Antagonistic gene transcripts regulate adaptation to new growth environments

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Cells have evolved complex regulatory networks that reorganize gene expression patterns in response to changing environmental conditions. These changes often involve redundant mechanisms that affect various levels of gene expression. Here, we examine the consequences of enhanced mRNA degradation in the galactose utilization network of Saccharomyces cerevisiae. We observe that glucose-induced degradation of GAL1 transcripts provides a transient growth advantage to cells upon addition of glucose. We show that the advantage arises from relief of translational competition between GAL1 transcripts and those of cyclin CLN3, a translationally regulated initiator of cell division. This competition creates a translational bottleneck that balances the production of Gal1p and Cin3p and represents a posttranscriptional control mechanism that enhances the cell’s ability to adapt to changes in carbon source. We present evidence that the spatial regulation of GAL1 and CLN3 transcripts is what allows growth to be maintained during fluctuations of glucose availability. Our results provide unique insights into how cells optimize energy use during growth in a dynamic environment.

Environmental change invariably elicits alterations in gene expression as a cell tunes its enzymatic repertoire to a new set of challenges (1–3). The time required to alter patterns of gene expression and create a new array of functional proteins limits the speed at which a cell can respond to changes in its growth environment. The concentrations of mRNA transcripts have a strong influence on the dynamics of gene networks. In dynamic environments, the rate of transcript turnover is one factor that controls the rapidity and duration of gene expression responses to stimuli (4, 5). In yeast, the transcripts of many glucose-repressed genes become unstable when cells are returned to growth on glucose. Sequences in the 5′-UTRs of several transcripts are known to convey glucose sensitivity (6–9).

The galactose network is a well characterized metabolic pathway that converts galactose to glucose 6-phosphate. In the presence of galactose, the genes of this pathway are highly expressed; however, like other secondary metabolic pathways, GAL genes are suppressed by glucose at multiple levels (10). In addition to being silenced at the transcriptional level, the half-life of GAL1 mRNA, which encodes the first enzyme in the pathway, is reduced fourfold when glucose is introduced into the medium (6). This observation is in contrast to that for the Gal1 protein, which is highly stable in both galactose and glucose environments (11). The mechanism behind the regulated degradation of GAL1 mRNA has not been reported. As part of this study, we found that, consistent with other glucose-sensitive genes, the 5′-UTR of GAL1 destabilizes the transcript in the presence of glucose.

The primary response to glucose availability is that yeast cells rapidly increase their growth rate. The decision to divide or not is contemplated during G1 and yeast cells increase their division rate by shortening the length of this cell cycle phase (12). Once past a certain point in G1, called START, yeast cells are committed to completing the division cycle. START was originally described as the point at which a threshold capacity for protein synthesis is reached (13, 14). This point is sensed by the translationally regulated transcript of CLN3, which encodes the earliest cyclin in the cascade that drives the cycle forward (15).

In this study, we analyzed the biological consequence of the glucose-mediated degradation of GAL1 mRNA. We first altered the 5′-UTR sequence of GAL1 to create a strain that expresses a glucose-resistant GAL1 transcript. We then used microfluidic technology (16) to measure the dynamics of the galactose network in single cells expressing this stabilized variant of GAL1 mRNA. Our results indicate that GAL1 mRNA is rapidly degraded in response to glucose to allow the cell to quickly increase its growth rate by shortening the length of G1. In subsequent experiments, we observed a reciprocally antagonistic relationship between the synthesis of Gal1p and Cin3p. When GAL1 translation was increased, CLN3 translation was reduced and vice versa, suggesting that these transcripts share a limited supply of translation factors. Finally, we show that the temporal coordination of Gal1p and Cin3p synthesis may arise from spatial regulation, a common mechanism in biological signaling pathways and an emerging theme in translational regulation.

