

Brief Report

Cycling without the Cyclosome

Modeling a Yeast Strain Lacking the APC

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ABSTRACT

The construction of viable *Saccharomyces cerevisiae* strains that lack the anaphase promoting complex (APC) was recently reported. The normally lethal deletions of APC genes were suppressed by the double deletion of the *PDS1* and *CLB5* genes in conjunction with the insertion of multiple copies of the *SIC1* gene controlled by its endogenous promoter. It was proposed that cyclic expression and degradation of Sic1 results in oscillations of Clb/CDK activity necessary for the cell cycle. We have used an updated version of a mathematical model of the yeast cell cycle to model strains that lack the APC. With a few modifications, the model accurately simulates the viability of Apc⁻ strains, as well as the phenotypes of 27 other previously characterized strains. We discuss a few minor inconsistencies between the model and experiment, and how these may inform future revisions to the model.

INTRODUCTION

When Tim Hunt and colleagues first reported their observation of proteins that rapidly and abruptly disappeared in dividing sea urchin eggs,¹ they were describing the handiwork of a ubiquitin ligase now known as the anaphase promoting complex (APC).^{2,3} The cell cycle is driven by cyclin-dependent kinases (CDKs) that are largely regulated by controlling levels of the associated cyclin subunits. The rapid drop of B-type cyclin/CDK activity that is required in each and every cell cycle is normally accomplished by the action of the APC, which ubiquitinates B-type cyclins and targets them for destruction by the proteasome. The inhibition of cyclin/CDK activity by cyclin proteolysis has been thought to be crucial for generating oscillations in CDK activity.

Since the genetic and biochemical identification of the APC in 1995,⁴⁻⁶ its role in the cell cycle has been intensely studied. The discovery of securins (Pds1 in budding yeast) demonstrated that the APC had at least one other essential function.^{7,8} Soon after, the roster of APC substrates grew rapidly. In addition to the B-type cyclins (Clb1-6) and Pds1, the list in budding yeast alone is thought to include a replication protein (Dbf4),⁹ three spindle-associated proteins (Cin8, Kip1, and Ase1),¹⁰⁻¹² four protein kinases (Cdc5, Hsl1, Ipl1 and Swe1),¹³⁻¹⁵ a mitotic exit regulator (Spo12),¹⁶ and one of its own regulatory subunits (Cdc20).^{14,17} The significance of APC-mediated destruction of most of these proteins has yet to be established.

Recently it was shown that yeast did not require the APC if two classes of proteins were simultaneously deleted or inhibited: securin and B-type cyclin/CDK.¹³⁻¹⁵ The gene encoding securin is nonessential and can thus be deleted (*pds1Δ*). The requirement for B-type cyclin degradation is circumvented by deleting one of the cyclins (*clb5Δ*) and over-expressing a Clb/CDK inhibitor (*SIC1^{10X}*).^{13,18} To explain how the cell cycle might continue in the absence of the APC, a simple model was proposed in which the obligatory oscillations in B-type cyclin/CDK activity are no longer driven by the synthesis and proteolysis of the cyclin subunit, but instead by the rise and fall of a stoichiometric inhibitor, Sic1.¹³ Since Sic1 is itself targeted for proteolysis by Clb/CDK phosphorylation, this forms a bi-stable switch that is at the heart of the cell cycle engine. Converting a switch into an oscillator requires two additional features: a built-in delay (due to transcriptional and translational lag, for example) and one or more negative feedback loops. Two negative feedback loops were proposed: first, rising Clb/CDK activity leads to the activation of an opposing phosphatase, Cdc14, triggering its own downfall; and second, reduced levels of Clb/CDK activity allow expression of the G₁ cyclins, which in turn can phosphorylate and trigger the proteolysis of Sic1, thus allowing Clb/CDK activity to rise again. This model

for a cell cycle lacking the APC is nearly identical to a previously described “relaxation oscillator”¹⁹ regulating mitosis in budding yeast, with the sole exception being that Sic1 now bears the entire burden of Clb/CDK inhibition.

