins. It is possible that one of the variables in a logical SUM operates on an intermediate link in the pathway rather than directly on the target variable. Thus, the logical SUM given in Eq. (7) can be realized in two ways: (1) x is inhibited by y only if z acts in a positive manner on one of the links of the corresponding pathway (Fig. 3A); or 2) z acts in a positive manner on x only if an intermediate link in the pathway is not inhibited by y (Fig. 3B). The same logical SUM can thus give rise to two different linkage patterns. To avoid this uncertainty, we will consider the order of operations in logical SUMs. By definition, the pathway corresponding to the right-hand operation in a logical SUM (\bar{y} in Eq. (7)) ends on the target variable, while the left-hand operation (z in Eq. (7)) acts on one of the intermediate links of this pathway. According to this definition, the logical SUM in Eq. (7) is represented by the linkage pattern shown in Fig. 3A, and the logical SUM that appears in Eq. (8) can be represented by a linkage pattern analogous to the one in Fig. 3B (see Discussion for choice of linkage patterns).

Fig. 4A illustrates the logical circuit defined by Eqs. (7)–(9) (circuit A). Each linkage in the graph represents an individual operation that appears in one of the logical functions X, Y and Z . The state table representation of the results of logical functions defined by Eqs. (7)–(9) is given in Table 1, circuit A. In general, graphical representations help to visualize the circuit linkages. On the other hand, as we shall see below, the state table representations are convenient for the determination of stable states when new circuits are obtained due to circuit ‘mutations’ (Thomas and d’Ari, 1990).

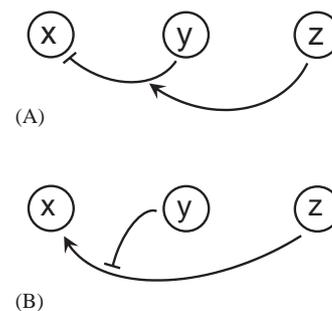


Fig. 3. Graphical representation of two possible operations of a logical SUM, in which linkages end anywhere in the middle of a signaling pathway represented by the last operation of the logical SUM. (A) Logical SUM $X = z\bar{y}$ (cf. Eq. (7)). The operation \bar{y} exerts its negative effect on variable x only if variable z is “on”. If z is “off” or removed, the operation \bar{y} is inactive. (B) Logical SUM $X = \bar{y}z$ (a corresponding situation is applied in Eq. (8)). The operation z exerts its positive effect on variable x only if variable y is “off”. If y is “on”, the operation z is inactive, but if operation \bar{y} is deleted, operation z is active. For the meaning of arrows and bars see Fig. 2.

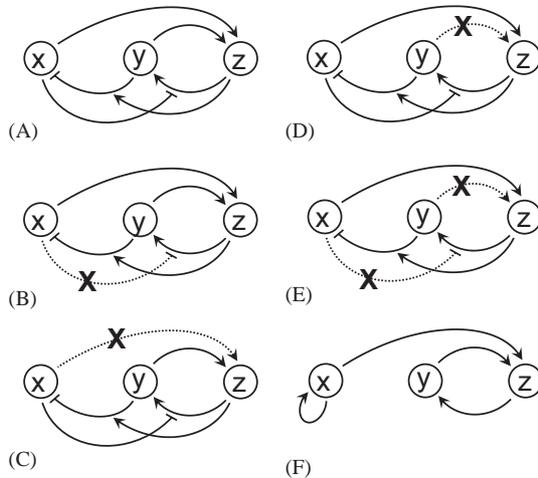


Fig. 4. Graphical representation of logical circuits defined by Eqs. (7)–(9) (A), 10–12 (B), 13–15 (C), 16–18 (D), 19–21 (E), and in the footnote of Table 1 (F). The deleted linkages are indicated by dashed lines and are also crossed. For the meaning of arrows and bars see Fig. 2.

