Checking cell size in yeast

Ivan Rupeš

To remain viable, cells have to coordinate cell growth with cell division. In yeast, this occurs at two control points: the boundaries between G1 and S phases, also known as Start, and between G2 and M phases. Theoretically, coordination can be achieved by independent regulation of growth and division, or by participation of surveillance mechanisms in which cell size feeds back into cell-cycle control. This article discusses recent advances in the identification of size-control modules in the two species, and how these mechanisms integrate with environmental stimuli. A comparison of the G1-S and G2-M size-control modules in the two species reveals a degree of conservation higher than previously thought. This reinforces the notion that internal sizing could be a conserved feature of cell-cycle control throughout eukaryotes.

Published online: 25 July 2002

How does a cell control its size? Two schools of thought exist: either a cell divides after it reaches a certain critical size, or cell growth and proliferation are regulated independently, with cell size emerging from a simple correlation of the two [1–5]. The most important difference between the two hypotheses is that cell size feeds back into the cell-cycle regulatory system in the former but not in the latter (Fig. 1a). Thus, the validity of the critical size theory depends on the existence of a ‘sizer’ – a molecule or set of molecules whose activity correlates with cell size. Nevertheless, the sizer is only one component that determines when cell division occurs; in addition, the extracellular environment influences the timing of the response to the changing activity of the sizer.

Two species of yeast, the budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe, provide genetic models in which to study cell-cycle control. The evolutionary divergence of these organisms is about the same as that between each of them and human [6]. Both yeast share cell-cycle characteristics with higher eukaryotes, such as G1, S, G2 and M phases, cyclin-dependent kinases (CDKs) and checkpoint controls [7–9]. Owing to the uncertain supply of nutrients in the wild, yeast cell-division rates must be coordinated with widely variable rates of cell growth, otherwise cells would get progressively smaller or larger. Although cell size is reduced in less favorable growth conditions, the range of growth rates exceeds the corresponding range of cell sizes for both yeast. Thus, a relationship between growth and division is a fact of life for yeast cells. The early studies have presented a compelling case for the existence of a critical size that is a prerequisite for progression through the cell cycle. In budding yeast, cell division is asymmetrical and produces cells of unequal size. To compensate for this asymmetry, which becomes more pronounced with increasing nutrient limitation, new daughter cells grow more before division than the mother cells. This additional growth occurs almost entirely in G1, before the reference point known as Start. Once Start is passed, the rest of the cell cycle is relatively constant in length [10] (Fig. 1b).

In fission yeast, G2-M is the primary cell-size control point [9]. The relative length of G2 varies greatly with growth conditions (Fig. 1b). Nitrogen limitation reduces cell size at division, and sudden shifts between different sources of nitrogen generate rapid acceleration or delay of mitosis in cells that are above or below the new cell-size threshold (i.e. the minimum size required for initiation of mitosis) [9]. Even during balanced growth, individual fission yeast cells can compensate for random fluctuations in their size at birth by adjusting their time spent in G2 [11].

Do fission yeast have a size control in G1, and do budding yeast have a control in G2? Start is defined in fission yeast as in budding yeast, although in favorable growth conditions it occurs almost immediately after exit from mitosis. A normally cryptic size-control point at Start is uncovered in mutants in which the G2-M size-control has collapsed and cells enter mitosis prematurely. These cells have an extended G1, suggesting that they initiate S phase only after reaching a certain minimum size [12] (Fig. 1b). Although the two yeast have traditionally been thought of as using different size-control strategies, the aim of this article is to show that they use similar mechanisms. What is different is their emphasis on the size-control points. Because these control mechanisms have been conserved over such long evolutionary distances,
it might be worth considering whether analogous systems operate in other eukaryotes.

Size control at Start: budding yeast

How does budding yeast measure its size, and how does this control Start? The G1–S size-control module is presented in Fig. 2. Its core consists of three G1 cyclins (Cln1–3) and two B-type cyclins (Clb5 and Clb6). These cyclins bind to a CDK1 homolog, Cdc28 (Table 1), and target its activity towards specific sets of substrates. The level of Cln3 is important for timely expression of the CLN1 and CLN2 genes. The resulting increase in the activity of Cln1– and Cln2–Cdc28 complexes is responsible for initiation of three major post-Start events: budding, spindle-pole duplication and, indirectly through
release of the inhibition of Clb5- and Clb6-Cdc28 by the CDK inhibitor Sic1, DNA replication [8,13].