Hypotheses such as these are easy to propose, but difficult to rigorously test. The mechanisms governing cell division have been well characterized over the years, perhaps nowhere as completely as in *S. cerevisiae*. Enough regulatory components are now catalogued that simply drawing the wiring diagram requires feats of acrobatics, and intuitively grasping the relationships between far-flung components is increasingly difficult. One way of handling this information is to adopt a more quantitative approach in describing the system, relying on mathematical descriptions of the components and their relationships. This approach, dubbed mathematical modeling, is growing in sophistication and usefulness. In this article, we present our efforts at incorporating an APC-less cell cycle into a budding yeast mathematical model. For more complete descriptions of modeling of biochemical systems, we direct the reader to recent reviews on the subject.^{20,21}

WHAT IS MATHEMATICAL MODELING?

The basic principle of mathematical modeling is deceptively simple. It rests on the assumption that a process being studied can be broken down into a set of mathematical relationships. In the case of modeling the cell cycle, these relationships are ones that experimental biologists are quite comfortable with, such as the rates of synthesis and degradation of a protein, or an association/dissociation reaction between two proteins. An example (the association and dissociation of Clb/CDK and Sic1) is shown in Figure 1A. Once the relationships are defined conceptually, they are converted into a set of differential equations, such as those shown in Figure 1B. Next, one must assign values to the kinetic constants (“parameters”) that appear on the right hand sides of each differential equation (in this case, k_a = association rate constant [$\text{nmol}^{-1}\text{min}^{-1}$] and k_d = dissociation rate constant [min^{-1}]). Finally, the variables (which are concentrations of proteins) are given initial values and the set of differential equations are solved numerically. That is to say, the present values of variables and parameters are plugged into the equations to compute how each variable will change in the next short interval of time. Each variable is then updated, and the differential equations are solved for the next time interval. This process is repeated iteratively and the outcome is presented by graphing concentration versus time (Fig. 1C). This can be done with software such as the freely available WinPP (<http://www.math.pitt.edu/~bard/xpp/xpp.html>) or, for simple models, with a standard spreadsheet program.

A model of the yeast cell cycle proposed by Chen et al.²² contains dozens of variables and over 50 kinetic parameters characterizing their interactions. Taken as a whole, the “wiring diagram” used in this model is far more complex than the example in Figure 1, but is composed of precisely this kind of simple kinetic relationship. Not only does this model reflect our theoretical understanding of the yeast cell cycle, it also incorporates biochemical rate constants that, whenever possible, are reasonable estimates based on the experimental evidence available in the literature.

The true test of such a model is how well it reflects empirical data. By changing or eliminating components in the system, one can determine how well this model predicts the cell cycle repercussions of mutations. The published version of the model accurately predicts the behavior of over 50 known mutants, and a newer version now accounts for the phenotypes of over 120 mutants (Chen KC,