2.3. Circuit A “mutants”

“Mutants” of circuit A can be modeled as deletions of at least one circuit linkage. “Mutants” can be represented graphically by removing the deleted linkages from the graph in Fig. 4A, or algebraically by correspondingly modified logical equations. Graphics of the “mutants” examined are shown in Fig. 4B–E; these “mutants” are also represented by their state tables in Table 1, circuits B to E, and the corresponding sets of logical equations are given below:

$$B: X = z\bar{y}, \quad (10)$$

$$Y = z, \quad (11)$$

$$Z = x + y, \quad (12)$$

$$C: X = z\bar{y}, \quad (13)$$

$$Y = \bar{x}z, \quad (14)$$

$$Z = y, \quad (15)$$

$$D: X = z\bar{y}, \quad (16)$$

$$Y = \bar{x}z, \quad (17)$$

$$Z = x, \quad (18)$$

$$E: X = z\bar{y}, \quad (19)$$

$$Y = z, \quad (20)$$

$$Z = y. \quad (21)$$

3. A logical circuit analysis of fission yeast cell polarity mutants

To analyse the logical circuit approach, we compare the “mutational” behavior of circuit A (Eqs. (7)–(9); Fig. 4A; Table 1) with known phenotypes of real cell polarity mutants in fission yeast. The goal of this analysis is to reveal the extent to which this circuit can predict known mutant classes. We also try to assign different polarity proteins to circuit variables and linkages.

Several “mutants” of circuit A fail to switch to bipolar growth, with no-growth and monopolar growth as the only stable states. Simple examples of such single ‘mutants’ are defined by the circuits B and C (Figs. 4B and C; Table 1). Circuit B is obtained by the removal of operation \bar{x} in Eq. (8). In the absence of this operation, the monopolar growth cannot be suppressed by the variable x , and bipolar growth is not induced. The behavior of circuit C is different, since the removed operation x in Eq. (9) represents the linkage through which x acts on the growth variable z . This ‘mutant’ can thus be interpreted as having a failure in the activation of growth. The “mutant” defined by circuit E (Fig. 4E; Table 1) includes both these features, i.e. failure in suppressing the monopolar growth as well as failure of variable x to induce growth. A straightforward further example of a bipolar growth “mutant” is obtained by the knock-out of variable x . In this case Eq. (7) becomes irrelevant and Eqs. (8) and (9) reduce into the simple positive feedback switch circuit described by Eqs. (1) and (2) (Fig. 2A). Because the described “mutants” represent different modifications of circuit A, it is expected that they exhibit different phenotypes.

Can the “mutants” of circuit A predict the properties of various fission yeast bipolar growth mutants? The *pom1* mutant shows expected properties for the removal of variable x . Induction of growth at the new cell end is correlated with up-regulation of Pom1p kinase activity, and cells can grow only in a monopolar mode in the absence of Pom1p (Bähler and Pringle, 1998; Bähler and Nurse, 2001). If Pom1p corresponds to x , it would be involved both in operation \bar{x} in Eq. (8) and in operation x in Eq. (9). The *teal* mutant exhibits a similar phenotype as the *pom1* mutant (Arellano et al., 2002; Chang and Verde, 2004). Tea1p could therefore also be involved in these two linkages, and the *teal* mutant could correspond to circuit E. In the absence of Tea1p, two functions of Pom1p described by these linkages would thus be abolished but its other functions (not explicitly represented by the present model) could remain intact. This would be consistent with the observation that the *teal* mutant shows less severe bipolar growth defects than the *pom1* mutant (Niccoli et al., 2003).

Tea1p acts upstream of Pom1p in that it is required for Pom1p localization (Bähler and Pringle, 1998; Bähler and Nurse, 2001; Behrens and Nurse, 2002; Niccoli et al., 2003). This relationship between the two proteins could be assigned to the signaling pathway represented in the model by operation x in Eq. (9). According to the previous assumption, both Pom1p and Tea1p are also involved in the pathway represented by operation \bar{x} in Eq. (8). In the latter pathway, Pom1p may act upstream of Tea1p. Tea1p is phosphorylated by Orb2p (Kim et al., 2003); the *orb2* mutant is also a bipolar growth mutant, and Orb2p therefore seems to act upstream of Tea1p in this pathway. Because an *orb2* mutant does not affect Pom1p kinase activity (Bähler and Nurse, 2001), Pom1p may act upstream of Orb2p and of Tea1p in this pathway. The specific defects in a pathway involving Pom1p, Orb2p, and Tea1p can be classified as circuit B-type mutations. Besides Orb2p, another possible candidate for this pathway is Tea3p, the major role of which is to efficiently activate growth at the second cell end (Arellano et al., 2002). Its position in the pathway might be downstream of Pom1p, and Tea3p could abolish some of the remaining Pom1p functions in the *tea1* mutant, thus leading to similar phenotypes in the *pom1* single mutant and the *tea1 tea3* double mutant (Niccoli et al., 2003).