Results

5′-UTR of GAL1 Conveys Glucose Sensitivity. We used the tet- transactivator (tTA) expression system (17) to achieve regulated galactose-independent expression of GAL1 and then measured the half-lives of variants of GAL1 mRNA in cells grown in either glucose or galactose by quantitative RT-PCR (6). We found that deletion of the 300 bp upstream of the first ATG of GAL1 (Δ5′-GAL1) was sufficient to stabilize GAL1 mRNA by threefold in cells grown on glucose (half-life increased from ~3 min to ~9 min) (Fig. 1A). Likewise, fusion of the same 300-bp sequence to the YFP gene (5′-UTRGL1-YFP) resulted in an mRNA that was threefold more stable in cells grown in galactose than in those grown in glucose (Fig. S1). These results suggest that the region immediately upstream of GAL1 is both necessary and sufficient for conferring glucose sensitivity, as is the case for other glucose-sensitive transcripts. We next used a PCR-based method to determine that the endogenous GAL1 transcript contains a 5′-UTR of ∼100 nt (Fig. S1). We then replaced the endogenous GAL1 gene with an allele harboring either the wild type or a randomized 100-bp sequence immediately upstream of the first ATG and a CFP tag at the 3′ end [strains WT and ST (stable), respectively]. We induced the expression of each allele from the native GAL1

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GAL1-CFP

and normalized to the number of cells in frame 1. The average sizes of the WT number of cells in each trap was estimated from the total area of the colony dynamic conditions. (The micrographs shown are monitored over time by measuring the total area of the cells in each trap.

Individual WT and ST colonies were grown in eight separate traps of a single microfluidic chip. There were three traps for each strain fed with galactose plus glucose-mediated mRNA degradation on the dynamics of the GAL1-CFP mRNA decayed with a half-life of mRNA concentration vs. time after addition of glucose to the growth medium. GAL1-CFP mRNA decayed with a half-life of ~3 min in WT and ~9 min in ST. (C) Individual WT and ST colonies were grown in eight separate traps of a single microfluidic chip. There were three traps for each strain fed with galactose plus 4-h pulses of glucose every 4 h and one trap each for the galactose-only conditions. (D) A schematic of the GAL1 transcript produced in WT and ST strains. The GAL1 mRNA levels measured by qRT-PCR in WT (blue) and ST (red) strains are plotted as normalized mRNA concentration vs. time after addition of glucose to the growth medium. GAL1-CFP mRNA decayed with a half-life of ~3 min in WT and ~9 min in ST. (C) Individual WT and ST colonies were grown in eight separate traps of a single microfluidic chip. There were three traps for each strain fed with galactose plus 4-h pulses of glucose every 4 h and one trap each for the galactose-only conditions. Each colony was started from 25 to 75 cells. The growth of each colony was monitored over time by measuring the total area of the cells in each trap. The micrographs shown are final frames for one WT (Upper) and one ST (Lower) trap, both started from 31 cells, and were grown for 30 h under the dynamic conditions. (D) Results of the dynamic growth experiment. The number of cells in each trap was estimated from the total area of the colony and normalized to the number of cells in frame 1. The average sizes of the WT and ST colonies were plotted vs. time; error bars = 1 SD.

Stable GAL1 mRNA Causes a Prolonged G1 Phase After a Shift to Glucose. Cells growing in galactose medium respond to glucose by inhibiting the expression of GAL genes and by increasing their growth rate. We began by studying the effect of glucose-mediated degradation of GAL1 mRNA on the inhibition of the galactose network in cells growing in a dynamic environment. We grew the WT and ST strains in a microfluidic chemostat and recorded the level of Gal1p-CFP in single cells using time-lapse fluorescence microscopy. Consistent with Gal1p being a highly stable protein in both glucose and galactose, in both strains Gal1p-CFP was depleted primarily through dilution via cell division. This process produced a step-like decrease of fluorescence in the single-cell trajectories (Fig. 2A). On average the Gal1p-CFP signal decayed ~2.5 times more slowly in ST cells than in WT cells (Fig. 2B). As highlighted in Fig. 2C (thick trajectories), the plateaus in the CFP trajectories show that the WT cells spent less time between cell divisions than ST cells during the glucose phase of the experiment (~20 min vs. ~90 min). Collectively, the results of the microfluidics experiments suggest that the primary difference between the strains is that WT cells divide more often in glucose than ST cells, causing them to deplete the Gal1p-CFP at a faster rate. On the basis of the observation that both WT and ST cells accumulated the same amount of Gal1p during growth in galactose and that Gal1p had not depleted in either cell type until the first cell division after glucose addition, we concluded that the ST phenotype was due to excess GAL1 mRNA, not protein.