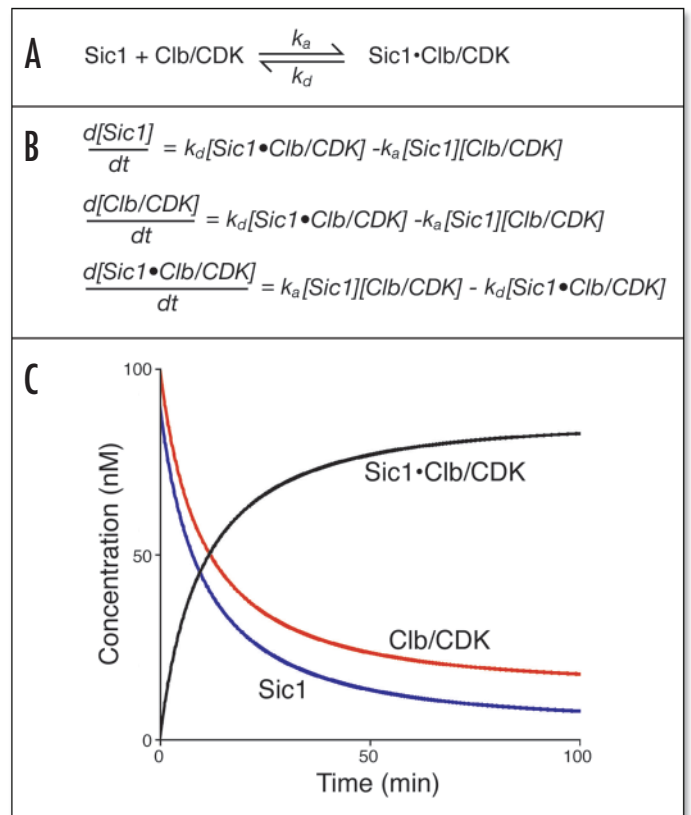


Figure 1. A simple example of mathematical modeling. (A) An association/dissociation reaction between Sic1, Clb/CDK, and Sic1•Clb/CDK. (B) differential equations describing the components in A. Since k_a is a second order rate constant, it will have the units ($\text{concentration}^{-1} \cdot \text{time}^{-1}$), while the first order constant k_d will have the unit (time^{-1}). (C) a graph of the results of a solution of the equations in B. For the purpose of this example, we have arbitrarily assigned $k_a = 0.001 \text{ nM}^{-1}\text{min}^{-1}$, $k_d = 0.001 \text{ min}^{-1}$, and initial values of $[\text{Sic1}] = 90 \text{ nM}$, $[\text{Clb/CDK}] = 100 \text{ nM}$, and $[\text{Sic1}\cdot\text{Clb/CDK}] = 0 \text{ nM}$. The equations were solved over 100 minutes with a step size of 0.1 minutes.

Calzone L, Csikasz-Nagy A, Cross FR, Novak B, Tyson JJ; unpublished data). While most mutants are accurately modeled, there are some mutants that do not behave in silico as they do in vivo. These failures are themselves informative, pointing us to portions of the wiring diagram that may need to be rewritten.

RESULTS AND DISCUSSION

We attempted to simulate Apc- cells (*apcΔ pds1Δ clb5Δ SIC1^{10X}*) using a current model of the yeast cell cycle (Chen KC, Calzone L, Csikasz-Nagy A, Cross FR, Novak B, Tyson JJ; unpublished data). The deletion of essential APC subunits was modeled by eliminating both Cdc20 and Cdh1 activity, which account for all APC activity. We modeled *clb5Δ* by reducing Clb5 expression 10-fold, to take into account the related but more weakly expressed Clb6 protein,²³ which is not included as a separate quantity in the model. Deletion of *PDS1* was modeled simply by reducing Pds1 expression to zero. Although 10 extra copies of *SIC1* were used to suppress APC mutants,¹³ Sic1 over-expression was modeled by increasing the expression only 6-fold. Our attempts at modeling 10-fold over-expression of Sic1 resulted in an effectively permanent block in G_1 . This inconsistency may suggest that in the model Sic1 synthesis rates are set too high or that the efficacy of Cln/CDK phosphorylation of Sic1 is set too low.