Among the mutants with defects in the establishment of bipolar growth, the *bud6* mutant could be assigned to circuit C-type ‘mutants’, because of the role of Tea1p in its localization (Glynn et al., 2001). The *ssp1* mutant could also belong to this category, because of the role of Ssp1p in actin relocalization (Rupeš et al., 1999), and because it acts downstream of Pom1p kinase activity (Bähler and Nurse, 2001). We conclude that the various fission yeast mutants which fail to induce bipolar growth can be accounted for by the “mutational” behavior of circuit A in a satisfactory manner.

In some of the described mutants, the growth pattern in daughter cells differs from the wild type. In *pom1*, *tea1*, and *tea3* mutants, the daughter that inherits the growing end will initiate growth at this end (Fig. 5A, left cell), whereas the daughter cell that inherits the non-growing end will initiate growth at its new end (Fig. 5A, right cell) (Glynn et al., 2001; Niccoli et al., 2003). This could reflect a failure of these mutants to mark the non-growing cell end. The daughter cell that did not inherit any previously growing cell end then as a “default” grows at its new end where the growth machinery is located from the previous cell division. The circuit B-type mutants may exhibit this phenotype as reflected by *orb2* mutants (Kim et al., 2003). Sawin et al. (1999) actually found that the *orb2* mutant uncovers a failure of one of the cell ends to maintain the molecular properties that identify an end. On the other hand, *bud6* mutants grow at the old ends in both daughter cells (Glynn et al., 2001), even if one of them did not grow

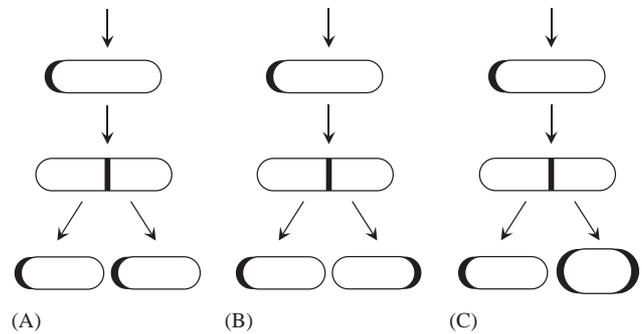


Fig. 5. Schematic representation of different growth behaviors in daughter cells from the various bipolar growth mutant classes as described in the main text. Growing regions are indicated as black areas.

during the previous cell cycle (Fig. 5B). Such a behavior supports the assignment of *bud6* as a C-type mutant and suggests Bud6p to be involved in the activation of growth. These examples demonstrate that different growth behaviors of daughter cells are also consistent with different defects in the underlying regulatory circuit A.

Another “mutant” of interest is the knock-out of the complete logical operation in Eq. (8), possibly as a result of the failure in the linkage modeled by operation z . In this case, the value of variable y is not under the control of the circuit and may thus depend on other conditions. If $y = 0$, the stable states are no-growth and bipolar growth, whereas with $y = 1$, they are no-growth and monopolar growth. The *for3* mutant may correspond to this situation, because its daughter cells have different fates: while one daughter cell grows in a monopolar fashion and has a normal shape (Fig. 5C, left cell), the other one grows in a bipolar fashion from the beginning of the cell cycle and has a more rounded shape (Fig. 5C, right cell) (Feierbach and Chang, 2001). This behavior suggests that in the daughter cell retaining the old end, conditions allow for y to be 1, while in the daughter cell without the old end, the value of y remains 0. This latter property is consistent with y being involved in the localized marking of the appropriate cell end, with the consequence that in the absence of y the growth machinery is distributed over a wider region and cells exhibit more oval shapes (Feierbach and Chang, 2001). It should be pointed out that the present analysis allows for the existence of the *for3* mutant phenotype, but it does not unravel its molecular basis.