The results of the microfluidics experiments indicated that ST cells had a growth defect following a galactose-to-glucose switch; however, it was difficult to extract cell cycle data from the trajectories presented in Fig. 2A. To analyze this phenomenon more precisely, we conducted flow cytometry experiments to determine the cell cycle profiles of both strains grown under conditions of sudden glucose availability. As expected, both WT and ST cultures had larger G1 fractions when grown in galactose (37% and 36%) than when grown in glucose (25%) (Fig. S3). We next measured the rate at which the G1 fraction of each galactose culture was reduced after glucose addition. Here, we observed a considerable difference in the growth characteristics of the two strains: Whereas the G1 cells of the WT culture responded to glucose immediately by entering the division cycle, the ST culture was slower to respond (Fig. 2C). We then confirmed this result in synchronized cultures (Fig. S3). These observations suggest that the ST cells have lost the ability to tightly regulate cell cycle entry in response to glucose when growing in these dynamic conditions. To identify the relevant aspects of GAL1 repression, we assayed the cell cycle response in cells expressing a variety of tTA-driven GAL1 alleles. We found that both transcriptional repression and enhanced mRNA degradation were required for the normal response to glucose; however, most of the phenotype could be attributed to the decay of mRNA transcripts. The cell cycle dynamics were sensitive to overexpression of GAL1 transcript, as well as to the length of the GAL1 ORF, but did not require that the transcript encode a functional Gal1 protein (Fig. S3). These results are consistent with the hypothesis that the GAL1 transcript, not its protein product, interferes with cell cycle entry when glucose becomes available.

ST Cells Have a Growth Disadvantage in a Dynamic Environment. Because glucose-sensitive transcripts are a part of multiple glucose-repressed pathways, it seems clear that the degradation of certain transcripts in response to glucose provides a selective advantage for cells. However, we found that the ST strain grew at least as well as the WT strain in conditions of constant glucose or constant galactose (Fig. S1). We hypothesized that the WT growth advantage would be realized only when cells were presented with a dynamic environment where glucose is only transiently available. To test this, we grew individual WT and ST colonies in separate chambers of a single microfluidic chemostat device that allows precise control over the growth medium (Fig. S2) (18), which was alternated between galactose only and galactose plus glucose medium every 4 h. We monitored the size of each colony by time-lapse microscopy as they grew in this dynamic environment. At regular time intervals, the area of each colony was measured (Fig. 1C) and the approximate number of cells in each frame was extrapolated on the basis of the average area of a single cell (the sizes of WT and ST cells were not found to be different in either static or dynamic growth conditions). WT and ST colonies grew at the same rate when fed only galactose (control colonies) (Fig. 1D). We found that WT cells grew as well as the controls under conditions of dynamic glucose availability. However, the periodic influx of glucose impaired the growth of ST cells. These results show that the selective advantage conferred by having a glucose-sensitive GAL1 transcript is realized only in conditions of transient glucose availability.