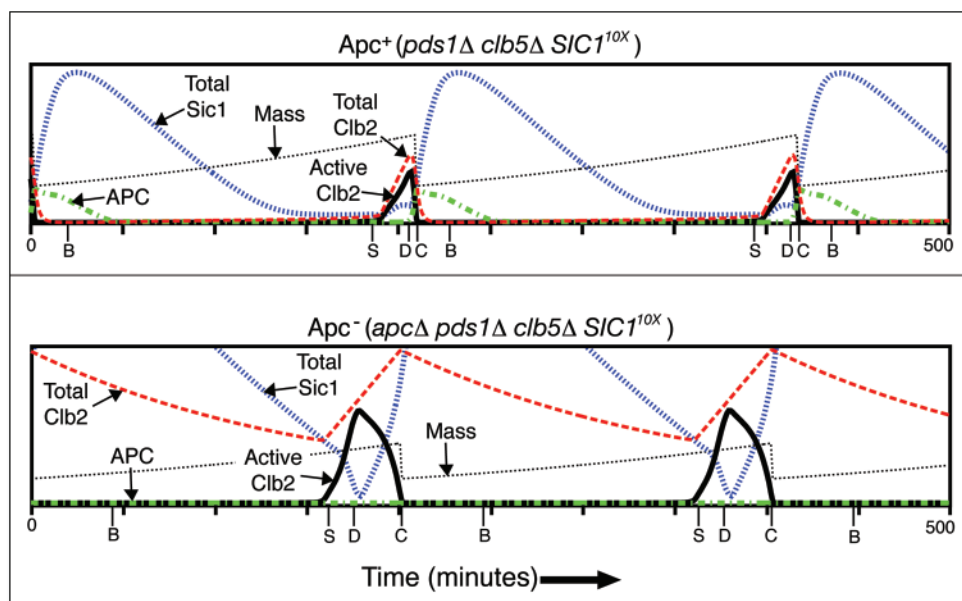


Figure 2. Mathematical modeling of the cell cycle in Apc^+ and Apc^- cells. In both cases, concentrations (in arbitrary units) of active Clb2/CDK ("Active Clb2"), total Clb2 protein ("Total Clb2"), total Sic1 protein ("Total Sic1"), and APC activity toward Clb2 ("APC") are graphed versus time (in minutes). Cell mass is also indicated ("Mass"). Landmark cell cycle events are indicated on the X-axis as follows: B = bud emergence, S = initiation of DNA replication, D = spindle alignment (and presumably chromosome segregation), and C = cell division. A detailed description of the wiring diagram used to generate this figure will be published elsewhere. Details about the changes made to model parameters for this article are available upon request.

On the other hand, there may not be an inconsistency, as it has not been verified that 10 extra copies of *SIC1* gene results in a 10-fold increase in *SIC1* expression in vivo. These mutations (*pds1Δ*, *clb5Δ* and *SIC1^{10X}*) were tested, alone and in combination, to see if their ability to rescue the *apcΔ* mutant in simulations was consistent with experimental results. As the model did not initially predict a viable cell cycle oscillator without the APC, model parameters were changed to see if some set of kinetic constants could bring all the results into agreement.

With a few modifications, the model accurately predicted the viability of Apc^- strains. The changes to the original parameters included a reduction in the mass doubling time (from 90 minutes to 150 minutes), reduced Clb2 stability (2-fold increase in APC-independent Clb2 degradation), a reduction in Clb2/CDK activity toward Sic1 (20% lower), and an increase in Cdc14 activity (several parameters were changed to affect Cdc14, none greater than 2-fold). These changes prevent Clb2 levels from climbing too rapidly for Sic1 to overcome them. We chose to reduce the mass doubling time because Apc^+ and Apc^- strains have been observed to have doubling times of 3.5 and 5 hours, respectively (Thornton B, Toczyski D; unpublished data). The fact that these strains are slow-growing is particularly interesting in light of observations that growth on poor carbon sources rescues mutations in certain genes controlling mitosis.^{19,24} We tested 31 other mutant combinations in this new model, and found that the phenotypes of some strains were no longer accurately simulated. For example, the reduction in Clb2 expression caused the model to predict that *SIC1^{10X}* cells would fail to inactivate Pds1 before exiting mitosis, resulting in mitotic catastrophe. We compensated for this by increasing the ability of APC^{Cdc20} to target Pds1 for destruction. In this manner we continued to adjust the parameters until the model was accurate for all 28 cases where the phenotypes are known (summarized in Table 1). We also used the model to simulate four untested mutant combinations, which generates the interesting and testable prediction that *cdh1Δ clb5Δ SIC1^{10X}* strains will be inviable due to mitotic catastrophe (Table 1).

