Report

Current Biology

Single-Cell Analysis of Growth in Budding Yeast and Bacteria Reveals a Common Size Regulation Strategy

Highlights

- Cell-cycle progression was measured for thousands of diploid *S. cerevisae* cells
- Daughter cell correlations of key cell-cycle variables are the same as in *E. coli*
- The incremental model—a constant volume added each cell cycle—explains the data
- The yeast data indicate that the volume is added between two budding events

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In Brief

Soifer et al. study growth of the budding yeast *S. cerevisiae* at the single-cell level and show that a model in which daughter cells add a constant volume between two budding events quantitatively explains all correlations and distributions of cell-cycle-related variables. Data on *E. coli* show remarkably similar correlations.

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Single-Cell Analysis of Growth in Budding Yeast and Bacteria Reveals a Common Size Regulation Strategy

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SUMMARY

To maintain a constant cell size, dividing cells have to coordinate cell-cycle events with cell growth. This coordination has long been supposed to rely on the existence of size thresholds determining cell-cycle progression [1]. In budding yeast, size is controlled at the G1/S transition [2]. In agreement with this hypothesis, the size at birth influences the time spent in G1: smaller cells have a longer G1 period [3]. Nevertheless, even though cells born smaller have a longer G1, the compensation is imperfect and they still bud at smaller cell sizes. In bacteria, several recent studies have shown that the incremental model of size control, in which size is controlled by addition of a constant volume (in contrast to a size threshold), is able to quantitatively explain the experimental data on four different bacterial species [4–7]. Here, we report on experimental results for the budding yeast *Saccharomyces cerevisiae*, finding, surprisingly, that cell size control in this organism is very well described by the incremental model, suggesting a common strategy for cell size control with bacteria. Additionally, we argue that for *S. cerevisiae* the "volume increment" is not added from birth to division, but rather between two budding events.

RESULTS

Correlations between Cell-Cycle Variables Support the Incremental Model

Cells of all kingdoms of life have to coordinate cell-cycle events and cell growth [1]. Here, we study cell size control in the budding yeast *Saccharomyces cerevisiae*, focusing on the diploid form prevalent in nature, and show that it bears much similarity with size control in *Escherichia coli*. To that end, we devised an imaging system that allows following the unperturbed cell-cycle dynamics of thousands of yeast cells, identifying sizes and various events of cell cycle with high temporal resolution (Figures S1A and S1B). Experiments were performed with cells growing in five different culture media. We focus on daughter cells (the first cell cycle of each cell after budding off mother cell; see Figure 1A), which are known to have stronger size control than mother cells [2]. Sizes at birth and division of daughter cells were strongly correlated in all conditions (Figure 1C). Upon averaging the data for all cells with a given size at birth (suppressing the effects of biological stochasticity), we found for all five growth conditions a linear correlation with a slope very close to 1 (slopes within 10% of 1 in all five growth conditions; see the Figure 1C legend), i.e.,

$$v_d = v_b + \Delta, \qquad \text{(Equation 1)}$$

where v_b is the cell size at birth and v_d is the cell size at division. See further details of the data analysis procedure in the Supplemental Experimental Procedures and Figures S1C–S1E. The slope of 1 under very different growth conditions is evidence to the robustness of our results and is supportive of the incremental model, recently shown to be the size control mechanism for four different species of bacteria [4–7]—but has not previously been shown to be applicable to budding yeast. In this model, a constant volume is effectively added from birth to division, as described by Equation 1. To further test the incremental model, we considered the *time* between cell birth and division, t_d. In the Supplemental Information, we show that growth at the single-cell level is exponential (Figure S2A; see also [10–15]). Therefore, the time needed in order to add a constant Δ between birth and division is given by

$$t_d = \frac{1}{\lambda}\log(1 + \Delta / v_b), \qquad \text{(Equation 2)}$$

where λ is the growth rate. This implies a specific correlation between interdivision time and size at birth, where all parameters can be independently determined: the growth rate in each medium is experimentally extracted independently, and the constant Δ is extracted from the size-size correlations of Equation 1 and Figure 1. The excellent agreement of this prediction with the data is shown in Figure 2, with *no fitting parameters*.