Cln3 functions to coordinate Start with cell growth and the supply of nutrients. The level of Cln3 varies dramatically with the available carbon [14] or nitrogen [15] source. The glucose-response elements in the CLN3 promoter are a target for Azt1, a transcription factor that stimulates CLN3 transcription in the presence of glucose [16,17]. Cln3, therefore, is associated with process 1 in Fig. 1a. Thus, Cln3 is also associated with process 3 in Fig. 1a. The translation efficiency of CLN3 is further controlled by the activity of the eukaryotic initiation factor (eIF) 4F mRNA-cap-binding complex. This complex appears to be a target for the target of rapamycin (TOR) phosphatidylinositol kinase pathway [19,20] and perhaps also for the cAMP-dependent protein kinase pathway [14]. Both these pathways are involved in nutritional sensing and affect several metabolic and growth-related functions, thus coordinating processes 1 and 2 (Fig. 1a). Finally, the Whi3 protein restricts Cln3 synthesis by localizing CLN3 mRNA to specific cytoplasmic loci with a possible function in nutritional sensing (Ref. [21] and B. Futcher, pers. commun.).

Is Cln3 a sizer? Cln3 is a low-abundance, constitutively unstable protein [22,23] and, therefore, its level depends primarily on its rate of synthesis. According to one model [13], the rate of Cln3 synthesis per cell increases with the volume of cytoplasm. Because Cln3 localizes to the nucleus [24,25], assuming that nuclear volume depends on the amount of DNA present, the effective concentration of Cln3 in G1 rises with increasing cell size. When a threshold level is reached, which takes longer in smaller daughter cells than in mother cells, suggesting that their timing might be linked to cell size [30]. However, it is not yet clear whether the timing of the peaks is crucial or whether the cln3ecb swi4ecb double-mutant cells delay Start simply because they fail to produce sufficient levels of Cln3 and Swi4.

If cells can sense their size, at least in part, through Cln3, what sets the critical threshold of its activity? Clearly, the threshold varies in different growth conditions. Part of the problem is that the exact link between Cln3 and Swi6–Swi4 is not known [31,32]. What is known, however, is that both synthesis and degradation of Cln1 and Cln2 are modulated by several other pathways. Somewhat counter-intuitively, the burst of CLN1 and CLN2 transcription is delayed in rich media, resulting in a larger size at Start. This response is, at least in part, mediated by cAMP [33,34]. Addition of glucose or cAMP represses transcription of CLN1, and this is sufficient to delay Start [35]. In addition, Grr1, a protein that promotes degradation of Cln1 and Cln2, is activated in response to glucose [36,37]. This further reduces the levels of Cln1 and Cln2 in cells. This type of regulation allows the response of Cln1 and Cln2 to be sensitized or desensitized to Cln3 activity. Cln1 and Cln2 thus make a specific contribution to the integration of environmental signals within the G1–S module (Fig. 1a, process 1) and, therefore, to the setting of a critical size.

**Size control at Start: fission yeast**

In fission yeast, Start is initiated by a CDK1 homolog, Cdc2, in association with a G1 cyclin, Puc1, and three B-type cyclins, Cig1, Cig2 and Cdc13. Two inhibitory mechanisms prevent premature initiation of S phase: one involves a CDK inhibitor, Rum1, and the other involves a component of the ubiquitin proteolysis machinery, Ste9. The wiring diagram reveals striking similarity between fission yeast and budding yeast (Fig. 3, Table 1) [9]. As already mentioned, the fission yeast G1–S size control is cryptic when cells grow in favorable conditions. Under limiting conditions, however, G1 is extended to a varying degree, with no simple correlation to G2–M regulation. This suggests that, as in budding yeast, there might be a minimum size requirement for progression through Start that varies with external conditions [38]. How cell size impinges on the G1–S module in fission yeast is still...
largely a mystery. Puc1 alone is insufficient to initiate S phase, indicating that it functions upstream of the B-cyclins. Puc1–Cdc2 phosphorylates and destabilizes Rum1, an inhibitor of Cdc13 and Cig2–Cdc2, paralleling the action of Cln1– and Cln2–Cdc2 complexes. In G1, Rum1 inhibits Cdc13–Cdc2 and, to a lesser extent, Cig2–Cdc2. In addition, the levels of Cdc13 and Cig1 are kept low by the action of Ste9. At Start, Puc1 and Cig1 phosphorylate and destabilize Rum1, possibly with increasing contribution from Cig1 as the level of Ste9 decreases. Cdc13 phosphorylates Ste9, resulting in Ste9 degradation. This creates a negative loop that contributes to gradual accumulation of Cdc13 during S phase. Transcription of cig2 is induced by a heteromeric complex that contains a Swi6 homolog, Cdc10, and two Swi4/Mbp1 homologs, Res1 and Res2. This transcription is not rate-limiting for Start but is present in this diagram as a feature conserved between the two yeast. Coloring reflects structural relationships as indicated.