Fig. 1. Cells expressing stable GAL1 transcripts do not grow well in a dynamic environment. (A) Decay of tTA-driven GAL1 transcripts, with (blue) and without (red) the 5′-UTR, in cells growing in glucose. Deletion of the 5′-UTR increased the half-life of the GAL1 transcript in glucose. (B) A schematic of the GAL1 transcript produced in WT and ST strains. The GAL1 mRNA levels measured by qRT-PCR in WT (blue) and ST (red) strains are plotted as normalized mRNA concentration vs. time after addition of glucose to the growth medium. GAL1-CFP mRNA decayed with a half-life of ~3 min in WT and ~9 min in ST. (C) Individual WT and ST colonies were grown in eight separate traps of a single microfluidic chip. There were three traps for each strain fed with galactose plus 4-h pulses of glucose every 4 h and one trap each for the galactose-only controls. Each colony was started from 25 to 75 cells. The growth of each colony was monitored over time by measuring the total area of the cells in each trap. The micrographs shown are final frames for one WT (Upper) and one ST (Lower) trap, both started from 31 cells, and were grown for 30 h under the dynamic conditions. (D) Results of the dynamic growth experiment. The number of cells in each trap was estimated from the total area of the colony and normalized to the number of cells in frame 1. The average sizes of the WT and ST colonies were plotted vs. time; error bars = 1 SD.
following changes in the cellular localization of Whi5p-YFP. Whi5p-YFP was readily detected in the nucleus of uninduced cells, and as expected, just before bud formation the signal was dispersed throughout the cytoplasm. We made WT and ST versions of this strain and grew them in our microfluidic device while monitoring the Whi5p-YFP signal by time-lapse fluorescence microscopy (Fig. 2E). The WT and ST strains behaved similarly under conditions of constant galactose (Fig. S4). In the dynamic glucose environment, the length of G1 in WT cells was strongly influenced by the presence of glucose. Under these conditions, G1 phases were long during the galactose phase (~70 min), but rapidly shortened when glucose was added (to ~10–20 min). In contrast, in ST cells the length of G1 did not respond to changes in carbon source. In both the galactose and the glucose phases, G1 was long and highly variable in duration. The scaled average Whi5p-YFP signal for each population is plotted over time in Fig. 2G. The results indicate that ST cells are defective in the growth response to glucose, up-stream of Whi5p in the pathway that promotes cell cycle entry. (See Fig. S4 and SI Text for complete dataset and further discussion.)

Stable GAL1 Interferes with CLN3 Translation. We next assayed the G1 pathway upstream of Whi5p (Fig. 2D). Cln3p activation leads to the transcription of the CLN2 gene, the product of which reinforces Whi5p nuclear export and S-phase entry. We found that 10 min after glucose was added to galactose cultures, WT cells expressed twofold more CLN2 mRNA than ST cells, as determined by qRT-PCR (Fig. 3A). When we raised the level of CLN3 expression in ST cells by deleting the endogenous gene and replacing it with a plasmid-borne copy, we found that the level of CLN2 mRNA detected in this assay was restored to that of WT cells. Increasing the expression of Cln3p even further by mutating the regulatory upstream ORF of the CLN3 plasmid (A315T-CLN3), which raises the translation efficiency of CLN3 without affecting the amount of mRNA, increased the level of CLN2 expression to twofold above the level measured in WT cells. These results show that the pathway connecting CLN3 to CLN2 expression is intact in ST cells. Because WT and ST cells had the same amount of CLN3 mRNA, and the pathway downstream of Cln3p appeared to be functional in ST, we concluded that the defect was in the up-regulation of CLN3 translation that occurs in response to glucose. We corroborated these findings by measuring the cell cycle response of ST + CLN3 cells in a flow cytometry assay (Fig. 3B and Fig. S5). The added CLN3 allowed the ST cells to respond to glucose as quickly as the WT cells. This result was then confirmed in synchronous cultures (Fig. S3A).

We next assayed the translation of CLN3 mRNA in WT and ST cells immediately after the addition of glucose, following the experimental design diagrammed in Fig. S5. First WT and ST cultures were grown for 2.5 h in galactose medium and then glucose was added. Immediately following the addition of glucose, cycloheximide and ice were added to the cultures to arrest translation. Cell lysates were prepared and fractionated over sucrose gradients by ultracentrifugation to separate translation complexes by density. The column graphs in Fig. 3C show the distribution of CLN3 mRNA in the fractionated WT and ST lysates, increasing in density from left to right, after glucose was added. The A315T traces show the polysome profiles for each strain and the polysome peaks, representing transcripts that were being translated by multiple ribosomes, are indicated (see Fig. S3D for direct comparison of WT and ST absorbance spectra). The results show that under these conditions, CLN3 transcripts exist in polysome complexes in WT cells, but in ST cells CLN3 messages are primarily associated with only single ribosomes. In contrast, the distribution of GAL1 transcripts was shifted toward higher ribosome occupancy in response to glucose in both strains, although the ST strain maintained high levels of this mRNA, whereas in WT cells GAL1 was quickly depleted (see Fig. S5 for GAL1 polysome distributions). These results confirm that the induction of CLN3

