Current Biology 22, R350–R359, May 8, 2012 ©2012 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2012.02.041

Cell Size Control in Yeast

Review

Jonathan J. Turner, Jennifer C. Ewald, and Jan M. Skotheim

Cell size is an important adaptive trait that influences nearly all aspects of cellular physiology. Despite extensive characterization of the cell-cycle regulatory network, the molecular mechanisms coupling cell growth to division, and thereby controlling cell size, have remained elusive. Recent work in yeast has reinvigorated the size control field and suggested provocative mechanisms for the distinct functions of setting and sensing cell size. Further examination of size-sensing models based on spatial gradients and molecular titration, coupled with elucidation of the pathways responsible for nutrient-modulated target size, may reveal the fundamental principles of eukaryotic cell size control.

Introduction

There is an intimate and often complex relationship between form and function in living organisms [1]. A simple and fundamental aspect of organismal form is size, which is an important determinant of cellular physiology. In unicellular organisms, adjusting the surface to volume ratio may be an adaptation to environmental conditions where surface transport is limiting. Surface-to-volume optimization is likely exhibited in the extensive fossil record of foraminifera, a group of unicellular marine protists: 60% of the variation in mean foraminifera size over the past 400 million years is accounted for by a linear model based solely on atmospheric oxygen concentration (J. Payne, personal communication and [2]). Thus, cell size can be an important selective trait for survival in changing, nutrient-limited environments.

Cell size also affects internal cellular architecture. Not only are the volumes of various organelles proportional to that of the cell [3,4], DNA content in eukaryotic cells scales linearly with cell size over nearly a million-fold range [5–7]. This is true within as well as between species: diploid yeast cells are about twice as large as haploids [8–10]. This constant ratio of DNA to cytoplasm suggests that cell size can adapt to support evolution of DNA content. Conversely, DNA content might adapt to accommodate physiologically-driven changes in cell size. Examples of the latter may be found in the yeast lineage, where a minimal genome could be viewed as an adaptation to small cellular size. With volumes as low as tens of femtoliters, yeast are among the smallest eukaryotes and have relatively few genes, as well as small intergenic regions. For example, whereas metazoan *cis*-regulatory elements are found thousands of base pairs away from the transcription start site, yeast elements are limited to ~800 base pairs upstream [11]. Thus, evolutionary pressures on cell size may influence mechanisms of transcriptional regulation via selection to maintain the appropriate DNA-to-cytoplasm ratio.

Consistent with the physiological importance of cell size, there is much evidence suggesting that cells have evolved molecular mechanisms for both monitoring and controlling size [12]. Cells of a given species typically vary little about their mean size [13], and this consistency requires tight co-regulation of cellular growth and division [14]. Single-cell studies have provided the most convincing evidence for cell size control. In these studies, variation in cell size at birth is harnessed to infer the presence or absence of cell size control: if size control is present, then cells that are smaller at birth will grow proportionally more than larger cells in the subsequent cell division cycle [15–17].

This co-regulation of growth and division has been studied in a variety of organisms, but the budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe* (Figure 1) have been favored model organisms for several reasons: a potentially simpler regulatory architecture, powerful systematic tools for genetic analysis, and simpler cell geometry [18]. More fundamentally, the regulation of growth and division is particularly tractable in yeast. In metazoans, both division and growth are subject to complex extrinsic signaling and multifarious mutual regulation. In yeast, on the other hand, preventing growth arrests the cell cycle, but growth proceeds apace when proliferation is halted [15,19]. Thus, yeast control size primarily by regulating division in response to growth. This requires detection of the nutrient concentrations that determine growth rate, growth rate itself, or cell size. Here, we review our current understanding of size control mechanisms in yeast, highlighting recent work and open questions.

Size and Growth

"the form of an organism is determined by its rate of growth in various directions; hence rate of growth deserves to be studied as a necessary preliminary to the theoretical study of form" D'Arcy Thompson, On Growth and Form [1].

Size is the most fundamental aspect of cellular form, and the basis of cell size control is the coupling of growth and division. The growth function f, relates a cell's current size, V, and cell cycle phase, ϕ, to the rate of size increase, dV/dt, so that $dV/dt = f(V, \phi)$. This relationship defines the requirements of cell size control. More specifically, whether cell growth is linear or exponential has been hotly debated and has important implications for size control [18]. For linear growth, cells increase their size at a constant rate C, so that $dV/dt = C$. In this case, specifying the period of the cell cycle specifies mean cell size without requiring a direct link between size and division, because the progeny of large or small cells asymptotically approach the target size over several generations [20]. Linearly growing cells could therefore maintain an average size simply by adjusting the length of the cell cycle. On the other hand, for exponential growth, the rate of growth is proportional to cell size, so that $dV/dt = \alpha V$. In this case, constant cell cycle periods allow growth to a constant multiple of birth size, so that slight fluctuations are not corrected in succeeding generations [21]. Therefore, exponentially growing cells must vary cell cycle duration to damp fluctuations. This is accomplished through size-dependent cell cycle progression.

Cell size and growth may be measured by a variety of methods, summarized in Table 1. In budding yeast, an exponential model of growth is supported by both single cell analysis using time lapse microscopy and bulk experiments using radioactive labeling [17,22,23]. Although it has recently

Department of Biology, Stanford University, Stanford, CA 94305, USA.
E-mail: skotheim@stanford.edu

been shown that arresting the cell cycle in various ways differentially shifts the growth rate [24], budding yeast adhere closely to exponential growth in an unperturbed cell cycle. The growth function for fission yeast, however, deviates from exponential (see [18] for a comprehensive review). Fission yeast growth includes two linear segments separated by a rate change point, followed by a period directly preceding division during which the cell does not increase its length. Despite this difference in growth function, budding and fission yeasts both rely on size control mechanisms to couple growth and division (Figure 2). This may occur because, even though active size controls are not required in linearly growing cells, they may provide a more efficient means of controlling size than relying on a growth rate-dependent cell cycle duration.

Additionally, exponential and linear growth functions have different implications for the effects of cell-to-cell variability and cell size mutants on fitness. Because the instantaneous rate of growth is proportional to cell size during exponential growth, mass doubling time is independent of cell size. Therefore, size control mutations shifting cell size, but not affecting metabolic processes, are not expected to affect population doubling time in exponentially growing cells. This is the case in budding yeast, where size mutants, for example *whi5Δ,* have wild-type population doubling times [25]. Similarly, although fluctuations may push sizes of individual cells significantly below or above the population mean, this is not expected to affect fitness for exponentially growing cells. Consistent with this argument, budding yeast exhibit much larger size variability than fission yeast, which does not exhibit exponential growth. For haploid cells, the coefficient of variation (standard deviation/mean) of *S. pombe* cell size at fission is ~0.06, while the coefficient of variation of *S. cerevisiae* cell size at budding is ~0.17 [16,17,26]. Yet, the noise in budding yeast is still bounded, which may reflect the limits over which growth is exponential. If cells get too large, then a single genome will be unable to support exponential growth of the cytoplasm [27]. Thus, we expect the degree of noise tolerated to be related to the range over which growth is exponential.

In addition to influencing the selective pressures on size control systems, growth functions may themselves be selected for. In particular, the growth functions of fission and budding yeasts may reflect the physiological requirements of symmetric and asymmetric division patterns. Upon nutrient limitation, budding yeast will produce daughter cells less than 20% of the mother cell size [15]. This asymmetric division may select for growth functions that, like exponential growth, are efficient over a larger range of cell sizes. In turn, efficient growth over a large size range lessens the pressure to have precise size control. Therefore, we expect to see the degree of cell-to-cell variation tolerated in size control increase with the degree of mother–daughter asymmetry in division size, illustrating the potential interdependence of the growth function, division pattern, and size control.

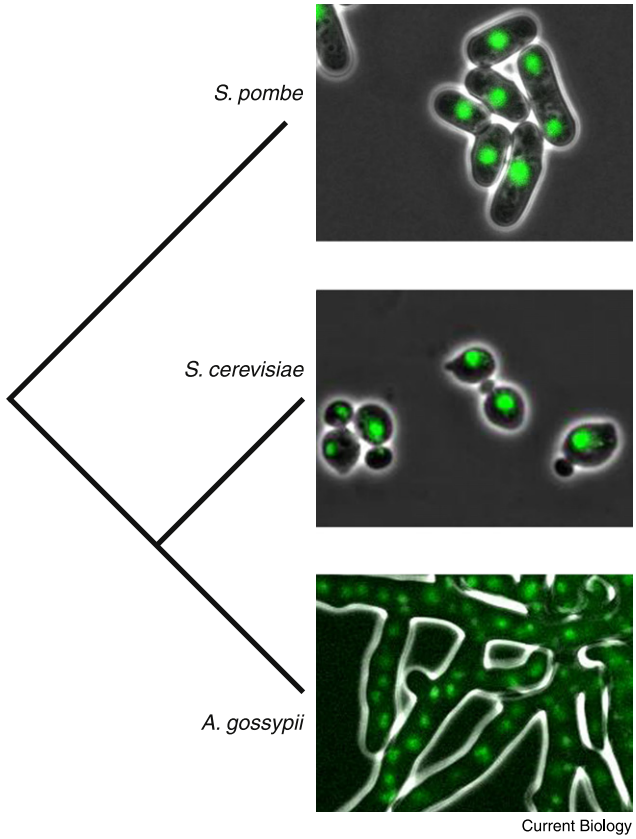

Figure 1. Yeast models for cell size control.
S. pombe and *S. cerevisiae* are the preeminent model organisms for cell size control studies. Interestingly, *S. cerevisiae* and *A. gossypii* are more closely related than either is to *S. pombe.* Despite their divergent morphologies, these two yeasts are regulated by similar cell cycle control networks governing the DNA to cytoplasm ratio.

