Minireview

A genomic approach to studying cell-size homeostasis in yeast
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Abstract

Using a complete set of budding-yeast mutants bearing deletions of all known open reading frames, a recent study has revealed multiple overlapping pathways that coordinately regulate cell-cycle progression with ribosome biogenesis and translation efficiency, providing new insights into the mechanisms governing cell-size homeostasis in eukaryotes.

Many students of biology may have been asked whether tall people have more cells than small people - or are their cells simply larger? Although this may seem a simple and naive question, the answer is far from obvious. Although cell size in multicellular animals (metazoa) depends on the organ and tissue type examined, cell size is generally invariant within a single tissue or organ. If the larger person simply has more cells, one has to conclude that individual cell size is invariant in humans and that our overall height and width is determined genetically by controls over cell proliferation. If this were true, individual cell size would have to be strictly regulated during development. Conversely, if large and small people have the same number of cells, one would have to conclude that genetic determinants over individual cell size must regulate our overall dimensions. So, what determines individual cell size? Mike Tyers and colleagues have tried to answer this question using a post-genomic approach in the unicellular budding yeast [1]. They have searched for both large and small cells from a complete collection of budding yeast strains, each of which carries a deletion of a single, distinct open reading frame (ORF). This analysis has revealed that cell size is regulated by multiple overlapping signal transduction pathways that coordinately regulate both ribosome biogenesis and progression through the cell-division cycle [1]. Amazingly, homologs of some of the identified components of these pathways have been implicated in size control in flies and mice.

The new findings form part of a story of understanding cell size that goes back several decades. Obviously, cells must grow in order to proliferate; otherwise they would become progressively smaller, a process that would not be sustainable. Pioneering work by Anders Zetterberg and colleagues [2] demonstrated that mammalian tissue-culture cells need to grow to a certain size before they undergo DNA replication. A key advance was made in the early 1970s when Paul Nurse and colleagues [3,4] showed that control over cell-cycle progression by cell size is genetically determined. These researchers identified classes of mutants of the fission yeast *Schizosaccharomyces pombe* that uncoupled cell growth from cell-cycle progression [3]. The first class comprised the cell-division cycle mutants (*cdc*), which continued cellular growth but halted cell-cycle progression and thus became very elongated [3]. The most famous of these, *cdc2*, was later found to be defective in a cyclin-dependent kinase, a conserved regulator of the transitions from both G1 to S phase and G2 to M phase in all eukaryotes. Mutants of the second class were *wee*, so called because they divide at a smaller size than wild-type cells, as judged by both total protein and RNA content [4]; only two of these mutants were identified - *wee1* and *wee2*.

These crucial observations led to the idea that entry into mitosis is regulated by a 'size sensor', which genetically determines the point at which cells have grown large enough to undergo nuclear division [3-5]. The cell size that triggers mitosis must be proportional to cell ploidy (the number of sets of chromosomes), given that diploid cells divide at precisely twice the size of haploids. Furthermore, given that cells undergo division at a smaller size in sub-optimal

growth conditions, Nurse and colleagues concluded that the size sensor is modulated by nutrient availability [6]. We now know that wild-type *wee1* encodes a tyrosine kinase that phosphorylates and inhibits a protein kinase complex made up of Cdc2 and Cdc13 (cyclin B) during G2 phase, and that *wee2* is a dominantly active allele of *cdc2*. Thus, it was proposed that the size of the cell is sensed by key regulators, such as the Wee1 kinase and the Cdc25 phosphatase, that determine the timing of Cdc2/cyclin B dephosphorylation and activation. In parallel studies, Hartwell and colleagues [7] showed that in the budding yeast *Saccharomyces cerevisiae* coordination of division with growth occurs not in G2 phase but at START, a critical point in G1 phase at which cells must have reached a minimum size if they are subsequently to enter S phase. This size threshold was also shown to increase in proportion to cell ploidy and nutrient status.