The Incremental Model Is Implemented at the G1/S Transition

It is appealing to interpret Equations 1 and 2 as indicating that the cell measures and controls the volume added between birth and

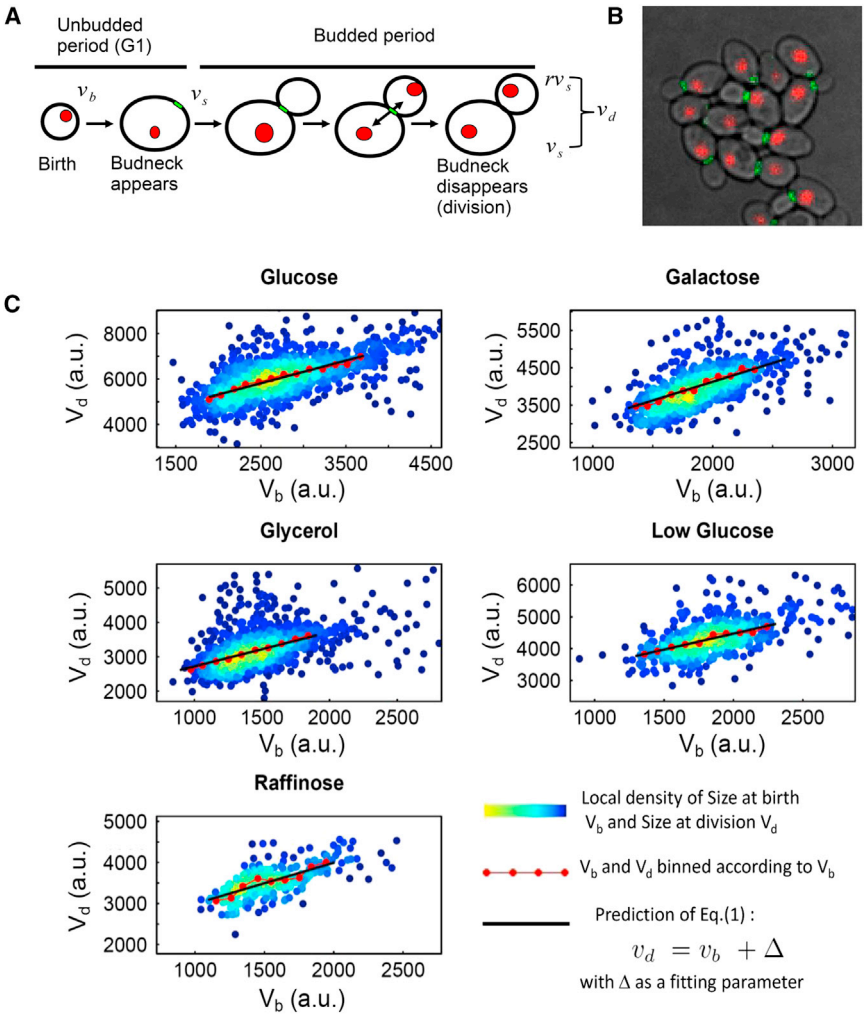

Figure 1. Correlations within the Budding Yeast Cell Cycle Support the Incremental Model

(A) Illustration of the budding yeast cell cycle. Fluorescent labeling of bud neck ring and the nucleus by the fusion proteins Cdc10-GFP [8] and Acs2-mCherry [9], respectively, enabled precise definition of cell birth, cell division, and the initiation of budding (see the Supplemental Experimental Procedures, section 1).The diagram shows the Start transition from G1 to the budded phase and defines the notation used in the text for the different cell-cycle variables.

(B) Growing microcolonies of *S. cerevisiae*. A typical image from our experiments on budding yeast: overlay of a bright-field picture and green and red fluorescent images. The strain used carries a fluorescent marker of the bud neck ring (green; indicates cell division) and a marker of the nucleus (red; assists the identification of cells in the image processing algorithm).