Perfect and Imperfect Size Thresholds

Despite different growth functions, size control is broadly similar in budding and fission yeasts. As indicated by early physiological studies, both yeasts implement continuous monitoring of a size-dependent signal and restrict cell cycle progression at specific stages in a size-dependent manner. In *S. pombe*, evidence for a size requirement for division came from experiments that tracked the growth and division of cells following synchronization by S-phase arrest [19]. Cells continued to grow during the arrest, so that they became much larger than normal cells. Upon release, the progeny of these cells returned to normal size after only a few division cycles, because each cycle took only about 60% of the normal time, preventing mass doubling before division. Cell division cycles returned to their normal duration once the cells had regained their normal size distribution. These shortened cycles indicated that the abnormally large arrested cells had surpassed a size threshold, manifested primarily at the G2–M transition [28,29]. Later work uncovered an additional size requirement at the G1–S transition, which is revealed by adverse growth conditions or mutations reducing cell size [30–34]. This secondary size checkpoint has been little-studied, so we do not discuss it here.

In *S. cerevisiae*, early single-cell studies showed that small cells spend a longer time in G1, which allows them to grow more than initially larger cells [15]. These experiments suggested a size threshold at the G1–S transition in *S. cerevisiae*, rather than at the G2–M transition, as in *S. pombe*. Additionally, the asymmetric division pattern of budding yeast has implications for size control. Mother cells fulfilled the size requirement when they first became mothers and bud at regular intervals [35]. Interestingly, even small mothers do

Table 1. Methods for measuring cell size.

Method	Measured property	Measurement principle	Advantages/disadvantages	References
Flow cytometry				
Resistive-pulse sensing (coulter counter)	Volume	Change in resistance due to volume displacement	Fast and accurate/does not track individual cells	[25,152,153]
Fluorescent flow cytometry and fluorescence activated cell sorting (FACS)	Volume and shape Protein content	Light scatter Total fluorescence after FITC staining	High-throughput, can be combined with different fluorescent labels/ indirect measurement of size, does not track individual cells	[154,155]
Imaging				
Bright field	Area	Volume estimate from area of focal plane	Single cell time course capability, difficult to accurately identify cell border	[156]
Fluorescent proteins	Protein content	Fluorescence of constitutively expressed fluorophores	Single cell time course capability/ indirect measurement	[17]
Interferometry	Dry mass	Phase shifts caused by biomolecules	Single cell time course capability, dry mass determination with subcellular resolution/complicated analysis	[157–159]
Microchannel resonators	Buoyant mass	Changes in mass affect resonance frequency	Single-cell time course capability/low throughput, specialized equipment needed	[23,49]

not exhibit size control and proceed through G1 rapidly, compared to similarly-sized daughter cells [36]. This difference in mother–daughter size control programs was later traced to the asymmetric distribution of the daughter-specific transcription factors Ace2 and Ash1, which regulate transcription of the G1 cyclin gene *CLN3* [37–41]. Thus, *S. cerevisiae* size control acts primarily on the G1–S transition in daughter cells.

Similar to the secondary size control at G1–S in fission yeast, there is evidence for additional size control in budding yeast during S/G2/M. This was initially suggested by the finding that halting bud growth by depolymerizing actin induces a G2/M delay in cells with buds smaller than a threshold size [42]. This effect depends on homologues of the key regulatory proteins for mitotic entry in fission yeast (see below). A subsequent study challenged the causal role of size by showing that stopping bud growth through disruption of vesicular transport does not cause a G2/M delay [43]. This study suggested that actin depolymerization engages the morphogenesis checkpoint [44], and that the apparent size dependence is in fact due to differences in G2/M progression. Nevertheless, single-cell studies show a weak dependence of G2/M duration on size at budding (S. Di Talia, personal communication), suggesting the need for further investigation.

It is important to note that, wherever it occurs in the cell cycle, size control can be more complex than a simple threshold. Typically, cell cycle control has been viewed in terms of 'sizers' and 'timers'. Sizers require that cells pass a size threshold, while timers require that cells wait a fixed amount of time, independent of size [45]. However, these concepts are insufficient to describe all size-dependent cell cycle progression. The degree of size control in a given cell cycle interval can be inferred by plotting a metric of cell growth within the interval versus the size of the cell upon entering the interval (Figure 3A). If the slope of a curve fit to the single cell data is –1, the amount of growth in the interval will exactly compensate the difference in initial sizes — a sizer. If the slope is 0, growth within the interval is uncorrelated with the entrance size and there is no size-dependent control — a timer. Imperfect size control will yield intermediate values. Haploid fission yeast cells exhibit a perfect sizer [16], whereas early frog embryos exhibit a perfect timer [46]. Budding yeast G1–S control has significant but imperfect size control, as exhibited by a slope of –0.7 (Figure 3B) [17]. Thus, eukaryotic cells exhibit distinct degrees of cell size control.

The intermediate degree of size control exhibited by budding yeast correlates with imperfect size compensation through a single cell cycle. In other words, it will typically take more than one cycle to damp size fluctuations. Additionally, imperfect size control means that the 'size threshold' for the G1–S transition is itself *size-dependent*, as, on average, cells that are smaller when they enter G1 will bud at smaller sizes than their larger brethren. This cannot be accounted for by a simple combination of sizer and timer modules. A full account of the molecular mechanism underlying budding yeast size control will be required to understand its complex size-dependence. Nevertheless, 'shifting the size threshold' up or down may be interpreted as shifting the size control curve (Figure 3B,C) right or left to generate larger or smaller cells at budding, respectively, for the same input birth size. G1 progression kinetics in budding yeast are thus imperfectly characterized by a single size-threshold and require a curve for accurate description.

Size-dependent cell cycle progression suggests that cells somehow sense their own size. A discussion of how cells sense their own size requires first a clarification of what we mean by size. At its most general, size conflates cellular mass and volume, which are often correlated. This correlation is imperfect, however, as cell density varies through the cell cycle significantly in *S. pombe,* and more subtly in *S. cerevisiae* [47–49]. Total protein or ribosome content may also represent relevant additional, correlated size metrics, which might be sensed by distinct molecular mechanisms. Two size-sensing mechanisms have emerged as favored models: a protein synthesis rate-based mechanism in budding yeast, and a gradient mechanism that directly detects cell length in fission yeast.

Geometric Size Sensing in *S. pombe*

Fission yeast are rod-shaped and grow highly asymmetrically, maintaining a constant width and increasing only in

length. This property has been exploited in size control studies, as it allows measurement of a single dimension, length, to indicate three-dimensional volume [47]. Intriguingly, *S. pombe* cells may exploit their own well-defined geometry by using a spatial gradient to sense cell length, as an indicator of total volume [50,51]. The principle is simple: a mitotic activator is localized to a region in the middle of the cell, and an inhibitor of the activator is arranged in a gradient originating from the cell poles. Thus, as the cell grows, the amount of inhibitor interacting with the activator decreases, leading to an increase in mitotic cyclin activity that drives mitosis.

The first protein specifically identified as a size controller was Wee1, which restrains cyclin-dependent kinase 1 (CDK1) activity [28] by phosphorylating CDK1 on Tyr15. Removal of this specific phosphate by Cdc25 is rate-limiting for mitotic entry [52–55]. The spatial size sensor model operates through the Wee1-inhibitory kinases Cdr1 and Cdr2 [56–60], which are themselves inhibited by Pom1, a kinase identified through its pleiotropic effects on cell morphology and growth [61]. While Pom1 is localized in a spatial gradient emanating from the cell poles [61,62], Cdr1 and Cdr2 are localized in cortical nodes in the middle of the cell [63]. As cells grow, their poles move apart, reducing medial Pom1 concentration. This leads to reduced Pom1-dependent Cdr1/2-inhibition, potentially yielding a gradient-based sizer mechanism (Figure 4A) [50,51]. The Pom1 gradient arises due to Tea4-dependent localization to the tips followed by autophosphorylation-induced membrane dissociation [64]. In support of the gradient model, mislocalization of Pom1 increases average cell length [50,51].

However, the pleiotropic effects of Pom1 perturbations raise some doubt as to the specificity of its role in size control. For instance, among other functions, Pom1 is required for proper positioning of the division plane [62,65]. Furthermore, the ability of the Pom1 gradient itself to accomplish size control has been insufficiently tested. Indeed, it has recently been shown that the Pom1 concentration on the membrane decays exponentially from the cell pole with a length scale of ~1.5 μm [66]. As pre-mitotic *S. pombe* cells have an average length of more than 10 μm, this finding suggests that the medial concentration of Pom1 will drop to extremely low levels well before mitotic entry. This is particularly relevant in *cdc11-119* mutants deficient in septation, which yield multinucleate cells containing a DNA to cytoplasm ratio similar to wild type (F. Neumann, personal communication). In addition, size scaling with ploidy suggests that non-spatial mechanisms also operate in fission yeast [8].

