SI Appendix

G.Charvin, F.R. Cross, E.D. Siggia

A PHENOMENOLOGICAL MODEL OF THE YEAST CELL CYCLE

Different types of models have been reported to describe the dynamics of the cell cycle. The first quantitative analysis of the cell cycle kinetics was done by Hartwell and Unger[1] in order to account for the average size and division timings of exponentially growing cells. Hartwell et al. assumptions were the following : 1) cell volume is increasing exponentially with time at a constant rate μ that is independent on the cell division cycle. 2) Division is morphologically asymmetrical, in such a way that daughter cells are born smaller than their mothers. Following a size control mechanism, the consequence of smaller daughter size is that the daughter division time T_D is larger that mothers' T_M. 3) Mother cell size is constant at each successive divisions, i.e. it doesn't increase with time. Consequently, according to the conservation of volume at division, one can write :

$$V_M + V_D = V_M e^{\mu T_M} \tag{1}$$

$$= V_D e^{\mu T_D} \tag{2}$$

where V_M and V_D are respectively the volume of mother and daughter cells at division. By combining equations (1) and (2), one can establish the following between growth rate and division times, as first obtained by Hartwell [1]:

$$e^{-\mu T_D} + e^{-\mu T_M} = 1 \tag{3}$$

An important consequence of it is that increasing the growth rate makes T_D and T_M converge towards the same value $T_\mu = log(2)/\mu$ (the mass-doubling time), i.e. the higher the growth rate the more symmetrical the division process is, an important phenomenon that was indeed observed experimentally[1].

In the 1970s, more elaborate models were not warranted by the data available. Since then, many of the genes involved in the control of the cell cycle were discovered. Chen et al. have integrated most of the molecular data available into a model which describes the time dependence of the genes that regulate the cell cycle[2]. This model uses effectively the same assumptions as Hartwell and Unger regarding the growth of individual cells, namely the biomass increases exponentially at a rate μ and the mother-daughter mass ratio is fixed at division, thus fixing

T_M and T_D according to Eq. 6. These assumptions contradict the experimental evidence that mother cells get bigger during successive divisions (see Fig. S3 *Upper Left*).

Recent methodological developments allowing the monitoring of single cell divisions have shown the possibility to precisely retrieve the timings associated to cell cycle events using specific fluorescent markers[3, 4]. Extending this methodology by tracking a large number number of cells in a custom microfluidic device has let us acquire large datasets of cell cycle events with less and less efforts. Based on this type of data, we propose to derive a simple yet qualitatively relevant description of the cell cycle whose agreement can directly be tested by comparing to single cell measurements. This model is a phenomenological description of the growth and division process of the yeast cycle, whose goal is to understand the dynamics of essential physiological phenomena (timings, sizes, size control) associated to the cell cycle. Furthermore, we show that such a description provides the necessary framework to understand the mechanism by which cells can lock on external periodic pulses of G_1 cyclins.

Principles and assumptions

The cell cycle oscillator (or clock) is described using one unique abstract variable ϕ, i.e the phase of the oscillator. ϕ takes values between 0 and 1, respectively meaning the beginning and the end of the cycle. The definition of ϕ implies no loss of generality if increases linearly with time (with a velocity ω). ϕ is reset to 0 when it reaches 1, which means that the cell divides. Cells are assumed to grow exponentially in volume with a rate μ. ω has no physical meaning at the molecular level, it is an abstract parameter -the speed of the cell cycle clock-, just like the growth rate μ is not simply connected to the molecular players involved in growth.

In order for the cells to achieve size homeostasis, the cell cycle clock is coupled to growth :

$$\frac{d\phi}{dt} = \omega \frac{V^m}{V^m + V_c^m} \tag{4}$$

$$\frac{dV}{dt} = \mu V \tag{5}$$

where m is a Hill coefficient that characterizes the strength of size control and V_c is the cell sizescale which sets the typical volume at which the cell cycle clock begins to tick. We define ϕ_1 as the phase at which the cell starts budding. For $\phi > \phi_1$, we assume that the newly created

mass goes to the daughter cell. Table I in this document summarizes the different parameters of the model, their meaning and numerical values (that are justified below).