The “deletion” of operation y in Eq. (9) leads to circuit D (Eqs. (16)–(18); Fig. 4D), in which the two stable states are no-growth and bipolar growth. The *cdc11* mutant that fails to undergo cell division is switching between these two growth modes. This reflects the coordination between cell cycle and growth regulation, the study of which is beyond the scope of this paper.

4. Discussion

To understand cellular functions, it is necessary to identify its molecular parts, as well as to recognize how they cooperate to form a highly regulated system. In this paper, aspects of cell regulation are treated on the basis of logical circuits. We have developed a regulatory circuit that is consistent with the available knowledge about fission yeast growth modes. Based on this analysis, we can also draw conclusions on general aspects of the usage of logical circuits. The discussion will start with the latter, before we point out specific insights from the logical circuit model to better understand the regulation and unknown properties of fission yeast growth patterns.

Logical circuits provide an adequate phenomenological description of biological regulatory systems involving feedback loops (Thomas and d'Ari, 1990; Huang, 1999). The “naïve” formulation of the asymmetrical logical method (Thomas and d'Ari, 1990) that we have adopted represents a convenient approach to analyse multiple, stable steady states in these systems. Moreover, the potential of the logical circuits approach also lies in possible generalizations such as multilevel logics where it is taken into account that variables which are involved in different pathways may become active (“on”) in these pathways at different concentration thresholds (Snoussi and Thomas, 1993; Thomas and Kaufman, 2001).

The search for logical circuits underlying cellular regulatory processes is compromised by the fact that often different circuits explain a given biological reality equally well. Not only are there tens of thousands of different logical circuits that give rise to the same number or even to the same set of stable states (i.e. to the same phenotype), but most of these circuits can also be realized through several different linkage patterns (Fig. 3). We have noted that circuits can be grouped into classes with similar characteristics. The circuit analysis can thus be simplified by only analysing properties of representative circuits of different classes. For practical reasons, it also seemed sensible to choose irreducible circuits as representatives of a class, i.e. circuits that involve the least number of linkages relative to other circuits of a given class. We focused on a logical circuit that can be phenomenologically considered as a good initial guess for growth regulation and then analysed its predictions in light of diagnostic experimental information. As an initial approximation, we chose the irreducible switch-like logical circuit A (Fig. 4A; Table 1) and showed that it represents basic features of the real fission yeast growth patterns. Our model on stable states assumes that monopolar and bipolar growths are triggered by two separate induction processes. Theoretical “mutants” of circuit A were then obtained by “deletion” of different circuit linkages. Notably, several

defects in bipolar growth can thus be predicted. Different “mutants” of circuit A also allow for the existence of different growth patterns observed in daughter cells of the various known mutants (Fig. 5). On the other hand, we did not try to explain the cause of the switch from one steady state to the other. This would require to take into account either external logical variables (Thellier et al., 2004) or to consider also the dynamical aspects of the circuit variables (Thomas and d'Ari, 1990).

Circuit A could have been designed in different ways. There are for instance four options for the linkage connections that correspond to the logical SUMs in Eqs. (7) and (8). In both equations, we had a choice between the linkage connections shown in Fig. 3. For Eq. (7), we chose the first option (Fig. 3A), because the alternative case would lead to many predicted “mutants” failing to grow in a monopolar way, which does not reflect the real situation. For Eq. (8), however, the alternative option was more attractive (Fig. 3B), because it increased the variety of possible relevant growth “mutants”. The advantage of circuit A can be further illustrated by comparison with another irreducible circuit F (Table 1; Fig. 4F). Circuit F has also three stable states and thus can exhibit similar growth phenotypes as circuit A, but its characteristics are not switch-like: the variables x and y cooperate for the induction of the bipolar growth mode in a negative way in circuit A, but in a positive way in circuit F. Circuit F cannot be considered a good approximation for fission yeast growth regulation, because the variety of its possible “mutants” is lower and less representative of biological reality. For the same reason, we did not consider circuits that involve only two elements.

