Glucose modulation of cell size in yeast

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Abstract
Saccharomyces cerevisiae cells grown in glucose have larger average size than cells grown in ethanol. Besides, yeast must reach a carbon source-modulated critical cell size in order to enter S phase at Start. This control is of utmost physiological relevance, since it allows us to coordinate cell growth with cell cycle progression and it is responsible for cell size homeostasis. The cell sizer mechanism requires the overcoming of two sequential thresholds, involving Cln3 and Far1, and Clb5,6 and Sic1, respectively. When both thresholds are non-functional, carbon source modulation of cell size at Start is completely abolished. Since inactivation of extracellular glucose sensing through deletion of either the GPR1 or the GPA2 gene causes a marked, but partial, reduction in the ability to modulate cell size and protein content at Start, it is proposed that both extracellular and intracellular glucose signalling is required for properly setting the cell sizer in glucose media.

Nutritional control of cell size in yeast
A tight and faithful coordination of cell growth and division is of utmost physiological relevance and it is responsible for cell size homeostasis [1]. In the budding yeast Saccharomyces cerevisiae, cells have to reach a critical cell size, called protein content at Start (Ps), to enter into S phase. Ps increases in proportion with ploidy and is modulated by nutrients [1,2]. In batch cultures, the average cell size remains at low and almost constant levels during growth on non-fermentable substrates, while the average size of the cells linearly increases with the specific growth rate only during growth on fermentable substrates [3]. Data from glucose-limited continuous cultures validate this observation, since the average cell size starts to increase after the critical dilution rate has been reached, i.e. after cells shift their metabolism from a fully oxidative to a respiro-fermentative one [4]. The Ps value, probably the more relevant parameter describing coordination between cell growth and the DNA division cycle, follows strictly that of the average protein content of the whole population [3]. The modulation of cellular metabolism, through addition of formate to the culture medium of chemostat growing cells, increases production of NADH, which in turn enhances the rate of ethanol production [4]. Significant increases in average protein content and Ps are observed following formate addition even at very low growth rates, thus linking the setting of cell protein content and Ps to actual metabolism and not to the specific growth rate [4]. In this review we summarize and comment on some recent findings from our laboratory further analysing these aspects.

Glucose sets cell size independently from its energy supply role
Both carbon source and cAMP are known to modulate Ps [1,2,5]. The addition of cAMP to permeable strains or the hyperactivation of the cAMP pathway by mutation almost doubles Ps [6]. Thus, it was of interest to investigate whether mutations in the glucose-sensing pathway that activates cAMP synthesis affect glucose modulation of cell size and Ps. Mutants carrying a deletion in either the Gpr1 receptor or the cognate Gα encoding gene (gpr1Δ or gpa2Δ strain, respectively) were analysed during exponential growth in synthetic complete medium supplemented with either 2% ethanol (SCE) or 2% glucose (SCD). gpr1Δ and gpa2Δ strains during exponential growth on SCD, but not on SCE, showed significantly altered protein distributions, resulting in a reduction of both average protein content and Ps. The duplication time and the length of the budding phase were unaffected, consistent with the notion that signalling through the Gpr1/Gpa2 pathway specifically modulates Ps setting [7].

Two thresholds control the G1 to S transition
As outlined above, there is general consensus that yeast cells have to reach a critical cell size (Ps) to enter S phase [1,2]. Recent work from our laboratory has allowed us to identify the molecular basis of the cell sizer in budding yeast. A threshold given by an activator, which interacts with an inhibitor blocking its action, is a very simple and effective biochemical switch mechanism [8,9]. We have put forward the hypothesis that Far1, the cyclin kinase-dependent inhibitor (Cki) long recognized to inhibit the G1 to S transition in response to mating pheromones [10], may also have a role in mitotic cycle cooperating with Cln3 in a nutritionally modulated threshold controlling entrance into S phase [8]. Therefore, a basic blueprint of the cell cycle has been designed...
Figure 1 | A simplified model of the cell size-dependent G1/S transition in budding yeast and its control by glucose

Two sequential growth-modulated thresholds involving cyclin-dependent kinases and their cognate inhibitors have to be overcome in order for a yeast cell to enter S phase. Several nested levels of control (not shown) modulate each involved protein, namely transcription of the encoding genes, translational efficiency, subcellular localization, post-translational modifications (i.e. phosphorylation), degradation and interaction with binding partners. Established and tentative roles of glucose (intracellular and extracellular) and signal transduction pathway(s) modulating the two thresholds are outlined.

based on a Cki•cyclin threshold function acting at Start, the overcoming of which activates waves of cyclins that set the timing for the onset of mitosis and cell division [8]. Direct molecular evidence giving strong support to the role of Far1 during the mitotic cycle has recently been obtained [7]. After overcoming the Cln3•Far1 threshold, a second one is operational [11]. It requires the Cki Sic1 and Clb5,6, the cyclins that interact with the kinase Cdc28 to activate DNA [1]. Threshold execution is made irreversible by degradation of Sic1, primed by a multiple phosphorylation dependent on Cln1,2•Cdc28 [11].

The two sequential Far1- and Sic1-dependent thresholds cooperate to modulate cell size in different carbon sources

Carbon source modulation of Ps is a hallmark response of the cell cycle to changing growth conditions. Carbon source affects the level of components of both thresholds, i.e. Cln3 and Far1 [7] and Sic1 and Clb5 (unpublished results from our laboratory), suggesting that both thresholds cooperate in glucose-dependent modulation of cell size. In fact we could show that if one or both components of each threshold are inactivated, yeast cells largely retain their ability to modulate cell size and Ps in the presence of glucose. In contrast, concurrent deletion of either CLN3 or FAR1 (first threshold) and SIC1 (second threshold), abolished glucose modulation of both P and Ps. The ratio for both parameters is in fact close to one in both cln3Δ sic1Δ and far1Δ sic1Δ cells, indicating that in exponentially growing cells, the glucose-dependent setting of cell size lies entirely on these two cyclin•Cdc28/Cki-mediated thresholds [7].

Conclusions and perspectives

The finding that inactivation of the Gpr1/Gpa2 pathway – that monitors extracellular glucose – does not affect the growth rate of the cells, while it partially abrogates glucose modulation of cell size, indicates that yeast cells are able to modify their cell cycle machinery in response to an external signal, showing a sort of growth factor/hormone-like control on cell cycle entry as observed in mammalian cells [2]. But, since the effect of mutations in the Gpr1/Gpa2 pathway on glucose modulation of cell size is only partial, extracellular glucose alone may not be enough to set Ps. Taken together with the previously reported data that indicate how the shifting of glucose metabolism to fermentation increases cell size and Ps, our data strongly support the notion that both extracellular and intracellular glucose cooperate to set Ps. Besides, other nutrient sensing pathways appear to cooperate with glucose sensing to set cell size in yeast. For instance, the TOR (target of rapamycin) pathway signals through the Ras/cAMP pathway [12] and has a role in modulating cell size [13].

In conclusion, we propose that the main regulatory circuits linking glucose sensing and utilization to the setting of Ps could be described by the model in Figure 1 (see the legend for details). The application of a modular systems biology approach [14], tested with success in [7], that requires
quantitative data (conventional as well as post-genomic) to be obtained on the components of each module of cellular regulatory circuits and their structuring in computer models [15], is expected to increase our understanding of cell cycle control.

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References

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