(C) Positive correlations between size at birth and division in *S. cerevisiae* daughter cells. Size at birth and division of daughter cells, grown in five different conditions. The color of the dots (blue to yellow) represents the local density. Red dots indicate data binned according to the size at birth. The black lines show the predictions of Equation 1—for all growth conditions, the slope of the plotted line is 1, and the offset Δ is taken as a fitting parameter (different for each growth condition, since the average cell size depends on the growth medium). A linear regression analysis on the raw data for the five different growth media yields a slope of 0.91, 0.99, 0.95, 1.01, and 1.1 for glucose, galactose, glycerol, low glucose, and raffinose, respectively, showing excellent agreement with prediction A.

See also Figure S1.

division. However, our data make this model unlikely. As explained in section 2 of the Supplemental Experimental Procedures and Figure S2B, our data show that the duration of the budded phase is uncorrelated with the cell size at birth. If the cue for division is the accumulation of a constant volume from birth, it is not clear how the cell could initiate budding such that division would occur a constant time later: this would be akin to measure a constant *negative* time from division. Moreover, previous research indicates that size control in budding yeast occurs at the G1/S transition (roughly speaking, the onset of budding) [2], rather than at division. We found that a solution to this seeming paradox can be achieved if we avoid the interpretation of Figure 1 as control of the volume accumulated between birth and division, and instead consider a model in which the control is over *budding*. Our model makes the following assumptions:

(1) Division occurs a constant delay after Start. This assumption is in agreement with the lack of correlations between the budded phase duration and size that we observed in our data (Figure S3).

(2) During the budded phase of yeast, almost all of the cell growth occurs in the bud—which describes the experimental observations well (the ratio of the mean cell size at budding to mean parent cell size at division is between 1.017–1.05 in our experiments).

(3) A constant volume increment is added between two budding events. In the Supplemental Experimental Procedures (sections 3 and 5), we show that if budding is triggered by accumulation of sufficient copies of an initiator protein, produced in proportion to volume growth and partitioned between the two cell bodies in relative proportion to their volume, then Equations 1 and 2 follow, as well as predictions C and D discussed below. However, we found that the model is also mathematically equivalent to an inhibitor model (Supplemental Experimental Procedures, section 4), in which budding occurs when the level of an inhibitor falls below a critical level: if a constant number of inhibitor molecules are produced in G2 and are partitioned between bud and parent cell in relative proportion to their volume, all correlations are identical to those of the initiator model (i.e., both models lead to a constant volume increment between two budding events). Interestingly, this model is similar to the model proposed in [16], where the dilution of the protein Whi5 as the cell grows is suggested to be responsible for size control. This