Beyond simple viability, the model makes specific predictions about the levels of cell cycle components and the timing of cell cycle events (Fig. 2). Several points are worth describing from the simulation results:

1. The mechanisms regulating the oscillation of Clb/CDK activity are different in the Apc^+ (*pds1Δ clb5Δ SIC1^{10X}*) and Apc^- (*apcΔ pds1Δ clb5Δ SIC1^{10X}*) strains. In the former case, the bulk of CDK activity is regulated through Clb proteolysis, as the amount of active Clb/CDK and the total amount of Clb are nearly identical. In the latter case, however, Clb/CDK regulation is through Sic1 inhibition. Although the active Clb/CDK peak is similar to what is seen in the Apc^+ cells, the total amount of Clb2 is much higher, and its pattern is completely different from that of the active Clb/CDK. Total Clb2 and total Sic1 fluctuate with a phase shift relative to each other to give rise to a burst of active Clb/CDK that drives the cell into DNA synthesis, mitosis and eventually exit from mitosis.

For Apc^- cells, the model predicts that the total amount of Clb2 should be high and show a 2-fold fluctuation during the cycle. Because Clb2 synthesis is stimulated each cycle as MCM is activated, if Clb2 were completely stable it would accumulate to higher levels each cycle and eventually would cause mitotic arrest. We therefore assume that there is a slow, constant background degradation rate of Clb2 (with a half-life of 100 min). The amount of total Clb2 would therefore fluctuate in a cycle, high when its synthesis is activated by MCM, and low when synthesis is turned off. In the experiments with Apc^- strains, Clb2 levels appeared completely stable;¹³ however, a 2-fold change might have escaped notice.

2. The model predicts that Apc^- cells will bud later than their Apc^+ counterparts (Fig. 2), and will exit mitosis with a delay relative to spindle alignment (and presumably chromosome disjunction), as is observed experimentally. However, the model also predicts that budding and DNA synthesis are uncoupled in both the Apc^+ and Apc^- strains, due to *clb5Δ* and *SIC1^{10X}*. S-phase is predicted to initiate 2 to 2.5 hours after bud emergence (Fig. 2, compare "S" to "B"), unlike experimental observations that in both strains they occur nearly simultaneously.¹³ The experimental observation is the more surprising of the two, since Sic1 is thought to inhibit B-type cyclin/CDK activity (thus blocking replication origin firing) but not Cln/CDK activity (thus allowing budding).²⁵⁻²⁷ In synchrony experiments, both Apc^+ and Apc^- cells took an unexpectedly long time to bud.¹³ This suggests that by some not yet understood mechanism the suppressing mutations (*pds1Δ clb5Δ SIC1^{10X}*) cause a delay in bud