Input parameters	Meaning	Remarks	Numerical value used
μ	Growth rate		Mass-doubling time $T_\mu \equiv log(2)/\mu = 84$min
ω	Cell cycle clock rate	when $V > V_c$, division time $T_M \equiv 1/\omega$	$1/\omega$= 71min
V_c	Cell size scale	Threshold volume involved in the size control mechanism	$V_c = 1000$ (arbitrary unit)
ϕ_1	Phase at budding		$\phi_1 = 0.25$ (determined from the fraction of the cycle time spent in G_1 in mother cells)
m	Strength of size control	m $= \infty$ corresponds to an ideal size control	$m = \infty$

TABLE I: Model parameters

case $m = \infty$

We can get a simple understanding of the evolution of cells in the limit of $m \to \infty$, since an analytical solution of the model can then be derived. This case corresponds to an ideal size control : the cell cycle phase of a small daughter cell (see blue lines on Fig. S4 *Lower* panel) is halted until the cell reaches a critical size V_c, at which it resumes and increases linearly. When $\phi = \phi_1$, the cell buds, then all the newly created mass goes into the daughter, so that the mother cell size stays constant from that time on (the total cell size, i.e mother + bud, keeps increasing exponentially during this time). At division, the cell has generated a daughter cell which experiences the same size-control delay, see red line on Fig. S4. On the other hand, the mother cell enters its new cycle without delay (green curve on Fig. S4) since its size is now larger than V_c.

We can calculate the various indicators, such as G_1 duration, S/G_2/M durations, division time and size at division and budding for both mothers and daughters that follow this set of rules. The cells behave differently depending on the initial volume V_n of a cell at division at the nth

cell cycle. Two cases are detailed below :

Case $V_n > V_c$

This is typically the case of an old mother cells (green curve on Fig. S4). Because $V_n > V_c$, the cells bypasses the size control mechanism. Since its size increases only during the unbudded period (of duration ϕ_1/ω), its size at the end of nth cycle V_{n+1} (division) is given by :

$$V_{n+1} = V_n e^{\mu\phi_1/\omega} > V_n$$

which means that the mother cell increases in size at each cell cycle (see blue and red circles on Fig. S5).

The division time of these cells is a constant and given by : $T_M = 1/\omega$. According to experiments (see Table I in this document for numeric value), ω is chosen such that T_M is smaller than the doubling time $T_\mu \equiv log(2)/\mu$. The G_1 and S/G_2/M durations (resp. τ_{G_1} and $\tau_{S/G_2/M}$) of these mothers are simply set by :

$$\tau_{G_1} = \phi_1/\omega$$

and :

$$\tau_{S/G_2/M} = (1 - \phi_1)/\omega$$

On the other hand, the volume of the newborn daughter at birth V_{n+1}^D is given by the conservation of volumes at division :

$$V_n e^{\mu\phi_1/\omega} + V_{n+1}^D = V_n e^{\mu/\omega}$$

so that :

$$V_{n+1}^D = rV_n$$

where r is :

$$r \equiv V_{n+1}^D/V_n = e^{\mu/\omega} - e^{\mu\phi_1/\omega} = 2^{T_M/T_\mu} - 2^{\phi_1 T_M/T_\mu}$$

Since $T_M < T_\mu$, we have : $r < 1$ for any 0¡ϕ_1¡1. In others words, the volume of the daughter cell at birth is smaller than its mother's at the beginning of the cycle. Therefore, successive daughters from big mother cells have decreasing sizes. Eventually, they must therefore reach a volume smaller than V_c at birth (and ultimately fall into the case described below).