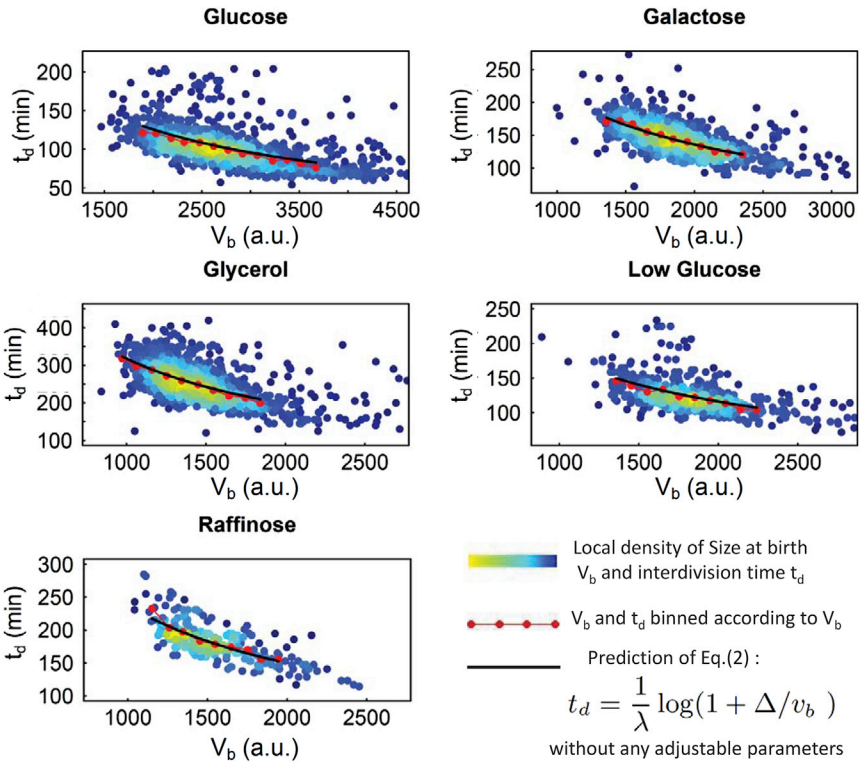

Figure 2. Negative Correlations between Size at Birth and Interdivision Time in *S. cerevisiae* Daughter Cells

Size at birth and interdivision time of daughter cells, grown in five different growth conditions. The color of the dots (blue to yellow) represents the local density. Red dots indicate data binned according to the size at birth. The black lines show the theoretical prediction B (Equation 2), without any adjustable parameters. See also Figure S2.

appears to be a plausible molecular mechanism to implement the incremental model in budding yeast.

In section 5 of the Supplemental Experimental Procedures, we show that assumptions 1–3 (in either interpretation, of an initiator/inhibitor model) lead to the following predictions:

(A) Adding a constant volume between two Start events leads, non-trivially, to Equation 1: hence, plotting of size at division versus size at birth is expected to produce a linear relationship with a slope of 1.

(B) Equation 1 implies a negative correlation between interdivision time and size at birth, specifically, Equation 2.

(C) The assumption of a budded phase of constant duration implies a division asymmetry (bud:parent cell ratio) independent of size at birth; thus, there would be no correlations when plotting the asymmetry against the size at birth.

(D) The model predicts that the volume increment during budding is *positively* correlated with the size at birth, with a slope of $r/1+r$, while that during G_1 is *negatively* correlated with it with a slope of $-(r/1+r)$, where r is the bud:parent cell volume ratio at cell division. These two contributions cancel during a full cell cycle, leading to a constant volume added between two budding events, as shown in Figure 3.

There is an important difference between the nature of predictions A and B as opposed to C and D: while the former cannot distinguish between adding the volume from birth to division versus adding it between two budding events, predictions C and D are inconsistent with the addition of volume between birth and division, and as such they are a useful way to distinguish between these two cases. It should also be emphasized that these predictions are different from those associated with a critical size model: for example, in that case, there would be no correlation between cell size at birth and division. Our results suggest that control acts at the G1/S transition, in agreement with other studies [2, 15, 17, 18], yet the particular mechanism that we suggest is different than the current paradigm.

Figures 1 and 2 show the excellent agreement of predictions A and B with our data. The agreement of prediction C with the data is shown in the Supplemental Information, where we found the division asymmetry r to be uncorrelated with the cell size at birth (Figure S3). The comparison of prediction D with our data is shown in Figure 3, showing the *lack* of correlations between volume added between two consequent budding events and size, and in Figure S3, showing the positive and negative correlations between volume increment and size when considering the G1 and the budded phase, respectively. The agreement of the experimental results with the model was good in four different culture media. It was poorer when cells grow on raffinose as a carbon source, for reasons that we do not understand.

The Incremental Model in Bacteria

In order to emphasize the striking similarity of the size control strategy in yeasts and bacteria, we performed the same analysis on data previously collected with *E. coli* by Stewart et al. [19] and Wang et al. [10] (details regarding the datasets and the analysis can be found in the Supplemental Experimental Procedures, section 7). Figure 4A shows that the same correlations as we showed for budding yeast in Figures 1 and 2 (corresponding to predictions A and B) also describes the data in *E. coli*. Section 3 of the Supplemental Experimental Procedures discusses a potential molecular mechanism which appears to be relevant for bacteria, where an initiator protein is accumulated between two DNA replication initiation events—akin to the budding-to-budding volume accumulation in budding yeast. In addition to reproducing the experimentally observed correlations, this model explains the known exponential dependence of size on growth rate, as shown in [4], and, importantly, regulates the number of multiple replication forks in addition to controlling size [20].