Prior to the discovery of the Pom1 gradient, *S. pombe* size control studies anticipated non-spatial mechanisms. It was suggested that accumulation of Cdc13 might drive the transition [67], but most studies focused on possible Wee1 or Cdc25-mediated mechanisms. Regulation of Wee1 by Cdr2 and Cdr1 kinases has been associated primarily with nutrient modulation of cell size [59,68,69]; size sensing was more commonly ascribed to Cdc25. An early study suggested that Cdc25 (rather than Cdc13) induced mitosis at a critical size through an accumulation mechanism [70], and the finding that Cdc25 levels increase during cell cycle arrest strengthened the link between cell growth and Cdc25 synthesis [71,72]. Finally, Cdc25 levels are disproportionately sensitive to the translation initiation rate, which is mediated by upstream open reading frames and additional 5′ regulatory sequences in the *cdc25* mRNA [73]. Although

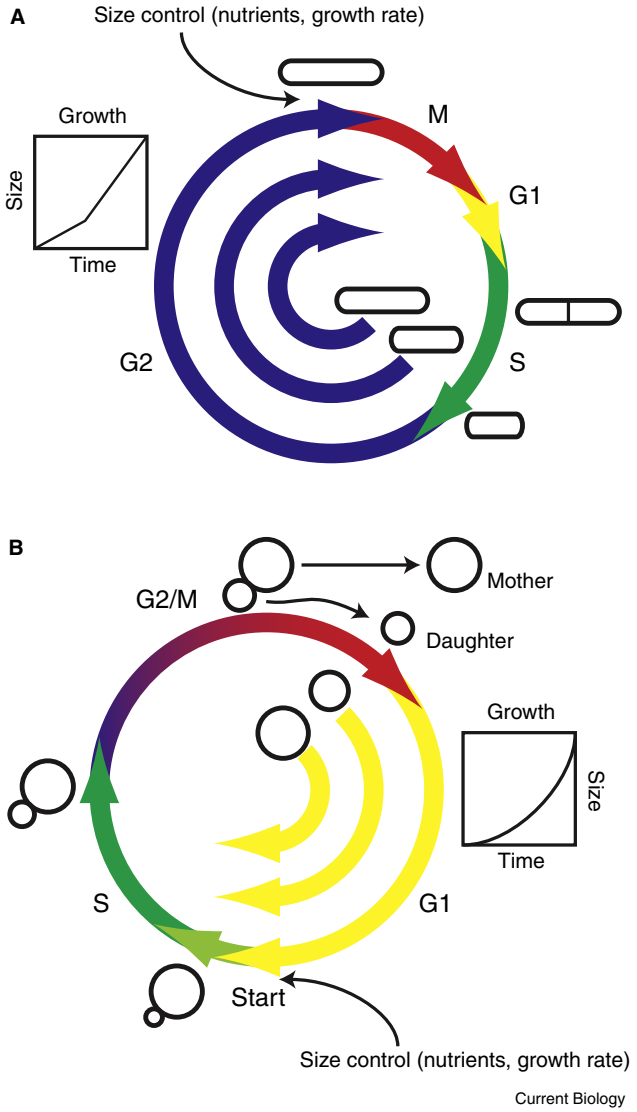

Figure 2. Size-dependent cell cycle progression in *S. pombe* and *S. cerevisiae*.

(A) *S. pombe* cells enter G2 at different sizes following S-phase. They grow in a bilinear fashion and enter mitosis upon reaching a threshold size, so that smaller cells spend more time in G2 than larger cells, as indicated. (B) *S. cerevisiae* daughter cells are born at different sizes and grow exponentially. Smaller cells spend more time in G1 prior to *Start* than larger cells (as indicated), which partially compensates for initial size variation. Size control is a function of nutrient conditions and growth rate and is exerted at G2–M in *S. pombe* and within G1 in *S. cerevisiae*.

these studies are far from conclusive, they suggest that a Cdc25-dependent translational sizer may act in concert with a Pom1–Cdr2–Wee1 spatial sensor. Furthermore, the maintenance of a consistent, albeit greater, division length in cells with both key phosphosites mutated in Cdc2 indicates that other pathways must be involved in mitotic size control [74]. Taken together, these data suggest that multiple size sensing mechanisms operate in fission yeast.

Synthesis Rate-Based Size Sensing in *S. cerevisiae*

A gradient-based geometric size sensing mechanism in budding yeast cells would require the formation of surface

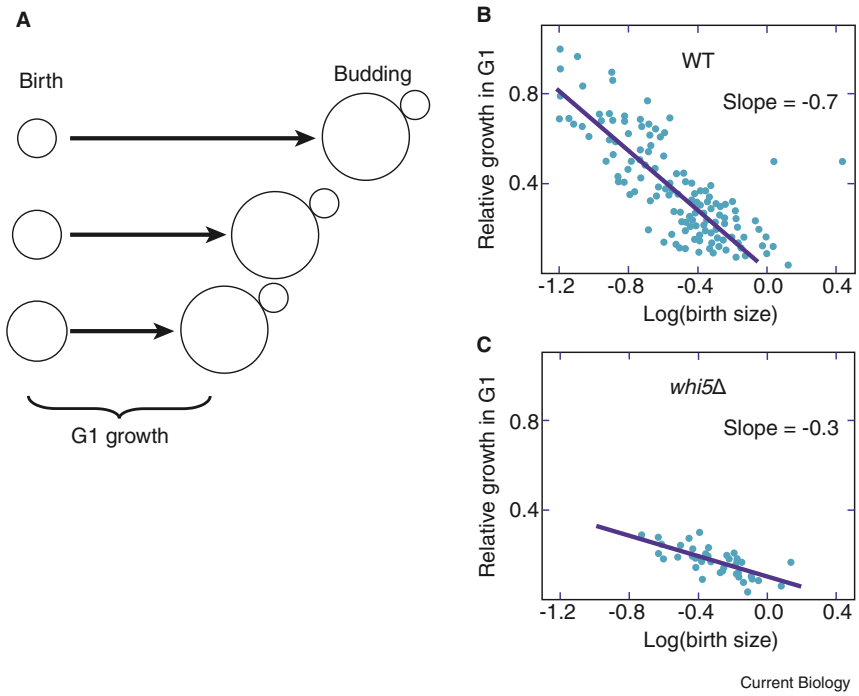

Figure 3. Single-cell size control assay as applied to *S. cerevisiae*.

(A) Size control leads to a negative correlation between cell size at birth and growth in G1. Live-cell imaging techniques allow cells to be tracked from birth to budding, which is concomitant with DNA synthesis. (B) Plotting the logarithm of size at birth vs. relative growth in G1 quantifies the efficiency of G1 size control. A slope of –1 would indicate perfect size control, whereas a slope of 0 indicates a lack of size control. Wild-type (WT) *S. cerevisiae* exhibit imperfect but significant size control, as indicated by the –0.7 slope relating birth size to growth in G1. (C) Deletion of the cell cycle control gene *WHI5* greatly diminishes the efficiency of size control, as indicated by the reduced slope of –0.3. (Data adapted from [17].)

gradients in nearly spherical G1 cells, or cytoplasmic gradients with length scales of a few microns. Although both mechanisms are feasible, as they have been observed in bacterial cells [75–77], there is strong evidence against a geometric size sensor operating in budding yeast. In particular, cell size increases linearly with ploidy over a six-fold range despite significant differences in geometrical characteristics, such as cell wall curvature, surface-to-volume ratio, and eccentricity [10,17]. It is therefore unlikely that geometric parameters other than size are responsible for initiating cell cycle progression. Furthermore, the filamentous fungus *Ashbya gossypii* presents an interesting case in comparison to budding yeast, because it exhibits similar G1–S control, despite its distinct geometry [78] (Figure 1). G1-S control mutations equivalent to those that affect cell size in budding yeast, such as *whi5* and *cln3* deletion mutations that make cells smaller and larger, respectively, have a similar effect on the amount of cytoplasm per nucleus in *A. gossypii* (A. Gladfelter, personal communication). This argues that size control, as manifested in the DNA to cytoplasm ratio, is unlikely to be operating on geometric considerations in species closely related to budding yeast.

A mechanism that has no geometric requirement and could maintain the DNA to cytoplasm ratio for cells of any shape is a protein synthesis rate-based sensor. Such a mechanism could explain the similar effects of mutations in *A. gossypii and S. cerevisiae* despite gross shape differences. At its most basic, this class of models posits that a division-promoting protein is synthesized at a rate proportional to that of overall protein synthesis, and that the cell enters the cell cycle when that factor reaches a threshold level. Such models were proposed early on to underlie the size threshold for division in bacteria and metazoans [79–81], as well as in *S. cerevisiae* and *S. pombe* [29,82,83]. Consistent with this model, reducing translation rate by incubation with low doses of cycloheximide extends G1 and increases the threshold cell size in *S. cerevisiae* [84–87]. Interestingly, although similar results were observed in *S. pombe* cells treated with pulses of cycloheximide [88], extended incubation of *S. pombe* cells in low levels of cycloheximide does not affect size at division, providing evidence against a synthesis rate mechanism in fission yeast (F. Navarro, personal communication).

Protein synthesis rate-based models rely on a sizer protein, whose abundance increases proportionally to the protein production rate. Such a sizer protein should exhibit three key properties. First, the sizer should be a dose-dependent activator of cell cycle progression; that is, it must be rate limiting and not merely a necessary component for cell cycle progression. Second, its expression should be nearly constitutive during the growth period, so that it consistently indicates overall protein synthesis rate. Finally, the sizer should be unstable, so that its activity reflects current protein production rate, rather than total synthesis [89–91]. The protein in *S. cerevisiae* that exhibits these three properties, and is thus the prime candidate for a sizer protein, is the G1 cyclin Cln3. *CLN3* is a dose-dependent activator of the G1–S transition [92–94], and unlike other cyclin genes, its expression oscillates only weakly through the cell cycle [40,94–98]. Finally, Cln3 is a highly unstable protein, with a half-life of less than five minutes [92–96]. Although the properties of Cln3, when examined using bulk assays, suggest that it acts as a synthesis rate sizer, its instability has prevented its analysis in live cells. This type of quantitative analysis is still required to demonstrate that, as predicted by the model, total amounts of the Cln3 protein correlate with size and determine precise timing of the G1–S transition.