Case $V_n < V_c$

In that case, the cell cycle clock is halted until the cell reaches a critical size V_c, at which the clock resumes. Depending on the initial cell size at division V_n of a cell, the duration of the G_1 period τ_{G_1} is given by :

$$V_{budding} = V_n e^{\mu \tau_{G_1}}$$

According to the rules defined above, $V_{budding} = V_c e^{\mu \phi_1 / \omega}$. Consequently, the duration of G_1 phase for these cells is given by :

$$\mu \tau_{G_1} = -log(V_n) + log(V_c) + \frac{\mu \phi_1}{\omega} \tag{6}$$

This equation shows that a cell that is smaller than V_c at division experiences a G_1 delay that depends on its size at division V_n. This is the essence of the size-control mechanism, which forces the cell to bud at a defined volume. Once budded ($\phi > \phi_{\ 1}$), the phase of this cell increases linearly. The duration of the S/G_2/M phase $\tau_{S/G_2/M} = (1 - \phi_1)/\omega$ is therefore identical to the case $V_n > V_c$ described above. Consequently, its division time T_n is :

$$T_n = \tau_{G_1} + \tau_{S/G_2/M} = \frac{1}{\omega} + \frac{1}{\mu} log(V_c/V_n) \tag{7}$$

T_n still depends on the initial volume V_n of this cell. However, we can show that the division time T^D_{n+1} and size at birth V^D_{n+1} of the daughter of this cell depend only upon input parameters. Indeed, the conservation of volumes at division of cell n implies that :

$$V_c e^{\mu \phi_1 / \omega} + V^D_{n+1} = V_n e^{\mu T_n}$$

Using equation (7), we deduce :

$$V^D_{n+1} = r V_c$$

and therefore :

$$T_{n+1}^D = T_D \equiv \frac{1}{\omega} + \frac{1}{\mu} log(\frac{1}{r}) \tag{8}$$

Thus, no matter what the size $V_n < V_c$ of a cell is, the size of its daughter V_{n+1}^D at birth and its subsequent division time is constant (see blue and red empty squares on Fig. S5). Since the mother division time is $T_M = 1/\omega$, equation (8) can rewrite :

$$e^{-\mu T_D} + e^{-\mu T_M (1-\phi_1)} = 1 \tag{9}$$

which is similar, although not identical, to Hartwell's relation described above.

Model implications, agreement with experimental data and limits of validity

Growth timings

This model uses two variables to describe the evolution of the growth and division processes. The volume V of the cell follows simple exponential growth. The clock ϕ of the cycle is strongly coupled to size, as small cells literally undergo a clock arrest. From this set of rules emerge two limit trajectories : big cells (typically mother cells) have a constant division time $T_M = 1/\omega$. Since the mean mother division time is 71 minutes, then $\omega \approx 1/71 min^{-1}$. The G_1 duration of these cells is about 18 minutes (see Fig. S2b), from which we can deduce $\phi_1 \approx 0.25$. Interestingly, according to the model, these mother cells keep increasing their size over successive generations (see Fig. S5). This is indeed observed experimentally (see Fig. S3 *Upper Left*) and is in striking contrast with preceding models of the cell cycle[1, 2].

Cells that are born small ($V < V_c$) due to asymmetric volume partitioning, undergo a delay in G_1, that makes them bud at a constant size $V_c e^{\phi_1 \mu/\omega}$. Consequently, their division time $T_D = T_M + \frac{1}{\mu} log(\frac{1}{r})$ is longer than T_M. Using $log(2)/\mu = 84$min (as determined by fitting the colony growth curve), the model predicts $T_D = 125$ min, whereas experimental data yield $T_D = 94$ min. This deviation originates from the simplicity of the model which assumes an ideal size control and a simplistic volume partitioning rule, see below.