So, why does cell size influence different cell-cycle transitions in different organisms? The answer to this was also resolved by the analysis of fission yeast *wee1* mutants [8]. During log-phase growth, fission yeast cells spend approximately 70% of their time in G2 phase. Wild-type cells undergo cytokinesis and separate only after they have not only completed one mitosis and chromosome separation but have also completed the DNA replication of the next cell cycle. In this manner newly born cells, which are approximately 7 μm in length, have a 2C DNA content (that is, they have replicated their chromosomes). On the other hand, newly born *wee1* cells are only 3.5 μm in length and have a 1C DNA content (in that they have not undergone DNA replication). The *wee1* cells were found to initiate S phase only after they had grown to approximately 5 μm. This led Nurse and colleagues to conclude that an additional size sensor in fission yeast governs the G1-to-S-phase transition; this additional sensor is analogous to that found in budding yeast (START) but is cryptic under optimal growth conditions [8].

The idea that mitosis in fission yeast may respond to a mass or size sensor prompted researchers to isolate *whi* (or *wee*) mutants in budding yeast that divide at a smaller cell size, in the hope of uncovering pathways that connect cell growth to progression through G1 phase of the cell cycle. The most famous of these, *whi1-1*, turned out to be a dominant allele of *cln3*, which encodes a cyclin that, in conjunction with its cyclin-dependent kinase (Cdk), Cdc28, regulates the timing of START [9]. The Cln3-Cdc28 complex and another protein of undefined biochemical function, Bck2, regulate the activity of two functionally overlapping transcription factors: SBF (a complex containing the Swi6 and Swi4 proteins) and MBF (containing the Swi6 and Mbp1 proteins). These transcription factors drive the expression of the G1 cyclins Cln1 and Cln2 which, in association with Cdc28, drive bud emergence, spindle pole body duplication and DNA replication, all of which are steps necessary for cell division. Deletion of *cln3*, *swi4*, *swi6* or *bck2* causes cells to undergo START at a larger cell size. Interestingly, Whi3 and Whi4 were found to be

RNA-binding proteins that sequester the *cln3* transcript, and Whi2 was identified as a regulator of Cln1-Cdc28 and Cln2-Cdc28 activities [10,11]. One could conclude from these early studies that cell-growth control is coupled to cell-cycle progression by the regulation of the activity of critical cyclin-Cdk complexes that control either commitment to the cell cycle (START) or entry into mitosis (see Figure 1). The question then becomes: what aspect of size are these cell-cycle regulators sensing?

Unfortunately, few *whi* mutants were analyzed in budding yeast because of the difficulties inherent in cloning genes that affect only size and not other characteristics, so progress in the area of cell-size homeostasis in yeast has been slow for many years. Completion of the *S. cerevisiae* genome sequence has opened an exciting new era in the systematic identification and analysis of gene function, however, and has provided a unique opportunity to examine the mechanisms governing cell-size homeostasis. As part of an international effort to understand gene function in budding yeast, a collection of strains has been constructed that collectively have deletions in each of the known ORFs [12-14]. Tyers and colleagues [1] made use of this resource to comprehensively screen for mutants that divide either at a smaller (*whi*) or larger (*lge*) cell size. Initially they classed the smallest and largest 5% of the 4,812 viable haploid deletions as either *whi* or *lge*, respectively. As some essential genes may affect cell size in a dose-dependent manner, the complete set of 1,142 diploid strains heterozygous for an essential gene deletion were also analyzed. As expected, the *lge* mutants included known activators of START, such as Cln3, Swi6, Swi4, and Bck2. Others were highlighted by a synthetic genetic array analysis of START components, a robotic process by which mutants can be crossed to an array of strains bearing deletions of the known ORFs, using regulators. In particular, this analysis revealed that cells lacking Lge1, a novel protein of previously unknown function, divide at larger cell size [1].