Although Cln3 fits the classical description of a sizer protein, there is a basic problem facing the Cln3 synthesis rate sizer model: given that the total amount of Cln3 is proportional to protein synthesis rate, which is proportional to size in exponentially growing budding yeast cells, the correlation between ribosome content and volume will render the concentration of Cln3 roughly constant. How, then, does the cell produce a signal whose activity increases with cell size, when the key activator remains at constant concentration? This problem is inherent to all synthesis rate sizer models

and suggests that cells normalize sizer protein abundance against a standard that does not change with cell growth.

Early formulations of this model proposed genomic DNA (or specific sites thereon, such as replication origins) as a non-growing standard [79,83]. In budding yeast, the nucleus was proposed as a proxy for the genome, as its volume was long assumed to depend on DNA content [99,100]. This mechanism is supported by the fact that Cln3's primary targets are the transcription factor SBF and its inhibitor Whi5 [101,102], and by Cln3's active nuclear localization [103,104]. However, the nucleus has recently been shown to grow in G1 and maintain a nearly constant nuclear to cytoplasmic volume ratio in both *S. cerevisiae* and *S. pombe* [8,9]. Thus, it is necessary to consider how Cln3 abundance might be compared directly to cellular DNA content. The recent finding that Cln3 is present at SBF-binding sites in the promoter of a key downstream target (*CLN2*) suggests a model in which Cln3 activity is titrated against the fixed number of SBF binding sites (called SCBs) in the genome [105]. Evidence for this model is currently limited to the finding that transformation of yeast with a high-copy plasmid containing several SCBs increases cell size at budding in a Cln3 and Whi5-dependent manner [105]. This promising model requires further study.

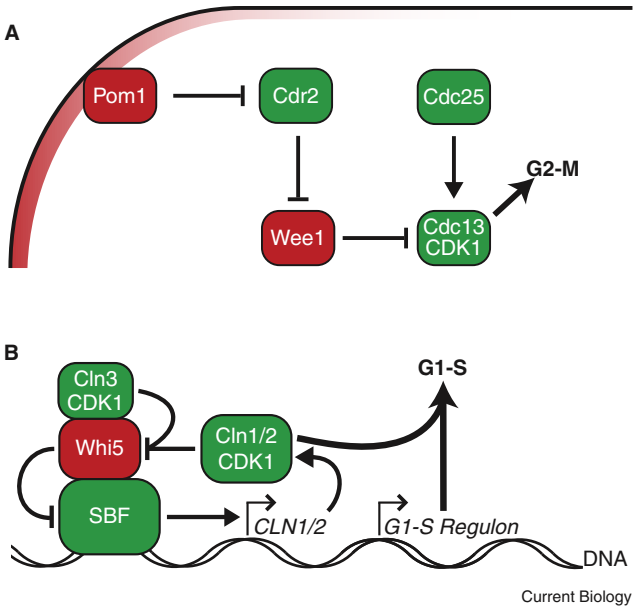

Figure 4. Models of size-dependent cell cycle regulatory networks.
Activators of cell cycle progression are colored green; inhibitors are colored red. (A) In *S. pombe*, size control acts at the G2–M transition. The mitotic inhibitory kinase Pom1, localized to a membrane gradient originating at the cell poles, inhibits the pro-mitotic kinase Cdr2, which is localized in cortical nodes at the midcell. Cdr2, in turn, inhibits the Wee1 kinase, which inhibits CDK1. The effects of Wee1 are opposed to those of the phosphatase Cdc25. As *S. pombe* cells grow in length, the medial concentration of Pom1 decreases, allowing activation of Cdc13-CDK1 and entry into mitosis. (B) In *S. cerevisiae*, size control acts at the G1–S transition. CDK1 in complex with the upstream G1 cyclin Cln3, whose activity is growth-dependent, activates the G1–S transcription factor SBF by direct phosphorylation and by inactivating the transcriptional inhibitor Whi5. This induces a transcriptional positive feedback loop that leads to a switch-like entry into S-phase. Importantly, Cln3 acts on promoter-bound SBF–Whi5 complexes, suggesting that Cln3 activity may be titrated against promoter-bound SBF.

Positive Feedback as the Basis of the Budding Yeast Threshold Response

Although the Cln3 synthesis rate model can provide a signal whose strength increases smoothly in proportion to cell size, it does not obviously provide a threshold mechanism. A threshold in G1 was first identified by Lee Hartwell and colleagues, who defined *Start* as the point of commitment to the mitotic cell cycle [106]. When exposed to mating pheromone, pre-*Start* cells immediately arrest their cell division cycle, whereas post-*Start* cells divide once more before arresting. Positive feedback loops have been shown to underlie threshold responses in both cell cycle and developmental transitions [107–109]. Indeed, in budding yeast, the threshold for *Start* is provided by a G1 cyclin positive feedback loop. The rate-limiting target of Cln3 in G1 is the SBF inhibitor Whi5, whose phosphorylation by Cln3-CDK initiates its dissociation from SBF and export from the nucleus [101,102,110]. Partial relief of SBF inhibition results in the transcriptional activation of the two downstream G1 cyclins, Cln1 and Cln2, completing the positive feedback loop (Figure 4B) [111–114]. Activating the Cln1- and Cln2-dependent positive feedback loop ensures rapid export of the remainder of the nuclear Whi5 and commits the cell to division [110]. It has been proposed that ultrasensitivity in Cln3 activity is a result of cytoplasmic retention mechanisms [115,116] or an upstream open-reading-frame limiting translation rate, rather than positive feedback [117]. However, these models are inconsistent with slow and steady export of Whi5 from the nucleus in *cln1Δcln2Δ* cells lacking the cyclin positive feedback loop but containing endogenous levels of Cln3 [114]. Importantly, progression through G1 becomes size-independent after Whi5 export [17], indicating that flipping the positive feedback-switch converts the gradually increasing size-dependent signal into a threshold response that marks the end of the size control program.

Increasing Cln-CDK activity effects S-phase entry by activating the related heterodimeric transcription factors SBF (Swi4–Swi6) and MBF (Mbp1–Swi6) to promote the coherent transcription of more than 200 genes [111,114,118–120]. SBF is activated via both the inhibition of Whi5 and a Whi5-independent mechanism [101,114]. MBF is also activated by G1-cyclin CDK activity, possibly through the Swi6 subunit shared with SBF [121]. Despite co-regulation, the transcription of the regulon through the mitotic cell cycle is temporally organized: the G1 cyclin *CLN1* is the earliest activated gene (*CLN2* is also relatively early) implying that G1 cyclin positive feedback is initiated prior to genome-wide changes to the transcriptional program [122]. The G1 cyclin promoters are therefore important determinants of the CDK-activity threshold required to trigger the positive feedback loop. Thus, we require a better understanding of the molecular mechanisms that determine the susceptibility of G1–S regulon promoters to Cln3-CDK activity to understand the molecular basis of cell size regulation.

Environmental Modulation of Cell Size

For any given environment, yeast maintain a consistent cell size by monitoring growth during the cell cycle and restricting proliferation in a size-dependent manner. However, it is well known that cell size can change dramatically as a function of extracellular conditions. Thus, the size control program is modulated according to nutrient conditions and growth rate. Typically, cells growing quickly on rich media

divide at a larger size than cells growing slowly under poor nutrient conditions.

Modulation of size control is particularly important for synthesis rate-based mechanisms. As protein synthesis rates for slow growing cells of a given mass are lower, these cells would be expected to require significant increases in cell size to overcome a static threshold [90]. However, the opposite is generally the case: although there is no linear relationship between the growth rate and the mean size of the population [25,123], slow growth on poor nutrients tends to reduce cell size [123]. Intriguingly, a change in the available nutrients immediately triggers an adjustment in size control. For example, when *S. cerevisiae* is shifted from ethanol (slow growth) to glucose (fast growth) containing media the budding index (percentage of cells in S/G2/M) temporarily decreases as cells rapidly adjust to a larger target size [87,124–126]. Upon a nutrient downshift, such as a shift from glutamate to proline [30,68,127], or sudden glucose starvation, *S. pombe* immediately undergoes several rounds of division without growth phases, to attain a much smaller average cell size [128,129]. These observations have led to the conclusion that, in addition to a size-sensing mechanism, there must be a mechanism that rapidly modulates the target size in response to nutrient availability [90,130].

Several important proteins involved in metabolism and growth have also been implicated in size control. Specifically, the highly conserved kinases target-of-rapamycin (TOR) and protein kinase A (PKA) respond to nutrient availability by sensing external cues through nutrient receptors [131], as well as internal cues such as glycolytic activity or amino acid concentrations (reviewed in [132–135]). The downstream targets of PKA and TOR include metabolic pathways and biosynthetic genes that promote growth. Several mutants of both the PKA and TOR pathways have been implicated in yeast size control [25,136]. As Yanagida *et al.* [136] and Shiozaki [137] have recently provided detailed reviews on size setting in *S. pombe*, which highlight the importance of TOR, we will focus our discussion on summarizing recent findings in *S. cerevisiae*.