The wee1 gene was isolated as the prototype cell-size mutant that enters mitosis precociously and loses the wild-type ability to modify cell size at mitosis in response to the availability of nitrogen [9]. As cells progress through S and G2, Cdc13 slowly accumulates, but the associated Cdc2 activity remains inhibited by Wee1-dependent tyrosine phosphorylation (Fig. 4a). A Wee1 inhibitory kinase, Cdr1, is probably a component of the nutrition-sensing module (Fig. 1a, process 1) as cdr1-deficient mutants are unable to adjust cell size over a range of nitrogen concentrations [9,45]. This property is shared with a related kinase, Cdr2, which also phosphorylates Wee1 in vitro [45–47]. The exact relationship between Cdr1 and Cdr2 and their possible upstream regulators is not known. Cdr1 localizes predominantly to the cytoplasm, whereas Wee1 is present mainly in the nucleus. Controlled accessibility of the two partners, therefore, is one possible mechanism by which Cdr1 could exert control over the setting of cell size [48]. Independent of Cdr1 and Cdr2, Wee1 is upregulated by inhibition of protein synthesis [49].

To initiate mitosis, cells must reverse Cdc2 tyrosine phosphorylation. The essential phosphatase for this is Cdc25 [9] (Fig. 4a). Switch-like activation of Cdc13–Cdc2 is ensured by a positive feedback loop between Cdc2 and Cdc25 [50] and probably also by a negative loop between Cdc2 and Wee1, the latter of which is related to the Cdc2–Wee1 loop identified in other eukaryotes [51]. The level of Cdc25, as well as that of Cdc13, is correlated with general synthetic activity through translational regulation involving the 5′-UTRs of the cig2 and cdc13 mRNAs, providing a potential link between growth rate and timing of mitosis [52] (Fig. 1a, process 3).
So, which components qualify for the size-dependent process (Fig. 1a, process 4)? One possibility is that gradual accumulation of Cdc13, which destabilizes the ‘off’ state of the switch, represents a sizing mechanism [53, 54]. However, there seems to be little experimental evidence supporting this because accumulation of Cdc13 in the nucleus appears to level off long before cells enter mitosis [55]. The same seems to be true for the pre-mitotic nuclear accumulation of Cdc25 [56]. Wee1 is also an unlikely candidate as Cdc2-tyrosine phosphorylation persists even after Wee1 and a related kinase, Mkh1, have been inactivated in a conditional mutant [57, 58]. That leaves us with a direct activation of the Cdc25–Cdc2 loop. Indeed, the sizing in G2–M has recently been shown to be mediated by Cdc25. Cells that have stopped growing because of interference with the actin cytoskeleton fail to initiate mitosis if their size is below the threshold, but do progress into mitosis unperturbed if they are oversized [58]. The inhibition is lost in cells in which Cdc25 has been replaced with a constitutive Cdc2-tyrosine phosphatase activity. Conversely, cells in which the critical size has been reduced by a nutritional down-shift accelerate their entry into mitosis even if their further growth has been interrupted by actin depolymerization. These cells suddenly become oversized relative to the new size threshold [58]. The size-related upstream regulators of Cdc25 (or Cdc13–Cdc2, as the presence of the positive loop makes it difficult to distinguish between direct activation of Cdc13–Cdc2 and Cdc25) remain to be identified. However, these results demonstrate that cell size impinges on cell-cycle regulation as a cell-cycle checkpoint. The identification of the checkpoint has an important consequence: it justifies the integration model of cell-size control by closing the loop between cell size and cell-cycle progression (Fig. 1a, process 4). It shows that the activity of the sizer does not simply coincide with cell growth but is directly determined by cell size. The checkpoint function cannot be executed without previous Cdc2-tyrosine phosphorylation. Consistently, wee1-deficient mutants are unable to control their size in G2–M. Furthermore, simultaneous collapse of the G1–S module in the wee1 background, owing to deletion of rum1 or ste9, results in a loss of viability [59–61].
Size control in G2–M: budding yeast