Because small size can result simply from reduced cell growth rate, many *whi* mutants were identified that were disrupted in genes involved in general respiration and nutrient uptake. To avoid this problem, Tyers and colleagues concentrated on only those viable haploid or heterozygous diploid *whi* mutants that had a normal growth rate. Only 25 mutants satisfied this criterion; these included known regulators of START, such as the RNA-binding protein Whi3 and Cdh1, an activator of the anaphase promoting complex that is necessary for the establishment and maintenance of G1 phase. An additional *whi* mutant, *ptk2*, was found to act upstream of *cdh1*. Remarkably, one of the smallest *whi* mutants identified was *sfp1*, which is defective in a gene encoding a zinc-finger transcription factor that itself controls the expression of genes involved in ribosome biogenesis and translation. Overexpression of *sfp1* causes a *lge* cell phenotype; together with the *whi* phenotype of *sfp1* mutants, this implies a dose-dependent role for ribosome
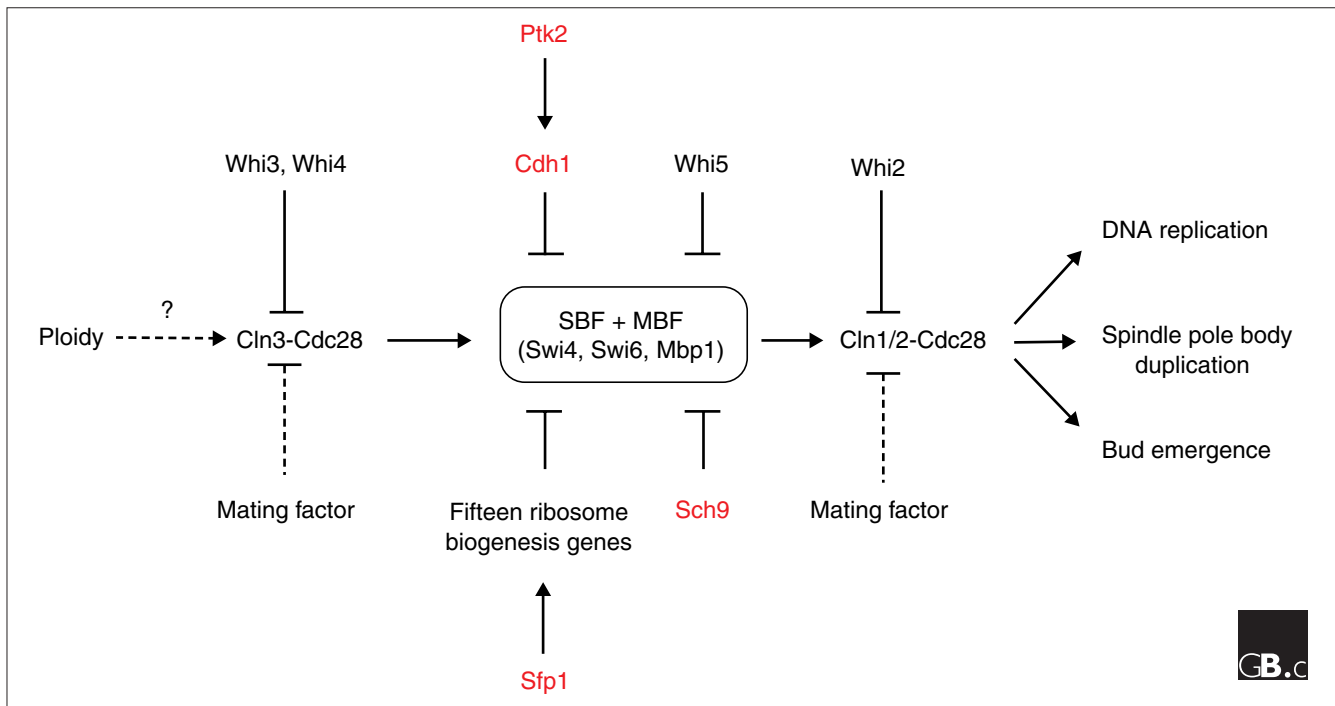
Figure 1
The genes controlling cell size in budding yeast (*S. cerevisiae*). Arrows indicate activation; lines with bars indicate repression; dotted lines indicate uncertainty. See text for further details. Proteins in red were implicated in this pathway for the first time by Tyers and colleagues [1]: Ptk2 is a serine/threonine protein kinase that regulates ion transport across the plasma membrane; Sch9 is a serine/threonine protein kinase (similar to Akt/PKB) that regulates longevity and stress resistance; Sfp1 is a zinc-finger-containing transcription factor that was initially described as an inhibitor of the G2/M transition; and Cdh1 is an activator of the anaphase-promoting complex.