Table 1 **APC MUTANT PHENOTYPES, EXPERIMENTAL AND SIMULATED**

#	Mutant	Experiment	Simulation
1.	wild-type	viable	viable
2.	<i>cdc20Δ</i>	metaphase arrest ¹⁴	metaphase arrest
3.	<i>cdh1Δ</i>	viable ^{32,33}	viable
4.	<i>clb5Δ</i>	viable ³⁴	viable
5.	<i>pds1Δ</i>	viable ³⁵	viable
6.	<i>SIC1^{10X}</i>	viable ¹³	viable
7.	<i>cdc20Δ cdh1Δ</i>	metaphase arrest ⁵	metaphase arrest
8.	<i>cdc20Δ clb5Δ</i>	metaphase arrest ³⁶	metaphase arrest
9.	<i>cdc20Δ pds1Δ</i>	telophase arrest ³⁶	telophase arrest
10.	<i>cdc20Δ SIC1^{10X}</i>	inviable ¹³	metaphase arrest
11.	<i>cdh1Δ clb5Δ</i>	viable ¹³	viable
12.	<i>cdh1Δ pds1Δ</i>	viable ³⁷	viable
13.	<i>cdh1Δ SIC1^{10X}</i>	ND	viable
14.	<i>clb5Δ pds1Δ</i>	viable ¹³	viable
15.	<i>clb5Δ SIC1^{10X}</i>	viable ¹³	viable
16.	<i>pds1Δ SIC1^{10X}</i>	viable ¹³	viable
17.	<i>cdc20Δ cdh1Δ clb5Δ</i>	inviable ³⁶	metaphase arrest
18.	<i>cdc20Δ cdh1Δ pds1Δ</i>	inviable ³⁶	telophase arrest
19.	<i>cdc20Δ cdh1Δ SIC1^{10X}</i>	inviable ¹³	mit. catastrophe
20.	<i>cdc20Δ clb5Δ pds1Δ</i>	viable ³⁶	viable
21.	<i>cdc20Δ clb5Δ SIC1^{10X}</i>	inviable ¹³	mit. catastrophe
22.	<i>cdc20Δ pds1Δ SIC1^{10X}</i>	viable ¹³	viable
23.	<i>cdh1Δ clb5Δ pds1Δ</i>	ND	viable
24.	<i>cdh1Δ clb5Δ SIC1^{10X}</i>	ND	mit. catastrophe
25.	<i>cdh1Δ pds1Δ SIC1^{10X}</i>	ND	viable
26.	<i>clb5Δ pds1Δ SIC1^{10X} = "Apc⁺"</i>	viable ¹³	viable
27.	<i>cdc20Δ cdh1Δ clb5Δ pds1Δ</i>	inviable ³⁶	telophase arrest
28.	<i>cdc20Δ cdh1Δ clb5Δ SIC1^{10X}</i>	inviable ¹³	mit. catastrophe
29.	<i>cdc20Δ cdh1Δ pds1Δ SIC1^{10X}</i>	inviable ¹³	dies at 6 th cycle
30.	<i>cdc20Δ clb5Δ pds1Δ SIC1^{10X}</i>	viable ¹³	viable
31.	<i>cdh1Δ clb5Δ pds1Δ SIC1^{10X}</i>	viable ¹³	viable
32.	<i>cdc20Δ cdh1Δ clb5Δ pds1Δ SIC1^{10X} = "Apc⁻"</i>	viable ¹³	viable

The viability and inviability of 32 strains are listed. Where known, the published and simulated terminal phenotypes of inviable mutant strains are described. The phenotypes of *cdc20Δ cdh1Δ* double mutants are assumed to be equivalent to loss of function of an essential APC subunit (such as *apc2Δ* or *cdc23-1* at the nonpermissive temperature). ND = phenotype not determined. For simulations, a "viable" result was indicated when the following events occurred in order: origin relicensing ($[(CLB2) + (CLB5) < 0.2]$), origin activation ($[(ORI) \geq 1]$), spindle alignment ($[(SPN) \geq 1]$), activation of Esp1 ($[(ESP1) \geq 0.1]$), and finally cell division ($[(CLB2) < 0.3]$). In addition, Esp1 had to be inactive prior to spindle alignment ($[(ESP1) < 1]$), budding ($[(BUD) \geq 1]$) had to occur prior to cell division, and an upper limit was set on cell size ($[(MASS) < 10]$). For simulations containing *pds1Δ*, the requirement for proper Esp1 activation was ignored. The results "metaphase arrest" or "telophase arrest" were indicated by cessation of division coupled with high Clb2/CDK activity and either low or high Esp1 activity, respectively. A result of "mit. catastrophe" was indicated by cell division prior to activation of Esp1.

emergence. This result could be explained if Sic1 has some minor ability to inhibit Cln/CDK activity, minor enough that it only becomes apparent when Sic1 is grossly over-expressed. However, this seems a remote possibility given the apparent specificity of Sic1.²⁵

3. Another difference between the model and the experimental results is the behavior of Sic1. The model predicts that Sic1 levels should reach much higher levels (about 3-fold higher when integrated across a cell cycle) in *Apc⁻* cells, because of an increased window of active Clb2/CDK triggering increased Mcm1 activity, which in turn activates transcription of Sic1's transcription factor, Swi5. In experiments, Sic1 levels in elutriated *Apc⁺* and *Apc⁻* cells are nearly

identical (ref. 13 and Fig. 2B; Thornton B, Toczyski D; data not shown). Simulations also predict that as cells transit *G₁* Sic1 levels should decline gradually, with a half-life well over an hour. In experiments, Sic1 levels decline abruptly, coincident with bud emergence and DNA replication.¹³ It has been shown that Sic1 must be phosphorylated on multiple sites to trigger degradation, which is predicted to result in ultrasensitivity to Cln/CDK levels.^{28,29} Incorporating this detail into the model might bring it into closer agreement with experimental results.