When screening the *S. cerevisiae* deletion collection for genes determining cell size, Jorgensen *et al.* [25] identified *SFP1* and *SCH9*, which encode previously poorly characterized components of the TOR and PKA pathways. Sfp1 and Sch9 activity is both modulated by nutrients and activates ribosome production through the ribosome biosynthesis (Ribi) and ribosomal protein (RP) genes [86]. Jorgensen *et al.* [25] proposed a model in which ribosome biosynthesis rates set the size threshold, while current ribosome activity (protein synthesis rate) enables passing that threshold.

Over the last decade, the pathways that connect nutrient sensing, Sfp1 and Sch9 activity, and ribosome biosynthesis have been investigated, and a more complete picture is beginning to emerge (reviewed in [134,138]). Briefly, Sfp1 and Sch9 are both directly phosphorylated by the TOR complex 1, with crosstalk to the PKA pathway [139,140]. Sfp1 activity is regulated by localization to the nucleus. Under unfavorable conditions, such as nutrient limitation or chemical stress, Sfp1 is retained in the cytoplasm by Mrs6, a component of the secretory pathway [138,141]. In the nucleus, Sfp1 activates the transcription of the Ribi and RP genes, likely by controlling the localization of the transcription factors Fhl1 and Ifh1 [86,142]. In turn, Sch9 activates Ribi, RP and rRNA gene expression by inactivating the transcriptional repressors Stb3, Dot6 and Tod6 [143].

Despite our increased understanding of the role of Sfp1 and Sch9 signaling in ribosome synthesis, there has been very little progress in determining how this contributes to size control. In fact, aspects of Jorgensen and Tyers's model have been challenged by several studies: Bernstein *et al.* [144] showed that disturbing the maturation of ribosomes, without inhibiting their transcription, delays *Start* in a Whi5-dependent manner and leads to *bigger* cells. Another study addressed the pleiotropic effects of *SFP1* deletion and showed that Sfp1 also directly targets metabolism [126]. Notably, the overexpression of Msr6, which leads to retention of Sfp1 in the cytoplasm, was shown to downregulate glycolysis [141]. Thus, Sfp1 may modulate cell size in a way that is independent of ribosome synthesis. Additionally, nutrient-dependent signaling may act on ribosome biosynthesis in a Sfp1/Sch9-independent way, possibly through transcriptional inhibitors, such as Crf1 [145]. A full understanding of the roles of Sfp1 and Sch9 at *Start* will likely require the identification of a molecular link between these two proteins and the core cell cycle regulatory network.

Additionally, there are multiple TOR- and PKA-independent pathways in nutrient signaling that may also play a role in size control [133]. For example, there is strong evidence for glucose metabolism regulating several cell cycle genes independently of PKA and TOR [146]. In this context, recent findings from Cai and Tu [147,148] appear especially interesting: the concentration of acetyl-CoA (the end product of glycolysis) directly modulates the acetylation of regulatory proteins, which in turn modify histone acetylation and thereby enhance transcription of many genes required for growth and proliferation. Another interesting link between metabolism, growth and the cell cycle was proposed by Futcher [149], who suggested that the accumulation of storage carbohydrates could be an important determinant of *Start* and cell size under nutrient limited growth conditions.

Although the specific connection remains unknown, metabolic regulation must interact with cell cycle control at the G1–S transition. Studies shifting cells from poor to rich media indicate that size resetting is downstream of Cln3. Several studies demonstrated that *CLN3*-deleted cells still show nutrient modulation of size [124,125,150]. Baroni *et al.* [124] and Tokiwa *et al.* [150] showed that upon either glucose or cAMP addition, transcription of the G1 cyclin *CLN1* (and to a lesser extend *CLN2*) is inhibited, whereas *CLN3* transcription is unaffected. Flick *et al.* [125] later mapped this inhibition to the *CLN1* promoter. Inhibition of downstream G1 cyclin transcription was not only found during transient size resetting, but also played a role during steady-state growth on glucose.

Because size resetting acts downstream of Cln3, we expect to find it acting on an element of the G1–S positive feedback loop. How is the activity of SBF modulated independently of Cln3? One likely candidate is the rate-limiting transcriptional inhibitor Whi5. While long-term steady state adjustments could simply be due to transcriptional regulation of Whi5, this would not account for immediate responses to glucose addition. We can thus speculate that modifications of Whi5 or of the Whi5–SBF interaction are responsible for rapid changes in target size. However, studies from Tyers and coworkers have shown that size adjustment is maintained in cells lacking either or both *CLN3* and *WHI5* [86,102]. This implies that either a Cln3/Whi5-independent mechanism or multiple interactions set the target size. We therefore anticipate the discovery of novel

transcriptional regulators that interact with SBF to fine tune the CDK requirement for passage through Start in response to nutrient conditions.

Concluding Remarks

The many years of work on cell size control in budding and fission yeasts have revealed the basic principles underlying the maintenance of a consistent cell size in these organisms: critical cell cycle transitions are delayed in a size-dependent manner, while the target size is modulated in response to environmental conditions. A large part of the protein regulatory networks that effect size sensing and cell cycle control have been elucidated in both budding and fission yeasts, but understanding the mechanisms underlying nutrient modulation of target size remains a major challenge. For size sensing, the remaining challenge is to determine how the various regulatory components work together to generate a size-dependent signal. In budding yeast, a protein synthesis rate model, based on titrating sizer molecules against genomic binding sites, shows promise. In fission yeast, a gradient-based spatial sensor may play an important role. The close homology between budding yeast and metazoan G1–S regulation, and between fission yeast and metazoan G2–M regulation [151], suggests conservation of the regulatory principles underlying size control. Thus, understanding size control mechanisms in yeast will help us answer the fundamental question of how eukaryotic cells sense and set their own size.

Acknowledgements

We thank F. Neumann and F. Cross for insightful comments on the manuscript. We thank A. Doncic for the images used in Figure 1. JS was funded by the Burroughs Wellcome Fund, the National Institutes of Health (GM092925), and the National Science Foundation (CAREER award #1054025). JT is supported by NIH training grant GM007276 and JE by a Swiss National Science Foundation Postdoctoral Fellowship (SNF PBEZP3-134441).