biogenesis in the regulation of START. Indeed, many haploinsufficient *whi* mutants were found to affect genes involved in either ribosome biogenesis or nucleolar function or genes encoding ribosomal subunits. Epistasis analysis indicated that Sfp1 acts upstream of Swi4 and Swi6 but independently of Cln3 and Bck2. Another *whi* mutant gene, *sch9*, whose product also acts upstream of SBF and MBF, encodes a protein kinase that is 49% identical to human Akt/protein kinase B (PKB) in its carboxyl terminus. This is particularly intriguing because PKB is part of a conserved signaling pathway, which includes phosphoinositide (PI) 3-kinase, the PI phosphatase PTEN, the kinase target of rapamycin (TOR), the tuberous sclerosis proteins TSC1 and TSC2 and the ribosomal S6 mitogen-activated protein (MAP) kinase, which has recently been implicated in cell-size control in flies and mice [15-17]. The *sch9 whi* mutant is also haploinsufficient, suggesting that Sch9 may be a dosage-dependent repressor of START. Sfp1 and Sch9 must be on distinct pathways, however, because double mutants are non-viable. These results suggest that commitment to the cell cycle is dynamically regulated by multiple signaling pathways that coordinately regulate ribosome biogenesis. This would provide an ideal mechanism by which the cell-size threshold for commitment to the cell cycle in yeast could be modulated by nutritional status, a key feature of the size sensor.

Most of the genes that affect size control in G1 phase directly or indirectly control the activity of the Cln1-Cdc28 and Cln2-Cdc28 kinases by modulating the activity of SBF or MBF transcription factors. In fission yeast, progression through G1 phase is regulated by a transcription-factor complex that is similar to the budding-yeast SBF and MBF complexes and contains the Cdc10, Res1 and Res2 proteins, homologs of budding yeast Swi4, Swi6 and Mbp1 proteins. Thus, one might expect the Cdc10 complex to be the focus of similar growth-control pathways in fission yeast to those that control SBF and MBF in budding yeast. But as previously stated, size control in fission yeast in optimal growth conditions operates at the G2-to-M-phase transition, which is not controlled by the Cdc10 complex. Although the identity of the mass sensor governing entry into mitosis remains unknown, Daga and Jimenez [18] have recently shown that the abundance of the Cdc13 mitotic B-type cyclin and of the Cdc25 phosphatase are under the control of the translation factor eIF4a, which is encoded by the *tif1* gene in fission yeast, and that translation of Cdc13 and Cdc25 is hypersensitive to reduction in *tif1* expression. The *cdc13* and *cdc25* mRNAs share unusual features in their 5′ untranslated regions, leading to the suggestion that control of their translation rates may provide a mechanism by which the activity of these mitotic regulators could be coupled to cell-growth control.

Thus, the common idea that emerges is that key regulators of cell-cycle progression are acutely sensitive to changes in the rate of ribosome biogenesis and/or translational efficiency. For this theory to be substantiated it would be necessary to compare the mechanisms governing cell-size homeostasis in other organisms. Now that the fission-yeast genome sequence has been completed, it is expected that a similar bank of haploid and diploid deletion mutants of all its ORFs will soon be constructed. A screen for regulators of size homeostasis in fission yeast should be a relatively straightforward exercise, because fission yeast grows by length extension. One can predict that mutations in at least one conserved signal-transduction pathway will be identified. Cells lacking Sty1/Spc1, a close structural homolog of the p38 and JNK stress-activated MAP kinases, divide at a larger size than wild-type cells [19,20]. As the Sty1/Spc1 pathway is activated by multiple environmental stresses, including nutrient deprivation, this may provide a way for the size sensor governing entry into mitosis to be modulated by the environment. The mechanism by which the Sty1/Spc1 pathway regulates cell-cycle progression is at present unknown.

So what do the recent studies of yeast tell us about cell-size homeostasis in metazoa? It is certainly intriguing that several components of the PI 3-kinase pathway that regulates cell-size homeostasis in mice, including PKB, TOR, TSC1, TSC2 and ribosomal S6 kinase, have close structural homologs in yeast and that some of these have been identified as regulators of size. Cell-size homeostasis in all eukaryotes may prove to be determined by a common ancestral pathway; in yeast this is modulated by nutritional status, whereas in metazoa it can be regulated by extracellular ligands, such as insulin. Does this tell us what determines the overall size of an animal? The answer, in fact, appears to be that neither cell size nor cell proliferation is the sole determinant of overall size, but that the two factors are coordinately regulated by the same signal-transduction pathways. Most notably, both diploid and polyploid variants of the salamander have the same overall size, even though the cells of the polyploid animals are larger but fewer in number [21]! We clearly have much more to learn about how cell size and organism size are regulated. The quest will hopefully be accelerated once the pioneering, post-genomic approaches that have been applied to budding yeast become available in other unicellular and multicellular model organisms.

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