Volume partitioning

According to the model, all the mass produced by the cell after budding is transferred to its bud (future daughter). The consequence of this assumption is that the ratio R of the size of a

daughter cell at birth to that of its mother at division is (it does not depend on cell size but upon model parameters only) :

$$R = 2^{\frac{T_M}{T_\mu}} - 1 = 0.55$$

Experimentally, we indeed observe a very good correlation of size between a mother and its daughter at birth, especially when its area is smaller than ≈ 1000 pixels (see Fig. S6). However, the ratio R retrieved from experimental data points yields 0.63 for experienced mothers and 0.69 for first time mothers. In other words, newborn cells receive more mass than the model assumes. The reason may be that not only the newly created mass is transfered to the bud but a given fraction of original mother mass is also transfered to the daughter ("mother feeding" effect, data not shown). A a consequence of it, daughter cells must have a shorter division time than expected (see above). This phenomenon of mother feeding was not included in the model for simplicity.

Cell to cell variability

Since the mother/daughter size ratio at division is fixed by model parameters, daughters of big old mothers inherit an area at birth that is larger than the one of a young mother. Therefore, interestingly a population of cells generated according to this model has an intrinsic variability in timings and sizes, independently of any source of noise[3]. It is beyond the scope of this study to investigate to what extent the observed variability stems from the growth and division process as in our model or represents true molecular variability or experimental error.

Size control

According to our model, successive daughters are born with a fixed size rV_c. However, daughters arising from aged mothers are born with variable sizes. These daughters should then experience variable G_1 delays, depending on their size at division. This manifestation of size control in G_1 has been previously observed experimentally in a WT background[1, 3] (see corresponding results in a *cln3* background in the Fig. S2c *Upper Left*), and is qualitatively well captured by the model. There is a negative correlation between the duration of G_1 and the size of a small cell at division, whereas big cell ($V > V_c$) don't display such a correlation (see Fig. S7) : plotting $\mu\tau_{G_1}$ as a function of the $log(V)$ yields a slope of -1 when $V < V_c$, as expected from an ideal size control[3]. Experimentally, this slope has been shown to be closer to -0.7 in wild-type cells. Here we find that $cln3$ cells have a slope of -0.3. However, we have

measured μ from tracking the volume of a single cell through time, instead of a more reliable fluorescent marker for size[3]. This procedure may introduce artifacts. In any case, it is likely that the size control mechanism operating in real cells does not match the case of an ideal size control. Using $m \to \infty$ therefore limits the quantitative agreement of our model to the data. A finite value for m would certainly be more appropriate, by loosening the coupling between growth and division.

Table II in this document provides a summary of the cell cycle indicators associated to the model described above.

Indicator	**Unforced cell cycle**	**Forced cell cycle**
Daughter ($V < V_c$)		
Division time	$T_D = 1/\omega + \frac{1}{\mu} log(\frac{1}{r})$	τ
G_1 duration	$\tau_{G_1} = \frac{\mu\phi_1}{\omega} + \frac{1}{\mu} log(\frac{1}{r})$	$\tau_{G_1} = \tau + \frac{1}{\mu} log(1 - e^{-\mu\tau})$
S/G_2/M duration	$\tau_{S/G_2/M} = (1 - \phi_1)/\omega$	$\tau_{S/G_2/M} = -\frac{1}{\mu} log(1 - e^{-\mu\tau})$
Size at division	rV_c	$V_c e^{\mu(1-\phi_1)/\omega} e^{-\mu\tau}$
Size at budding	$V_c e^{\mu\phi_1/\omega}$	$V_{eq} = V_c e^{\mu(1-\phi_1)/\omega} (1 - e^{-\mu\tau})$
Size control in G_1	$\mu\tau_{G_1} = -log(V_{div}) + log(V_c) + \frac{\mu\phi_1}{\omega}$	none
Size control in S/G_2/M	none	$\mu\tau_{S/G_2/M} = -log(V_{bud}) + log(V_c) + \frac{\mu(1-\phi_1)}{\omega}$
Mother ($V > V_c$)		
Division time	$T_M = 1/\omega$	$T_M = 1/\omega$
G_1 duration	$\tau_{G_1} = \phi_1/\omega$	$\tau_{G_1} = \phi_1/\omega$
S/G_2/M duration	$\tau_{S/G_2/M} = (1 - \phi_1)/\omega$	$\tau_{S/G_2/M} = (1 - \phi_1)/\omega$
Size at division	variable	variable
Size at budding	variable	variable
Size control in G_1	none	none
Size control in S/G_2/M	none	none