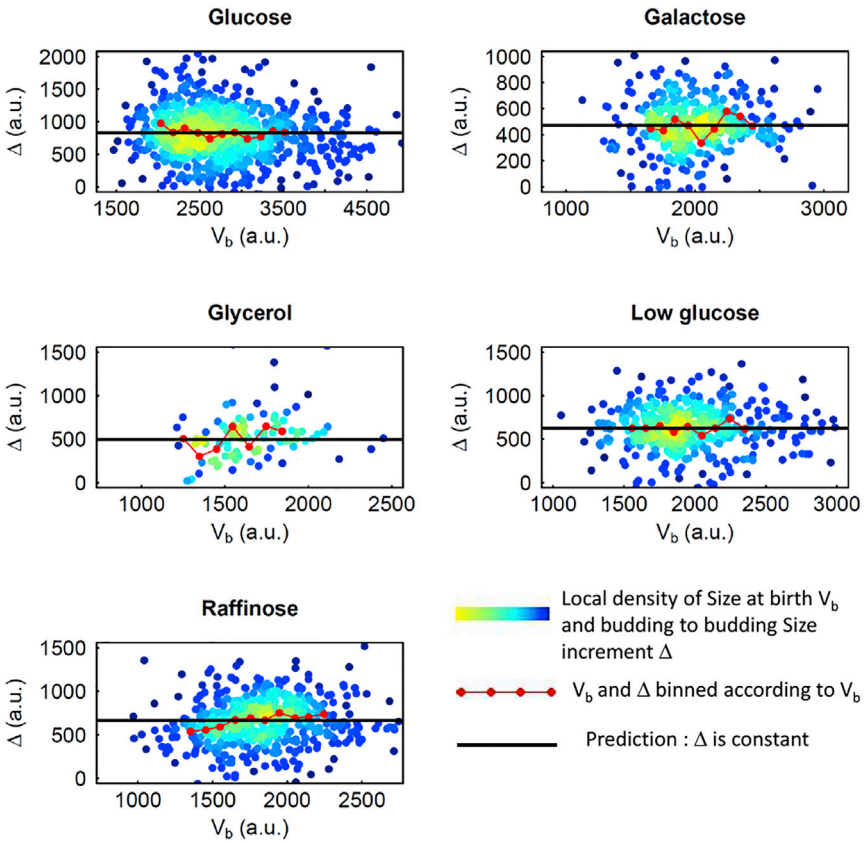

Figure 3. No Correlations between Budding to Budding Volume Increment and Size at Birth

We found no correlation between the total volume added between two budding events and the size at birth, as expected from our model: the positive correlations of the increment during the budded phases (Figure S3B) and the negative ones during G1 (Figure S3A) cancel out to give the incremental model between two budding events. Note that in light of the mechanisms proposed in sections 3 and 4 of the Supplemental Experimental Procedures, the volume increment during the budded phase is assumed to divide between the bud and parent cell in proportion to their relative volume. See also Figure S3.

Size and Time Distributions Can Be Collapsed for Bacteria and Yeast

An additional prediction of our model is that a *single* parameter describing the noise magnitude will determine both the size and interdivision time distributions, as derived in the Supplemental Experimental Procedures (section 6). This allows us to scale the experimentally measured distributions for size and interdivision time. Figure 4B (left) compares the theory with the experimental results for budding yeast growing in glucose, showing the excellent scaling collapse obtained in this way. An important property of these distributions is their coefficient of variation (CV). A priori, one may think that the CV of the size distribution is independent of that of the interdivision time distribution. However, since a single source of stochasticity is responsible for the widths of both distributions in our model, the ratio of the CVs is uniquely determined. For *E. coli*, the assumption of nearly symmetric division simplifies the calculations and allows us to obtain analytic formulas that are not possible for asymmetric division. As predicted in [4], the distribution of size at birth is relatively narrow and its CV is log(2) ≈ 0.69 smaller than that of the interdivision times. In agreement with this prediction, we estimated the ratio of CVs of size at division and interdivision time in the dataset from Wang et al. [10] and found 0.69 ± 0.03 for three independent experiments. The theory thus predicts that the distribution of the normalized logarithm of size at birth, $\log_2(v_b/v_0)$ (with v_0 being the average size at birth), should collapse on the distribution of interdivision time appropriately rescaled, (t / τ_d − 1). Figure 4B (right) shows the distributions of size at birth and interdivision time normalized according to the theory, the excellent collapse of the curves supporting the validity of our stochastic model.