In G2, Cdc28 is phosphorylated by the Wee1 homolog Swe1, and dephosphorylated by the Cdc25 homolog Mih1 [8] (Fig. 4b, Table 1). Unlike their counterparts in fission yeast, however, neither of these components are essential in budding yeast. It has been proposed that instead of size control, Clb2–Cdc28 tyrosine phosphorylation evolved a specialized function in execution of a morphogenesis checkpoint. The checkpoint is activated when cells fail to form a bud or when the integrity of the actin cytoskeleton is perturbed [62]. Part of this signal is believed to be mediated by inhibition of Hsl1, one of the three Cdr1 and Cdr2 homologs in budding yeast (Table 1), and its interacting partner, Hsl7. Inhibition of Hsl1 and Hsl7 is thought to result in stabilization of Swe1 and inhibition of mitosis [63]. However, a more sensitive assay revealed that Swe1 is, in fact, normally present in cells until the end of mitosis, just as in other eukaryotes [64]. This revives the question as to whether other functions of Wee1 and Cdc25 homologs, such as size control, might be conserved in budding yeast. Overexpression of SWE1 results in pre-mitotic arrest, and deletion of MIH1 causes subtle but consistent reduction of cell size (D. Kellogg, pers. commun.). This alone does not tell us whether Swe1 and Mih1 actively participate in size control, but indirect evidence strongly suggests that G2–M size control is, indeed, present in budding yeast, although it normally remains cryptic. Reversing the situation seen in fission yeast, budding yeast cells that have their G1–S size-control compromised, such as Cln3 overproducers, are small, but their generation time (i.e. time between two subsequent cell divisions) remains largely unaffected, suggesting that the second control-point becomes active and prevents catastrophic regression of cell size [65, 66]. Conversely, deletion of SWE1 causes subtle but consistent reduction of cell size (D. Kellogg, pers. commun.). This alone does not tell us whether Swe1 and Mih1 actively participate in size control, but indirect evidence strongly suggests that G2–M size control is, indeed, present in budding yeast, although it normally remains cryptic. Reversing the situation seen in fission yeast, budding yeast cells that have their G1–S size-control compromised, such as Cln3 overproducers, are small, but their generation time (i.e. time between two subsequent cell divisions) remains largely unaffected, suggesting that the second control-point becomes active and prevents catastrophic regression of cell size [65, 66]. (Fig. 1b). At the molecular level, Clb2–Cdc28 and Swe1 are engaged in a strong negative feedback loop, suggesting the presence of the same switch-like mechanism of Cdc28 activation that is conserved throughout eukaryotic cells (D. Kellogg, pers. commun.). In light of these results, the concept of a morphogenesis checkpoint in budding yeast requires reexamination to separate the specific response to failed bud formation from a simple manifestation of cell-size control. The effect of cell size has not been directly addressed in this context. It seems, however, that the inability of cells to reach a sufficient size for G2–M might explain the pre-mitotic arrest that is caused by perturbation of actin integrity, as occurs in fission yeast [58]. It would be premature to speculate about the identity of the G2–M sizer in budding yeast, but it would be intriguing to test whether some of the Cdr1 and Cdr2 homologs (Table 1) can mediate nutritional signals. If the G2–M module is conserved, what makes Mih1 non-essential? One explanation is that, in the absence of Mih1, Cdc28 inhibition is eventually overturned by continued accumulation of Clb2, activating more and more free Cdc28 molecules [23] until the switch is flipped.

Concluding remarks

What is 'cell size'? Entirely in accord with Pringle and Hartwell [10], I use this deliberately vague expression instead of more specific terms simply because we are still far from fully understanding what represents cell size within cells. Production of Cln3 is clearly dependent on the total number of ribosomes in the cytoplasm, which increases as cells grow, but other possible sizers do not offer such clear-cut solutions. Protein mass, the number of ribosomes and cell volume are obvious candidates, and one or another blend that includes these, and perhaps other structurally based determinants, might constitute an integrated measure of size. So, why did cells bother to evolve such a complicated system of size monitoring and feedback controls if, intuitively, a simple correlation of growth and proliferation could suffice? One possible answer is that cells need it to boost robustness of the cell-cycle regulatory network. There is always variation in the size at which cell–cycle transitions occur. If unchecked, the variation would increase from generation to generation until the cells at the tails of the distribution would enter territory that is incompatible with survival. The cell-size homeostasis phenomenon that prevents this from happening has been demonstrated elegantly in fission yeast [11]. Stochastic processes, to which low-abundance constituents are especially vulnerable, are inherent to biological systems. Even in eukaryotic cells, gene expression can occur in erratic bursts rather than smoothly [69]. Eroding components of the regulatory network causes increased heterogeneity in cell size and unpredictable behavior [30, 70]. This leads to the argument that cell-size feedback mechanisms or cell-size checkpoints are a conserved solution to the noise problem. They might underlie more complex mechanisms that have evolved from preexisting building blocks such as the ones that constitute processes 1–3 in Fig. 1a to ensure proper development and differentiation in multicellular organisms.

References

20 Danaie, P. 
21 Garí, E. 
22 Tyers, M. 
17 Newcomb, L.L. 
30 MacKay, V.L. 
29 McInerny, C.J. 
28 Chen, K.C. 
19 Berset, C. 
26 Dirick, L. 
11 Sveiczer, A. 
11, 2522–2531 
Saccharomyces cerevisiae 

sufficient to restore G1-to-S-phase progression in 
G1 cyclin CLN3 to modulate cell fate in budding 

yeast revisited. 

http://tig.trends.com