TABLE II: Summary of cell cycle indicators as described by the model. We distinguish the two cases with or without cell cycle forcing

RESPONSE OF THE CELL CYCLE TO PERIODIC FORCING

Principles

The framework introduced in the previous section can be used to describe the response of the cells to periodic forcing. The forcing can be simply modeled by an extra term to the set of equations (4) and (5) :

$$\frac{d\phi}{dt} = \omega \frac{V^m}{V^m + V_c^m} + g(\phi)\Delta(t) \tag{10}$$

$$\frac{dV}{dt} = \mu V \tag{11}$$

where $g(\phi) = \phi_1 - \phi$ if $0 < \phi < \phi_1$ and 0 otherwise. Δ is a periodic spike train (each spike with time integral one) of period τ. Therefore, cells experiencing a G_1 cyclin pulse while they are in G_1 instantly reset their phase to $\phi = \phi_1$. This assumption simplifies the ≈ 30 min time from pulse initiation to budding observed in the experimental data, and amounts to the idea that the 'instantaneous' pulse actually 'occurs' 30 min after pulse initiation. This change simplifies the modeling but has no effect on the interpretation; in any case, it is clear that production of a sufficient level of Cln2 after induction will require more than zero time.

case $m = \infty$

In this limit case, we can derive an analytical solution of the problem, showing that successive daughters should lock to the external pulse. We can calculate how the locking affects the various aspects of cell physiology and what is the range of locking.

In the previous section (unforced cell cycle), the n index was used to describe successive cell cycles. In the following, n refers to the sequence of pulses. Let V_n (resp. T_n) be the volume (resp. the division time) of a daughter cell when it is hit by the n-th pulse . To test if cells lock on the external pulse, we calculate the evolution of V_n and T_n as a function of n. We must distinguish two cases : if the pulse arises when $\phi > \phi_1$, then it has no effect on the dynamics of the cycle. In that case, if τ is strictly smaller than the division time of daughter cells T_D, then eventually one of the following pulses will arise during the G_1 phase, which constitute the

second case : $0 < \phi < \phi_1$. This latter case can be subdivided in two further cases : $V_n > V_c$ and $V_n < V_c$.

$\underline{V_n > V_c}$

If $V_n > V_c$ and the pulse arises before $\phi = \phi_1$, the conservation of volume between successive cycles gives the relation between V_{n+1} and V_n :

$$V_{n+1} = V_n e^{\mu\tau}(1 - e^{-(1-\phi_1)\mu/\omega}) \tag{12}$$

For $\tau < T_D$, we get : $V_{n+1} < V_n$, so that the volume decreases and ultimately the cells belong to the case described in the next paragraph.

$\underline{V_n < V_c}$

In this case, again the conservation of volume at division between successive cycles gives the relation between V_{n+1} and V_n :

$$V_{n+1} = V_n e^{\mu\tau}(1 - e^{-(1-\phi_1)\mu/\omega}\frac{V_n}{V_c}) \tag{13}$$

This equation indicates that V_n converges to an equilibrium value V_{eq} (see Fig. 4) given by :

$$V_{eq} = V_c \frac{1 - e^{-\mu\tau}}{e^{-\mu(1-\phi_1)/\omega}}; \tag{14}$$

The cell division time T_n also converge to τ :

$$T_n = \tau + \frac{1}{\mu} log(\frac{V_n}{V_{n+1}}) \tag{15}$$

This implies that successive daughters lock to the external trigger signal of period τ.