One potentially powerful application of modeling is the ability to predict interesting phenotypes that might not have been intuitively expected. In the qualitative model described at the start of this paper, two negative feedback loops were predicted to be important for the cell cycle in *Apc⁻* cells: transcriptional regulation of the *G₁* cyclins by Clb/CDK, and Clb/CDK regulation of Cdc14 activation. Does the mathematical model make these same predictions? Simulations do in fact predict that *Apc⁻* strains will be sensitive to increased Cdc14 activity (modeled by eliminating *BUB2*, which normally helps keep Cdc14 sequestered until after chromosome segregation) but not to constitutive expression of the *G₁* cyclin Cln2. The mathematical model includes a third negative feedback loop: Clb/CDK stimulation of Swi5 expression (and therefore Sic1) via Mcm1. The model predicts that constitutive Swi5 expression should also be lethal in *Apc⁻* cells. Experimentally testable predictions such as these are one of the most useful outcomes of modeling a biological system.

Models of the type described here do not currently take stochastic effects into account; survival is an all-or-none affair and, with one exception, all cells are considered identical. The exception is

the case of mother versus daughter cells. Due to asymmetric division in *S. cerevisiae*, the bud will form a smaller cell (the daughter), whereas the original cell body (the mother) inherits slightly more of the cell mass and grows larger with each division until it dies.³⁰ The results displayed in Figure 2 follow the daughter cell from each division. *Apc⁻* mothers only complete two cycles before permanently arresting due to high levels of Clb2 (data not shown). Mother-specific lethality is an interesting prediction of the model, and could be tested by performing a pedigree analysis on *Apc⁻* cells. Elutriated *Apc⁻* cells die in their first cell cycle 3% of the time, and 20% fail to form microcolonies with greater than 32 cells (5 doublings).¹³ Rapid

senescence of mothers may be partly responsible for this phenotype.

We found that the simulation of *Apc⁻* cells requires a precise set of parameters to work properly (data not shown), indicating a lack of robustness. In this case, “robustness” refers to the ability of a system to weather changes to values of its parameters and still function.³¹ The wild-type model has been shown to be quite robust,¹⁹ capable of withstanding very large changes to most of the model parameters and still maintaining its basic behavior. Unlike the smooth curves of a computer simulation, a living organism is expected to encounter stochastic variations in its environment that might present a challenge to a nonrobust system. So, while this model cannot directly account for low plating efficiency of the sort we see in *Apc⁻* cells, the lack of robustness of the *Apc⁻* model might lead one to predict such a phenotype. It would be interesting to explore whether there are correlations between poor model robustness and poor plating efficiency amongst yeast mutants.

CONCLUSION

It is important to note that mathematical modeling is meant to be a valuable companion to experimentation, not a replacement. Mathematical modeling provides a fixed framework into which new experimental observations can be fitted, and in turn makes quantitative predictions that can be tested experimentally. As new results are obtained, the parameters of the model are tweaked until the model accommodates them. The ability to fit new results into an existing model argues for the validity of the wiring diagram upon which the model is built. When irreconcilable differences arise from incorporating new data, not only do they hint at how the model should be revised, but they also provide clues for future experiments. The fact that “fiddling” with model parameters is still required should not be taken as an indictment of the approach; instead, it should be recognized that modeling is valuable as a process, not just as an end result. It is through the back-and-forth between simulation and experiment that increasingly accurate models are generated, which, in turn, are better able to make interesting and testable predictions. As modeling becomes increasingly accessible to the nonspecialist, the intimate coupling of quantitative modeling and experimentation will become a more prevalent, and therefore all the more useful, tool.

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