References

1. Thompson, D.A.W., and Whyte, L.L. (1942). On Growth and Form (Cambridge: Cambridge University Press).
2. Payne, J.L., Groves, J.R., Jost, A.B., Nguyen, T., Myhre, S., Hill, T.M., and Skotheim, J.S. (2012) Late Paleozoic fusulinoidean gigantism driven by atmospheric hyperoxia. Evolution, in press.
3. Marshall, W.F. (2011). Origins of cellular geometry. BMC Biol. *9*, 57–65.
4. Chan, Y.-H.M., and Marshall, W.F. (2010). Scaling properties of cell and organelle size. Organogenesis *6*, 88–96.
5. Gregory, T.R. (2001). Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol. Rev. Camb. Philos. Soc. *76*, 65–101.
6. Cavalier-Smith, T. (2005). Economy, speed and size matter: evolutionary forces driving nuclear genome miniaturization and expansion. Ann. Bot. *95*, 147–175.
7. Petrov, D.A. (2002). Mutational equilibrium model of genome size evolution. Theor. Popul. Biol. *61*, 531–544.
8. Neumann, F.R., and Nurse, P. (2007). Nuclear size control in fission yeast. J. Cell Biol. *179*, 593–600.
9. Jorgensen, P., Edgington, N.P., Schneider, B.L., Rupes, I., Tyers, M., and Futcher, B. (2007). The size of the nucleus increases as yeast cells grow. Mol. Biol. Cell *18*, 3523–3532.
10. Mortimer, R.K. (1958). Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of Saccharomyces cerevisiae. Radiat. Res. *9*, 312–326.
11. Dobi, K.C., and Winston, F. (2007). Analysis of transcriptional activation at a distance in Saccharomyces cerevisiae. Mol. Cell. Biol. *27*, 5575–5586.
12. Mitchison, J.M. (1971). The Biology of the Cell Cycle (Cambridge University Press).
13. Adolph, E.F. (1931). The Regulation of Size as Illustrated in Unicellular Organisms (Springfield, IL; Baltimore, MD: C.C. Thomas).
14. Prescott, D.M. (1956). Relation between cell growth and cell division. III. Changes in nuclear volume and growth rate and prevention of cell division in Amoeba proteus resulting from cytoplasmic amputations. Exp. Cell Res. *11*, 94–98.
15. Johnston, G.C., Pringle, J.R., and Hartwell, L.H. (1977). Coordination of growth with cell division in the yeast Saccharomyces cerevisiae. Exp. Cell Res. *105*, 79–98.
16. Sveiczer, A., Novak, B., and Mitchison, J.M. (1996). The size control of fission yeast revisited. J. Cell Sci. *109*, 2947–2957.
17. Di Talia, S., Skotheim, J.M., Bean, J.M., Siggia, E.D., and Cross, F.R. (2007). The effects of molecular noise and size control on variability in the budding yeast cell cycle. Nature *448*, 947–951.
18. Mitchison, J.M. (2003). Growth during the cell cycle. Int. Rev. Cytol. *226*, 165–258.
19. Mitchison, J.M., and Creanor, J. (1971). Induction synchrony in the fission yeast. Schizosaccharomyces pombe. Exp. Cell Res. *67*, 368–374.
20. Conlon, I., and Raff, M. (2003). Differences in the way a mammalian cell and yeast cells coordinate cell growth and cell-cycle progression. J. Biol. *2*, 7.
21. Tyson, J.J., and Hannsgen, K.B. (1985). Global asymptotic stability of the size distribution in probabilistic models of the cell cycle. J. Math. Biol. *22*, 61–68.
22. Elliott, S.G., and McLaughlin, C.S. (1978). Rate of macromolecular synthesis through the cell cycle of the yeast Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *75*, 4384–4388.
23. Godin, M., Delgado, F.F., Son, S., Grover, W.H., Bryan, A.K., Tzur, A., Jorgensen, P., Payer, K., Grossman, A.D., Kirschner, M.W., *et al*. (2010). Using buoyant mass to measure the growth of single cells. Nat. Methods *7*, 387–390.
24. Goranov, A.I., Cook, M., Ricicova, M., Ben-Ari, G., Gonzalez, C., Hansen, C., Tyers, M., and Amon, A. (2009). The rate of cell growth is governed by cell cycle stage. Genes Dev. *23*, 1408–1422.
25. Jorgensen, P., Nishikawa, J.L., Breitkreutz, B.-J., and Tyers, M. (2002). Systematic identification of pathways that couple cell growth and division in yeast. Science *297*, 395–400.
26. Lord, P.G., and Wheals, A.E. (1981). Variability in individual cell cycles of Saccharomyces cerevisiae. J. Cell Sci. *50*, 361–376.
27. Zhurinsky, J., Leonhard, K., Watt, S., Marguerat, S., Bähler, J., and Nurse, P. (2010). A coordinated global control over cellular transcription. Curr. Biol. *20*, 2010–2015.
28. Nurse, P. (1975). Genetic control of cell size at cell division in yeast. Nature *256*, 547–551.
29. Fantes, P., and Nurse, P. (1977). Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. Exp. Cell Res. *107*, 377–386.
30. Nurse, P., and Thuriaux, P. (1977). Controls over the timing of DNA replication during the cell cycle of fission yeast. Exp. Cell Res. *107*, 365–375.
31. Carlson, C.R., Grallert, B., Stokke, T., and Boye, E. (1999). Regulation of the start of DNA replication in Schizosaccharomyces pombe. J. Cell Sci. *112*, 939–946.
32. Forsburg, S.L., and Nurse, P. (1991). Identification of a G1-type cyclin puc1+ in the fission yeast Schizosaccharomyces pombe. Nature *351*, 245–248.
33. Forsburg, S.L., and Nurse, P. (1994). Analysis of the Schizosaccharomyces pombe cyclin puc1: evidence for a role in cell cycle exit. J. Cell Sci. *107*, 601–613.
34. Martín-Castellanos, C., Blanco, M.A., de Prada, J.M., and Moreno, S. (2000). The puc1 cyclin regulates the G1 phase of the fission yeast cell cycle in response to cell size. Mol. Biol. Cell *11*, 543–554.
35. Hartwell, L.H., and Unger, M.W. (1977). Unequal division in Saccharomyces cerevisiae and its implications for the control of cell division. J. Cell Biol. *75*, 422–435.
36. Lord, P.G., and Wheals, A.E. (1983). Rate of cell cycle initiation of yeast cells when cell size is not a rate-determining factor. J. Cell Sci. *59*, 183–201.
37. Colman-Lerner, A., Chin, T.E., and Brent, R. (2001). Yeast Cbk1 and Mob2 activate daughter-specific genetic programs to induce asymmetric cell fates. Cell *107*, 739–750.
38. Weiss, E.L., Kurischko, C., Zhang, C., Shokat, K., Drubin, D.G., and Luca, F.C. (2002). The Saccharomyces cerevisiae Mob2p-Cbk1p kinase complex promotes polarized growth and acts with the mitotic exit network to facilitate daughter cell-specific localization of Ace2p transcription factor. J. Cell Biol. *158*, 885–900.
39. Cosma, M.P. (2004). Daughter-specific repression of Saccharomyces cerevisiae HO: Ash1 is the commander. EMBO Rep. *5*, 953–957.
40. Di Talia, S., Wang, H., Skotheim, J.M., Rosebrock, A.P., Futcher, B., and Cross, F.R. (2009). Daughter-specific transcription factors regulate cell size control in budding yeast. PLoS Biol. *7*, e1000221.
41. Laabs, T.L., Markwardt, D.D., Slattery, M.G., Newcomb, L.L., Stillman, D.J., and Heideman, W. (2003). ACE2 is required for daughter cell-specific G1 delay in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *100*, 10275–10280.
42. Harvey, S.L., and Kellogg, D.R. (2003). Conservation of mechanisms controlling entry into mitosis: budding yeast wee1 delays entry into mitosis and is required for cell size control. Curr. Biol. *13*, 264–275.
43. McNulty, J.J., and Lew, D.J. (2005). Swe1p responds to cytoskeletal perturbation, not bud size, in S. cerevisiae. Curr. Biol. *15*, 2190–2198.