![Cell cycle in yeast](https://example.com/cell_cycle_yeast.png)

**Fig. 2.** Cells expressing stable GAL1 transcripts are impaired in the cell cycle response to glucose. (A) The cells were grown in a microfluidic chemostat under conditions of constant 0.5% galactose, supplemented with a 5-h square wave impulse of 0.25% glucose every 5 h. Single-cell Gal1p-CFP trajectories were recorded in individual cells by time-lapse fluorescence microscopy. The period of glucose addition is represented by the light blue shaded region. For each strain, the trajectories of five cells are highlighted in blue (WT) or red (ST). Cell divisions can be detected in the individual trajectories as sharp dips in fluorescence. The time elapsed between divisions (t) is indicated for a representative trajectory for each strain, shown as a thick line. (B) The average Gal1p-CFP level for a population of cells (n = 25) after glucose addition is plotted vs. time (min). (C) The plot of the change in the relative size of the G1 population over time for a population of cells (Fig. 2C). The average Whi5p-YFP signal plotted against time for ST strain, the trajectories of glucose addition is represented by the light blue shaded region. For each population is plotted over time in Fig. 2G. The results indicate that ST cells are defective in the growth response to glucose, up-stream of Whi5p in the pathway that promotes cell cycle entry. (See Fig. S4 and SI Text for complete dataset and further discussion.)

The events leading to cell cycle entry in yeast have been well characterized and involve the activation of a pathway that leads to the nuclear export of a cell cycle inhibitor, Whi5p (19–21) (Fig. 2D). We decided to monitor cell division in our WT and ST cells by

![Whi5p-YFP expression](https://example.com/whi5p_yfp.png)
translation that normally occurs in response to glucose is impaired in the ST strain. We conclude that this is the source of the cell cycle defect exhibited by these cells when they are grown under dynamic conditions.

**CLN3 Translation Interferes with Gal1p Synthesis.** Although overexpression of CLN3 was able to hasten the response of ST cells to glucose, it had the unexpected consequence of slowing the accumulation of Gal1p-CFP in cells during growth in galactose (Fig. S6). QRT-PCR results showed that the level of GAL1 mRNA was not decreased in this strain (Fig. S6), suggesting that the ability to accumulate Gal1p-CFP in cells during growth in galactose (Fig. S6). qRT-PCR results showed that the level of GAL1 mRNA was not decreased in this strain (Fig. S6), suggesting that the ability to accumulate Gal1p-CFP in cells during growth in galactose (Fig. S6). 

We considered the possibility that GAL1 translation may be subject to cell cycle position and that, by decreasing the G1 fraction of the cell population, increased CLN3 expression may have indirectly prevented Gal1p-CFP accumulation. However, the single-cell trajectories do not support this hypothesis. In both WT and WT + CLN3, the cell cycle was responsive to Gal1p levels rather than the other way around (Fig. S6 and SI Text). To confirm that CLN3 expression was not inhibiting Gal1p accumulation through its effect on cell cycle position, we replaced the CLN3 plasmid with one encoding a nonfunctional protein, Cln3p-NLS, which is incapable of regulating cell cycle entry (22). The dynamics and steady-state levels of Gal1p-CFP in these cells were similar to what we observed in WT + CLN3 cells (Fig. S6).