DISCUSSION

In this work, we showed that size control in budding yeast relies on an incremental strategy, leading (effectively) to the addition of a constant volume between birth and division. This is in contrast to the long-standing paradigm in which the Start transition occurs when the cell size reaches a threshold value. Our study of correlations shows that the incremental strategy is likely to be implemented at the G1/S transition: the cell adds a constant volume between two Start transitions, not between birth and division.

Despite the differences in morphology, DNA replication and growth of *S. cerevisiae* and *E. coli*, we showed that these two organisms control their size using an identical strategy—described mathematically by the incremental model, in which a constant volume is added between two events in the cell cycle. The correlations between size at birth and at division and between size at birth and interdivision time are quantitatively predicted by this model and agree well with our experimental data for both organisms. In bacteria, DnaA is known to be a key regulator of the cell cycle, as it triggers initiation of new rounds of DNA replication. Similarly, in budding yeast, Whi5 seems to be a leading candidate in cell-cycle regulation. Thus, similar cell-cycle control in both organisms is most likely a result of convergent evolution, rather than of an identical molecular mechanism. In fact, even the principles by which the molecular mechanisms operate may be different in the two. In the implementation of the incremental model first introduced by Sompayrac et al. to describe the bacterial cell cycle [21], a size increment is added between two successive events of DNA replication initiation, through the accumulation of an initiator. Division then occurs after a constant delay, leading to a constant increment of volume between birth and division. On the other hand, the molecular mechanism in

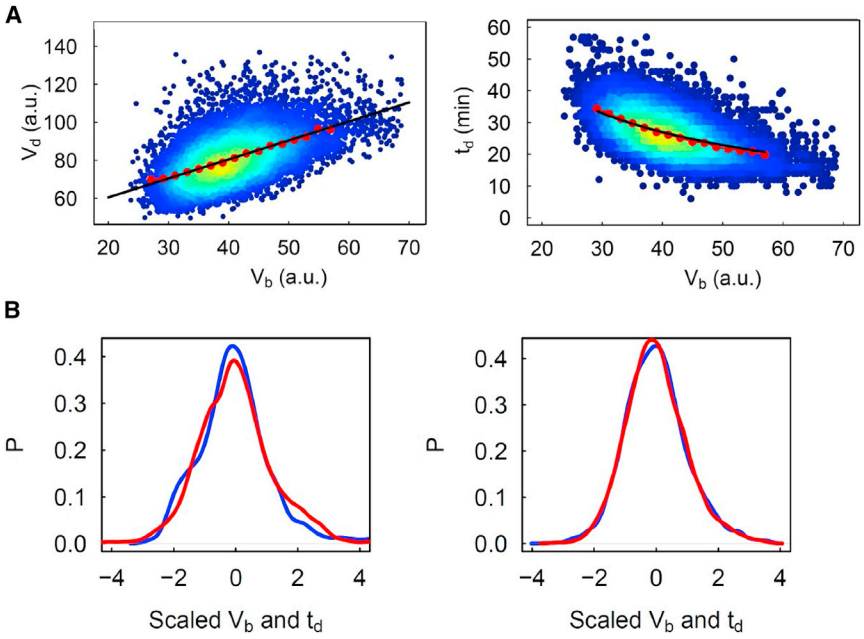

Figure 4. Size and Time Distributions Can Be Collapsed for Both *E. coli* and *S. cerevisiae*

(A) Correlations between size at birth, size at division, and interdivision time support incremental model in *E. coli*. Left: size at birth and division of single cells in fast growth conditions (Stewart et al. dataset [19]; see the Supplemental Experimental Procedures for more information). The color of the dots (blue to yellow) represents the local density. Red dots indicate data binned according to the size at birth. The black lines show the predictions of Equation 1, with a slope of 1. In this case, the offset Δ is equal to the average cell size at birth, since the division is approximately symmetric. Right: size at birth and interdivision time of single cells in fast growth conditions. The favorable comparison of the binned data (red points) with the prediction of Equation 2 (solid line), with no fitting parameters, strongly supports the incremental model for size control in *E. coli*.