44. Howell, A.S., and Lew, D.J. (2012). Morphogenesis and the cell cycle. Genetics *190*, 51–77.
45. Donnan, L., and John, P.C. (1983). Cell cycle control by timer and sizer in Chlamydomonas. Nature *304*, 630–633.
46. Wang, P., Hayden, S., and Masui, Y. (2000). Transition of the blastomere cell cycle from cell size-independent to size-dependent control at the midblastula stage in Xenopus laevis. J. Exp. Zool. *287*, 128–144.
47. Mitchison, J.M. (1957). The growth of single cells. I. Schizosaccharomyces pombe. Exp. Cell Res. *13*, 244–262.
48. Hartwell, L.H. (1970). Periodic density fluctuation during the yeast cell cycle and the selection of synchronous cultures. J. Bacteriol. *104*, 1280–1285.
49. Bryan, A.K., Goranov, A., Amon, A., and Manalis, S.R. (2010). Measurement of mass, density, and volume during the cell cycle of yeast. Proc. Natl. Acad. Sci. USA *107*, 999–1004.
50. Moseley, J.B., Mayeux, A., Paoletti, A., and Nurse, P. (2009). A spatial gradient coordinates cell size and mitotic entry in fission yeast. Nature *459*, 857–860.
51. Martin, S.G., and Berthelot-Grosjean, M. (2009). Polar gradients of the DYRK-family kinase Pom1 couple cell length with the cell cycle. Nature *459*, 852–856.
52. Russell, P., and Nurse, P. (1987). Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. Cell *49*, 559–567.
53. Gautier, J., Solomon, M.J., Booher, R.N., Bazan, J.F., and Kirschner, M.W. (1991). cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2. Cell *67*, 197–211.
54. Gould, K.L., and Nurse, P. (1989). Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis. Nature *342*, 39–45.
55. Nurse, P. (1990). Universal control mechanism regulating onset of M-phase. Nature *344*, 503–508.
56. Coleman, T.R., Tang, Z., and Dunphy, W.G. (1993). Negative regulation of the wee1 protein kinase by direct action of the nim1/cdr1 mitotic inducer. Cell *72*, 919–929.
57. Parker, L.L., Walter, S.A., Young, P.G., and Piwnica-Worms, H. (1993). Phosphorylation and inactivation of the mitotic inhibitor Wee1 by the nim1/cdr1 kinase. Nature *363*, 736–738.
58. Wu, L., and Russell, P. (1993). Nim1 kinase promotes mitosis by inactivating Wee1 tyrosine kinase. Nature *363*, 738–741.
59. Breeding, C.S., Hudson, J., Balasubramanian, M.K., Hemmingsen, S.M., Young, P.G., and Gould, K.L. (1998). The cdr2+ gene encodes a regulator of G2/M progression and cytokinesis in Schizosaccharomyces pombe. Mol. Biol. Cell *9*, 3399–3415.
60. Kanoh, J., and Russell, P. (1998). The protein kinase Cdr2, related to Nim1/Cdr1 mitotic inducer, regulates the onset of mitosis in fission yeast. Mol. Biol. Cell *9*, 3321–3334.
61. Bähler, J., and Pringle, J.R. (1998). Pom1p, a fission yeast protein kinase that provides positional information for both polarized growth and cytokinesis. Genes Dev. *12*, 1356–1370.
62. Celton-Morizur, S., Racine, V., Sibarita, J.B., and Paoletti, A. (2006). Pom1 kinase links division plane position to cell polarity by regulating Mid1p cortical distribution. J. Cell Sci. *119*, 4710–4718.
63. Morrell, J.L., Nichols, C.B., and Gould, K.L. (2004). The GIN4 family kinase, Cdr2p, acts independently of septins in fission yeast. J. Cell Sci. *117*, 5293–5302.
64. Hachet, O., Berthelot-Grosjean, M., Kokkoris, K., Vincenzetti, V., Moosbrugger, J., and Martin, S.G. (2011). A phosphorylation cycle shapes gradients of the DYRK family kinase Pom1 at the plasma membrane. Cell *145*, 1116–1128.
65. Padte, N.N., Marting, S.G., Howard, M., and Chang, F. (2006). The cell-end factor Pom1p inhibits Mid1p in specification of the cell division plane in fission yeast. Curr. Biol. *16*, 2480–2487.
66. Saunders, T., Pan, K., Angel, A., Guan, Y., Shah, J., Howard, M., and Chang, F. (2012). Noise reduction in the intracellular pom1p gradient by a dynamic clustering mechanism. Dev. Cell, in press.
67. Sveiczer, A., Tyson, J.J., and Novak, B. (2001). A stochastic, molecular model of the fission yeast cell cycle: role of the nucleocytoplasmic ratio in cycle time regulation. Biophys. Chem. *92*, 1–15.
68. Young, P.G., and Fantes, P.A. (1987). Schizosaccharomyces pombe mutants affected in their division response to starvation. J Cell Sci. *88*, 295–304.
69. Belenguer, P., Pelloquin, L., Oustrin, M.L., and Ducommun, B. (1997). Role of the fission yeast nim 1 protein kinase in the cell cycle response to nutritional signals. Biochem. Biophys. Res. Commun. *232*, 204–208.
70. Moreno, S., Nurse, P., and Russell, P. (1990). Regulation of mitosis by cyclic accumulation of p80cdc25 mitotic inducer in fission yeast. Nature *344*, 549–552.
71. Kovelman, R., and Russell, P. (1996). Stockpiling of Cdc25 during a DNA replication checkpoint arrest in Schizosaccharomyces pombe. Mol. Cell. Biol. *16*, 86–93.
72. Rupes, I., Webb, B.A., Mak, A., and Young, P.G. (2001). G2/M arrest caused by actin disruption is a manifestation of the cell size checkpoint in fission yeast. Mol. Biol. Cell. *12*, 3892–3903.
73. Daga, R.R., and Jimenez, J. (1999). Translational control of the cdc25 cell cycle phosphatase: a molecular mechanism coupling mitosis to cell growth. J. Cell Sci. *112*, 3137–3146.
74. Coudreuse, D., and Nurse, P. (2010). Driving the cell cycle with a minimal CDK control network. Nature *468*, 1074–1079.
75. Chen, Y.E., Tropini, C., Jonas, K., Tsokos, C.G., Huang, K.C., and Laub, M.T. (2011). Spatial gradient of protein phosphorylation underlies replicative asymmetry in a bacterium. Proc. Natl. Acad. Sci. USA *108*, 1052–1057.
76. Corbin, B.D., Yu, X.-C., and Margolin, W. (2002). Exploring intracellular space: function of the Min system in round-shaped Escherichia coli. EMBO J. *21*, 1998–2008.
77. Huang, K.C., and Wingreen, N.S. (2004). Min-protein oscillations in round bacteria. Phys. Biol. *1*, 229–235.
78. Nair, D.R., D'Ausilio, C.A., Occhipinti, P., Borsuk, M.E., and Gladfelter, A.S. (2010). A conserved G_1 regulatory circuit promotes asynchronous behavior of nuclei sharing a common cytoplasm. Cell Cycle *9*, 3771–3779.
79. Donachie, W.D. (1968). Relationship between cell size and time of initiation of DNA replication. Nature *219*, 1077–1079.
80. Rossow, P.W., Riddle, V.G., and Pardee, A.B. (1979). Synthesis of labile, serum-dependent protein in early G1 controls animal cell growth. Proc. Natl. Acad. Sci. USA *76*, 4446–4450.
81. Brooks, R.F. (1977). Continuous protein synthesis is required to maintain the probability of entry into S phase. Cell *12*, 311–317.
82. Unger, M.W., and Hartwell, L.H. (1976). Control of cell division in Saccharomyces cerevisiae by methionyl-tRNA. Proc. Natl. Acad. Sci. USA *73*, 1664–1668.
83. Fantes, P.A., Grant, W.D., Pritchard, R.H., Sudbery, P.E., and Wheals, A.E. (1975). The regulation of cell size and the control of mitosis. J. Theor. Biol. *50*, 213–244.
84. Popolo, L., Vanoni, M., and Alberghina, L. (1982). Control of the yeast cell cycle by protein synthesis. Exp. Cell Res. *142*, 69–78.
85. Moore, S.A. (1988). Kinetic evidence for a critical rate of protein synthesis in the Saccharomyces cerevisiae yeast cell cycle. J. Biol. Chem. *263*, 9674–9681.
86. Jorgensen, P., Rupes, I., Sharom, J.R., Schneper, L., Broach, J.R., and Tyers, M. (2004). A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes Dev. *18*, 2491–2505.
87. Kief, D.R., and Warner, J.R. (1981). Coordinate control of syntheses of ribosomal ribonucleic acid and ribosomal proteins during nutritional shift-up in Saccharomyces cerevisiae. Mol. Cell. Biol. *1*, 1007–1015.
88. Polanshek, M.M. (1977). Effects of heat shock and cycloheximide on growth and division of the fission yeast, Schizosaccharomyces pombe. With an Appendix. Estimation of division delay for S. pombe from cell plate index curves. J. Cell Sci. *23*, 1–23.
89. Shilo, B., Riddle, V.G., and Pardee, A.B. (1979). Protein turnover and cell-cycle initiation in yeast. Exp. Cell Res. *123*, 221–227.
90. Schneider, B.L., Zhang, J., Markwardt, J., Tokiwa, G., Volpe, T., Honey, S., and Futcher, B. (2004). Growth rate and cell size modulate the synthesis of, and requirement for, G1-phase cyclins at start. Mol. Cell. Biol. *24*, 10802–10813.
91. Schneiderman, M.H., Dewey, W.C., and Highfield, D.P. (1971). Inhibition of DNA synthesis in synchronized Chinese hamster cells treated in G1 with cycloheximide. Exp. Cell Res. *67*, 147–155.
92. Nash, R., Tokiwa, G., Anand, S., Erickson, K., and Futcher, A.B. (1988). The WHI1+ gene of Saccharomyces cerevisiae tethers cell division to cell size and is a cyclin homolog. EMBO J. *7*, 4335–4346.
93. Cross, F.R. (1988). DAF1, a mutant gene affecting size control, pheromone arrest, and cell cycle kinetics of Saccharomyces cerevisiae. Mol. Cell. Biol. *8*, 4675–4684.
94. Tyers, M., Tokiwa, G., Nash, R., and Futcher, B. (1992). The Cln3-Cdc28 kinase complex of S. cerevisiae is regulated by proteolysis and phosphorylation. EMBO J. *11*, 1773–1784.
95. Tyers, M., Fitch, I., Tokiwa, G., Dahmann, C., Nash, R., Linskens, M., and Futcher, B. (1991). Characterization of G1 and mitotic cyclins of budding yeast. Cold Spring Harb. Symp. Quant. Biol. *56*, 21–32.
96. Tyers, M., Tokiwa, G., and Futcher, B. (1993). Comparison of the Saccharomyces cerevisiae G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. EMBO J. *12*, 1955–1968.
97. McInerny, C.J., Partridge, J.F., Mikesell, G.E., Creemer, D.P., and Breeden, L.L. (1997). A novel Mcm1-dependent element in the SWI4, CLN3, CDC6, and CDC47 promoters activates M/G1-specific transcription. Genes Dev. *11*, 1277–1288.
98. MacKay, V.L., Mai, B., Waters, L., and Breeden, L.L. (2001). Early cell cycle box-mediated transcription of CLN3 and SWI4 contributes to the proper timing of the G1-to-S transition in budding yeast. Mol. Cell. Biol. *21*, 4140–4148.
99. Cavalier-Smith, T. (1982). Skeletal DNA and the evolution of genome size. Annu. Rev. Biophys. Bioeng. *11*, 273–302.
100. Futcher, B. (1996). Cyclins and the wiring of the yeast cell cycle. Yeast *12*, 1635–1646.

101. de Bruin, R.A., McDonald, W.H., Kalashnikova, T.I., Yates, J., and Wittenberg, C. (2004). Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5. Cell *117*, 887–898.

102. Costanzo, M., Nishikawa, J.L., Tang, X., Millman, J.S., Schub, O., Breitkreuz, K., Dewar, D., Rupes, I., Andrews, B., and Tyers, M. (2004). CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast. Cell *117*, 899–913.

103. Miller, M.E., and Cross, F.R. (2000). Distinct subcellular localization patterns contribute to functional specificity of the Cln2 and Cln3 cyclins of Saccharomyces cerevisiae. Mol. Cell. Biol. *20*, 542–555.

104. Edgington, N.P., and Futcher, B. (2001). Relationship between the function and the location of G1 cyclins in S. cerevisiae. J. Cell Sci. *114*, 4599–4611.

105. Wang, H., Carey, L.B., Cai, Y., Wijnen, H., and Futcher, B. (2009). Recruitment of Cln3 cyclin to promoters controls cell cycle entry via histone deacetylase and other targets. PLoS Biol. *7*, e1000189.

106. Hartwell, L.H., Culotti, J., Pringle, J.R., and Reid, B.J. (1974). Genetic control of the cell division cycle in yeast. Science *183*, 46–51.

107. Ferrell, J.E. (1996). Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. Trends Biochem. Sci. *21*, 460–466.

108. Ferrell, J.E., and Machleder, E.M. (1998). The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes. Science *280*, 895–898.

109. Justman, Q.A., Serber, Z., Ferrell, J.E., El-Samad, H., and Shokat, K.M. (2009). Tuning the activation threshold of a kinase network by nested feedback loops. Science *324*, 509–512.

110. Doncic, A., Falleur-Fettig, M., and Skotheim, J.M. (2011). Distinct interactions select and maintain a specific cell fate. Mol. Cell *43*, 528–539.

111. Nasmyth, K., and Dirick, L. (1991). The role of SWI4 and SWI6 in the activity of G1 cyclins in yeast. Cell *66*, 995–1013.

112. Dirick, L., and Nasmyth, K. (1991). Positive feedback in the activation of G1 cyclins in yeast. Nature *351*, 754–757.

113. Cross, F.R., and Tinkelenberg, A.H. (1991). A potential positive feedback loop controlling CLN1 and CLN2 gene expression at the start of the yeast cell cycle. Cell *65*, 875–883.

114. Skotheim, J.M., Di Talia, S., Siggia, E.D., and Cross, F.R. (2008). Positive feedback of G1 cyclins ensures coherent cell cycle entry. Nature *454*, 291–296.