Different classes of bipolar growth “mutants” predicted by circuit A can be distinguished with respect to their severity. The most severe is the mutant obtained by the knock-out of the system element represented by the variable x . Less severe are mutants that arise due to the loss of specific roles of this element. The “mutant” deficient in bipolar growth that arises due to the loss of operation \bar{x} in Eq. (8) (circuit B) corresponds to a defect in the inhibition of the monopolar growth mode that is normally mediated by activation of the molecule represented by x . The loss of operation x in Eq. (9) (circuit C) which is involved in the activation of growth is less severe than the loss of operation \bar{x} in Eq. (8): in the latter case, operation x becomes irrelevant, whereas the loss of operation x has no effect on operation \bar{x} . This is consistent with the observations: assuming that the *pom1* mutant corresponds to a deletion of variable x , the *tea1* mutant to circuit E, the *tea3* mutant to circuit B, and the *bud6* mutant to circuit C, it is possible to predict the phenotypic differences and similarities between these mutants as well as between different combinations of double mutants (Niccoli et al., 2003; Glynn et al., 2001).

The switch from no-growth to growth is promoted by recruiting the growth machinery to the cell ends. This process seems to be based on an interplay between molecules that are transported by microtubules or actin filaments to the growth sites and cell end markers accumulated at the growth sites (e.g. Marks and Hyams, 1985; Mata and Nurse, 1997; Behrens and Nurse, 2002; Chang and Verde, 2004). In terms of circuit A, z could represent the process of growth material delivery to cell ends, while x and y could represent the activity of cell end markers. The vector $(y, z) = (1, 1)$ thus defines the state of the system in which the delivery is active and the respective marker is located at the proper cell end, whereas the vector $(y, z) = (0, 0)$ defines the state of no delivery and delocalized cell end marker. This is consistent with a positive feedback mechanism between Mod5p and Tea1p (Snaith and Sawin, 2003); Tea1p, after localization to the cell ends, forms a complex involving Mod1p that plays a role in marking the ends for growth.

For the monopolar to bipolar transition, circuit A suggests the existence of two different cell end markers, one for monopolar growth and one for bipolar growth, with the activated marker inhibiting the other marker. An active cell end marker could guide the growth machinery to the site of polarized growth. Several phenomena can be explained by assuming that for normal growth behavior, the corresponding marker has to be both properly localized and properly activated. This helps to interpret the memory properties of the cell ends, which determine the site of growth initiation in daughter cells after division. In wild-type cells, growth begins at the ends that previously grew, which seem to 'remember' their growth history by retaining markers. Based on circuit A, it is also possible to discuss mutants, in which the new cell end is marked but growth is not induced. Some data suggest that the non-growing cell end is marked before growth begins, possibly by a different marker. The induction of bipolar growth could therefore be understood as a switch from the activated state of the monopolar end marker to the activated state of the bipolar end marker. It seems that the induction of bipolar growth requires a preparatory phase needed to make the new cell end competent for growth. Tea1p appears to play a role in this preparatory phase while Bud6p appears to function in the induction of growth. Accordingly, in the presence of Tea1p and absence of Bud6p (see circuit C) the new end is marked but is not growing (Glynn et al., 2001).

With this analysis of the regulation of fission yeast growth behavior, we tried to point out the utility of the logical circuit approach in studying principles of biological regulation. More specifically, we have shown that even a simple, switch-like and irreducible logical circuit can describe several features of fission yeast growth patterns, for both wild-type and mutant cells.

Some of these features are already well understood at the molecular level. The present analysis indicates that for molecular studies of biological regulatory processes, a parallel analysis of corresponding logical circuits may represent a useful complementary approach for the identification of key properties of the system and to reveal the relative importance of various cellular players.

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