Physiological properties of locked cells - Agreement with experimental data

Size control

If the volume of the cell at division is small enough that it is still smaller than V_c when the cell is hit by the pulse, cell budding is triggered by the external cyclin pulse. In this case, there is no longer any size control of the duration of G_1, and it simply depends on μ and τ only (no matter what cell volume at division is) :

$$\tau_{G_1} = \tau + \frac{1}{\mu} log(1 - e^{-\mu\tau})$$

This disruption of G_1 size control is indeed observed experimentally (compare *Upper Left* and *Upper right* panels on Fig. S2c).

However, this doesn't mean that any size control has been abolished. Indeed, the model predicts that the duration of S/G_2/M of triggered cells is now controlled by its size at budding V_{bud} :

$$\mu\tau_{S/G_2/M} = -log(V_{bud}) + log(V_c) + \frac{\mu(1-\phi_1)}{\omega} \tag{16}$$

In other words, a cell that is submitted to forcing (but which hasn't necessarily reached the fixed point V_{eq}) should adjust its S/G_2/M duration according to its size at budding . Qualitatively, such behavior is manifest in the experiments when comparing the locked case to the unlocked case, where G_2 is almost independent on cell size at budding (compare *Lower* panels in Fig. S2c). Yet, the observed S/G_2/M size control in locked cells is far from being ideal.

Therefore, this shows that cell locking using G_1 cyclin forcing displaces the apparent size control effect from G_1 to S/G_2/M. It also suggests that a common mechanism, that we model as the rate of phase increase with volume, that is most apparent during G_1 in WT cells, may be latent during the entire cell cycle and become visible in G_2 when it is bypassed in G_1. Also, it is interesting to notice that, if the coupling to growth were restricted to the G_1 phase in equation (10), then no fixed point would appear in equation (13) and the cell would not lock at all. Indeed, in this case, one can show that equation (12) would then describe the evolution of size at trigger of successive daughters (in the case $V_n < V_c$), which would become smaller and smaller. Therefore, in the frame of this model, an ubiquitous size control mechanism is required to lock the cells.

Range of locking

The range of locking is defined as follows: τ must be smaller than the daughter natural limit period : $\tau_{max} = T_D$. Otherwise cells tend go faster than the external pulse. On the other hand, the minimal locking period τ_{min} is obtained when the G_1 period is reduced to zero, which yields (see Table II in this document): $\tau_{min} = T_\mu$.

The locking period thus can't be smaller than the mass doubling time. The case $\tau = T_\mu$ corresponds to the ideal case where mother and daughter cells divide symmetrically and have

the same size, since mother cells do not increase their size (mother cells have zero G_1 in that case).

Experimentally, we indeed see that no locking is observed when $\tau = 99\text{min} > T_D = 94\text{min}$. On the other hand, the fraction of locked cells is still pretty high at $\tau = 78$ min, which is slightly smaller than the mass doubling time $T_\mu = 84$ min. However, in this case, looking at trajectories of chains of daughters reveals that cells eventually skip a pulse every once in a while ("intermittent-locking", see main text). Overall, the agreement between the range observed experimentally and what is predicted by the model is therefore satisfying.

Cell size

According to the model, cell size of successive daughters at trigger V_{eq} depends on the forcing period, see equation (14). Using parameters values of Table I in this document, the model predicts a 20% variation of size over the whole range of forcing period, in a good agreement with experimental measurement, see Fig. 3e in main text.

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[4] Bean J, Siggia E, Cross F (2006) Coherence and timing of cell cycle start examined at single-cell resolution. *Mol Cell*, 21(1):3–14.

STRAIN LIST

Strains (all W303)	Genotype	Origin
GC84-35B	MAT a cln3::LEU2 CDC10-YFP::LEU2 WHI5-GFP::KanMx TRP1::MET3-CLN2	this study
GC99-14D	MAT a cln3::LEU2 CDC10-YFP::LEU2 ADE2	this study
GC12-12C	MAT α CDC10-YFP::LEU2 WHI5-GFP::KanMx TRP1::MET3-CLN2 ADE2	this study

TABLE III: Strain table