115. Wang, H., Garí, E., Vergés, E., Gallego, C., and Aldea, M. (2004). Recruitment of Cdc28 by Whi3 restricts nuclear accumulation of the G1 cyclin-Cdk complex to late G1. EMBO J. *23*, 180–190.

116. Vergés, E., Colomina, N., Garí, E., Gallego, C., and Aldea, M. (2007). Cyclin Cln3 is retained at the ER and released by the J chaperone Ydj1 in late G1 to trigger cell cycle entry. Mol. Cell *26*, 649–662.

117. Polymenis, M., and Schmidt, E.V. (1997). Coupling of cell division to cell growth by translational control of the G1 cyclin CLN3 in yeast. Genes Dev. *11*, 2522–2531.

118. Andrews, B.J., and Herskowitz, I. (1989). Identification of a DNA binding factor involved in cell-cycle control of the yeast HO gene. Cell *57*, 21–29.

119. Koch, C., Moll, T., Neuberg, M., Ahorn, H., and Nasmyth, K. (1993). A role for the transcription factors Mbp1 and Swi4 in progression from G1 to S phase. Science *261*, 1551–1557.

120. Ferrezuelo, F., Colomina, N., Futcher, B., and Aldea, M. (2010). The transcriptional network activated by Cln3 cyclin at the G1-to-S transition of the yeast cell cycle. Genome Biol. *11*, R67.

121. Wijnen, H., Landman, A., and Futcher, B. (2002). The G(1) cyclin Cln3 promotes cell cycle entry via the transcription factor Swi6. Mol. Cell. Biol. *22*, 4402–4418.

122. Eser, U., Falleur-Fettig, M., Johnson, A., and Skotheim, J.M. (2011). Commitment to a cellular transition precedes genome-wide transcriptional change. Mol. Cell *43*, 515–527.

123. Brauer, M.J., Huttenhower, C., Airoldi, E.M., Rosenstein, R., Matese, J.C., Gresham, D., Boer, V.M., Troyanskaya, O.G., and Botstein, D. (2008). Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast. Mol. Biol. Cell *19*, 352–367.

124. Baroni, M.D., Monti, P., and Alberghina, L. (1994). Repression of growth-regulated G1 cyclin expression by cyclic AMP in budding yeast. Nature *371*, 339–342.

125. Flick, K., Chapman-Shimshoni, D., Stuart, D., Guaderrama, M., and Wittenberg, C. (1998). Regulation of cell size by glucose is exerted via repression of the CLN1 promoter. Mol. Cell. Biol. *18*, 2492–2501.

126. Cipollina, C., van den Brink, J., Daran-Lapujade, P., Pronk, J.T., Porro, D., and de Winde, J.H. (2008). Saccharomyces cerevisiae SFP1: at the crossroads of central metabolism and ribosome biogenesis. Microbiology *154*, 1686–1699.

127. Petersen, J., and Nurse, P. (2007). TOR signalling regulates mitotic commitment through the stress MAP kinase pathway and the Polo and Cdc2 kinases. Nat. Cell Biol. *9*, 1263–1272.

128. Costello, G., Rodgers, L., and Beach, D. (1986). Fission yeast enters the stationary phase G0 state from either mitotic G1 or G2. Curr. Genet. *11*, 119–125.

129. Pluskal, T., Hayashi, T., Saitoh, S., Fujisawa, A., and Yanagida, M. (2011). Specific biomarkers for stochastic division patterns and starvation-induced quiescence under limited glucose levels in fission yeast. FEBS J. *278*, 1299–1315.

130. Jorgensen, P., and Tyers, M. (2004). How cells coordinate growth and division. Curr. Biol. *14*, R1014–R1027.

131. De Virgilio, C., and Loewith, R. (2006). The TOR signalling network from yeast to man. Int. J. Biochem. Cell Biol. *38*, 1476–1481.

132. Otsubo, Y., and Yamamato, M. (2008). TOR signaling in fission yeast. Crit. Rev. Biochem. Mol. Biol. *43*, 277–283.

133. Zaman, S., Lippman, S.I., Zhao, X., and Broach, J.R. (2008). How Saccharomyces responds to nutrients. Annu. Rev. Genet. *42*, 27–81.

134. Smets, B., Ghillebert, R., De Snijder, P., Binda, M., Swinnen, E., De Virgilio, C., and Winderickx, J. (2010). Life in the midst of scarcity: adaptations to nutrient availability in Saccharomyces cerevisiae. Curr. Genet. *56*, 1–32.

135. Loewith, R., and Hall, M.N. (2011). Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics *189*, 1177–1201.

136. Yanagida, M., Ikai, N., Shimanuki, M., and Sajiki, K. (2011). Nutrient limitations alter cell division control and chromosome segregation through growth-related kinases and phosphatases. Philos. Trans. R. Soc. Lond., B *366*, 3508–3520.

137. Shiozaki, K. (2009). Nutrition-minded cell cycle. Sci. Signaling *2*, pe74.

138. Lempiäinen, H., and Shore, D. (2009). Growth control and ribosome biogenesis. Curr. Opin. Cell Biol. *21*, 855–863.

139. Urban, J., Soulard, A., Huber, A., Lippman, S., Mukhopadhyay, D., Deloche, O., Wanke, V., Anrather, D., Ammerer, G., Riezman, H., *et al*. (2007). Sch9 is a major target of TORC1 in Saccharomyces cerevisiae. Mol. Cell *26*, 663–674.

140. Lempiäinen, H., Uotila, A., Urban, J., Dohnal, I., Ammerer, G., Loewith, R., and Shore, D. (2009). Sfp1 interaction with TORC1 and Mrs6 reveals feedback regulation on TOR signaling. Mol. Cell *33*, 704–716.

141. Singh, J., and Tyers, M. (2009). A Rab escort protein integrates the secretion system with TOR signaling and ribosome biogenesis. Genes Dev. *23*, 1944–1958.

142. Rudra, D., Zhao, Y., and Warner, J.R. (2005). Central role of Ifh1p-Fhl1p interaction in the synthesis of yeast ribosomal proteins. EMBO J. *24*, 533–542.

143. Huber, A., French, S.L., Tekotte, H., Yerlikaya, S., Stahl, M., Perepelkina, M.P., Tyers, M., Rougemont, J., Beyer, A.L., and Loewith, R. (2011). Sch9 regulates ribosome biogenesis via Stb3, Dot6 and Tod6 and the histone deacetylase complex RPD3L. EMBO J. *30*, 3052–3064.

144. Bernstein, K.A., Bleichert, F., Bean, J.M., Cross, F.R., and Baserga, S.J. (2007). Ribosome biogenesis is sensed at the Start cell cycle checkpoint. Mol. Biol. Cell *18*, 953–964.

145. Martin, D.E., Soulard, A., and Hall, M.N. (2004). TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. Cell *119*, 969–979.

146. Newcomb, L.L., Diderich, J.A., Slattery, M.G., and Heideman, W. (2003). Glucose regulation of Saccharomyces cerevisiae cell cycle genes. Eukaryot. Cell *2*, 143–149.

147. Cai, L., and Tu, B.P. (2011). Acetyl-CoA drives the transcriptional growth program in yeast. Cell Cycle *10*, 354–346.

148. Cai, L., Sutter, B.M., Li, B., and Tu, B.P. (2011). Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol. Cell *42*, 426–437.

149. Futcher, B. (2006). Metabolic cycle, cell cycle, and the finishing kick to Start. Genome Biol. *7*, 107.

150. Tokiwa, G., Tyers, M., Volpe, T., and Futcher, B. (1994). Inhibition of G1 cyclin activity by the Ras/cAMP pathway in yeast. Nature *371*, 342–345.

151. Cross, F.R., Buchler, N.E., and Skotheim, J.M. (2011). Evolution of networks and sequences in eukaryotic cell cycle control. Philos. Trans. R. Soc. Lond., B, Biol. Sci. *366*, 3532–3544.

152. Coulter, W. (1953). Means for counting particles suspended in a fluid. US Patent no 2656508.

153. Zhang, J., Schneider, C., Ottmers, L., Rodriguez, R., Day, A., Markwardt, J., and Schneider, B.L. (2002). Genomic scale mutant hunt identifies cell size homeostasis genes in S. cerevisiae. Curr. Biol. *12*, 1992–2001.

154. Herzenberg, L.A., Parks, D., Sahaf, B., Perez, O., Roederer, M., and Herzenberg, L.A. (2002). The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford. Clin. Chem. *48*, 1819–1827.

155. Cipollina, C., Vai, M., Porro, D., and Hatzis, C. (2007). Towards understanding of the complex structure of growing yeast populations. J. Biotechnol. *128*, 393–402.

156. Gordon, A., Colman-Lerner, A., Chin, T.E., Benjamin, K.R., Yu, R.C., and Brent, R. (2007). Single-cell quantification of molecules and rates using open-source microscope-based cytometry. Nat. Methods *4*, 175–181.

157. Popescu, G., Park, Y., Lue, N., Best-Popescu, C., Deflores, L., Dasari, R.R., Feld, M.S., and Badizadegan, K. (2008). Optical imaging of cell mass and growth dynamics. Am. J. Physiol. Cell Physiol. *295*, C538–C544.

158. Wang, Z., Millet, L., Mir, M., Ding, H., Unarunotai, S., Rogers, J., Gillette, M.U., and Popescu, G. (2011). Spatial light interference microscopy (SLIM). Opt. Express *19*, 1016–1026.

159. Barer, R. (1952). Interference microscopy and mass determination. Nature *169*, 366–367.