(B) Distributions of size at birth and interdivision time can be scaled. Left: distribution of newborn size of yeast daughter cells in glucose and the interdivision time distribution are scaled according to the theory (see the Supplemental Experimental Procedures for details). The only fitting parameter in the theory is the magnitude of a stochastic noise, σ_T, which accounts for the coefficient of variation of *both* distributions. Right: similarly, for symmetric divisions, the incremental model predicts that the size distribution is narrower than that of the interdivision time distribution by log(2). This implies that the distribution of $\log_2(v_b/v_0)$ (with v_0 being the average size at birth), should collapse when plotted against the distribution of $(t-\tau_d)/\tau_d$ (and τ_d the doubling time). This is shown in the figure, where the blue line is the scaled size distribution, $(1/C)\log_2(v_b/v_0)$, and the red line is the scaled time distribution, $(1/C)\,(t-\tau_d)/\tau_d$, with $C = 0.2$ (note that the collapse is independent of the choice of C). All distributions were generated from the data using a kernel density estimation.
See also Figure S4.

S. cerevisiae may be due to the dilution of the inhibitor Whi5, a model supported by recent experiments [16]. We have shown here that this model implements the incremental model, if the inhibitor is shared between the parent cell and the bud in proportion to their volume. The simple mechanism that we propose explains both the correlations that we observed and is in accord with the widespread view that in budding yeast size control occurs via control at the G1/S transition [2, 3].

An appealing feature of our model is that it offers a coordination of different events in the cell cycle—namely, growth, division, and DNA replication. DNA replication is coupled to growth and size control acting at the level of initiation of replication/Start and is also coupled to division. Our work paves the way for an improved molecular level understanding of size control, and combining our phenomenological observations with the molecular techniques as used in [15, 16, 22] is a promising direction. It would also be interesting to repeat the analysis that we applied here for *E. coli* and *S. cerevisiae* to other organisms and find the regime of applicability of the incremental model, which will shed new light on the cell cycle for both eukaryotes and prokaryotes.

EXPERIMENTAL PROCEDURES

We used high-throughput time lapse microscopy with a built-in auto-focusing apparatus [23] and developed automatic software that enabled tracking individual cells over multiple division cycles. Our analysis identified timings of cell-cycle transitions and respective cell volumes. We grew yeast cells at different growth rates by changing the carbon source in the medium (glucose at high or low concentrations, galactose, glycerol, and raffinose). Yeast strains were as described in [24].

Yeast Time-Lapse Microscopy

Yeast cells were pre-grown for around 24 hr in synthetic complete (SC) medium to OD600 of about 0.5. The carbon sources used were as follows: 2% glucose, 2% galactose, 0.05% glucose, 2% raffinose, and 2% glycerol plus 2% ethanol. The cells were then prepared for imaging growing on agar pads with the respective SC as previously described. We observed growth of microcolonies at 30°C using fully automated Olympus IX71 inverted microscope equipped with a motorized xy and z stage, external excitation and emission filter wheels (Prior), and an infrared-based fast laser autofocus [23]. Fluorescent proteins were detected using EXFO X-Cite light source at 12.5% intensity and Chroma 89021 mCherry/GFP ET filter set. Exposure time for the detection of eGFP and mCherry was 120 ms. Imaging was done by cooled EMCCD camera (Andor). The microscopic setup allowed simultaneous imaging of 60 fields of view for 6 hr. Bright-field and red and green fluorescence images were collected every 3 min for the fermentable and every 5 min for the non-fermentable carbon sources.

E. coli Data Analysis

We analyzed the results of video microscopy experiments performed by Stewart et al. [19] and Wang et al. [10]. See section 7 in the Supplemental Experimental Procedures for the details of data analysis.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.11.067.

AUTHOR CONTRIBUTIONS

I.S. designed and conducted the experiments. A.A., L.R., and I.S. analyzed the data, developed the model, and wrote the paper.

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