**GAL1 and CLN3 Transcripts Are Spatially Coregulated in Dynamic Environments.** The GAL1 and CLN3 transcripts were each exquisitely sensitive to the expression level of the other in cells that were growing in dynamic glucose conditions. Considering that the genetic changes that revealed this relationship would not significantly impact the overall level of mRNA in the cell, it is unlikely that this sensitivity was due to a general phenomenon of protein synthesis. We began to contemplate possible scenarios that might cause these transcripts to be specifically influenced by each other. The first clue came from live cell images of WT and ST cells growing in galactose medium. We frequently observed one or two bright spots in the CFP channel in these cells as they induced the expression of Gal1p-CFP (WT images in Fig. S5 and Fig. 4A). This observation suggested that Gal1p synthesis may be spatially regulated. The number of cells with Gal1p-CFP foci was sensitive to the history of growth conditions before galactose induction. In cultures that had received only galactose in the past 24 h, cells with Gal1p-CFP foci were rare (6.5% vs. 42%) (Fig. 4B).

We used fluorescence in situ hybridization (FISH) to determine whether the observed Gal1p-CFP spots could result from the spatial regulation of GAL1 transcripts. We found that GAL1 transcripts were often clustered into one or two large foci in the cytoplasm (43% of cells that showed any TRITC hybridization); however, in this case there was little signal outside of foci. In two-color FISH experiments where we simultaneously probed for Gal1p (TRITC) and CLN3 (FITC), we found near perfect overlap in 99% of the cells that displayed both GAL1 and CLN3 foci (Fig. 3D). On the other hand, the transcript of another galactose-induced gene, GAL2, did not colocalize with GAL1 spots (Fig. 4E). Consistently, the expression of Gal2p was not inhibited by CLN3 overexpression (Fig. S6E).

We repeated the two-color FISH experiment in the WT and ST strains, but modified the protocol so that the three-dimensional shape of the cells was better preserved. We found that 95% of WT cells in which both GAL1 and CLN3 foci were detected had near perfect fluorescence overlap (Fig. 4G), whereas 77% of the CLN3 foci overlapped with GAL1 in ST cells. WT + CLN3 cells also displayed colocalizing GAL1 and CLN3 foci; however, we did not

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**Fig. 3.** GAL1 and CLN3 transcripts are mutually antagonistic. (A) Summary of CLN3 and CLN2 mRNA levels, as measured by qRT-PCR in WT, ST, ST + CLN3, and ST + A315T-CLN3 strains 10 min after glucose addition. (B) xy plot of the change in the relative size of the G1 population over time after glucose addition, as measured by flow cytometry. (C) Polysome distribution of CLN3 mRNA in WT (Left) and ST (Right) cells just after glucose addition; error bars indicate ±1 SD. The sucrose gradient increases from left to right. A315 signal identifies the fractions containing translation complexes with increasing numbers of ribosomes. These graphs are representative of results obtained from at least three independent experiments. See SI Text for polysome distributions of cells growing in galactose. (D) Average Gal1p-CFP accumulation in WT (Left) and WT + CLN3[A315T] (Right) cells growing in a microfluidic chemostat; error bars are ±1 SD.

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mRNA (determined by qRT-PCR), WT + CLN3 cells were delayed in Gal1p-CFP expression by almost 50 min. In addition, the steady-state levels of Gal1p-CFP were fivefold higher in WT cells relative to WT + CLN3 cells. Furthermore, WT cells reached steady-state levels in half the amount of time (75 min vs. 150 min). Eliminating the translational regulation of the plasmid-derived CLN3 (WT + A315T) had the same effect on the average Gal1p-CFP accumulation, but dramatically increased the variation between individual cells, illustrated by the error bars in Fig. 3D. In fact, >10% of these cells never induced expression of Gal1p-CFP over the course of the experiment (300 min).
that had been growing in galactose for dynamic conditions. In contrast, Gal1p-CFP foci were rarely seen in WT cells. Gal1p-CFP was detected in one to two foci in WT and ST cells growing under Fig. 4. smaller foci distributed throughout the cytoplasm. CLN3 mRNA was mostly mRNA was detected as one to two large brightly stained foci, with several (parent strain), 2.5 h after being switched from glucose to galactose. GAL1 mRNA was mostly detected in K699 cells, 2.5 h after being switched from glucose to galactose. GAL1 mRNA was detected as one to two large brightly stained foci, with several smaller foci distributed throughout the cytoplasm. CLN3 mRNA was mostly detected in one to two large foci. (D) Two-color FISH of GAL1(TRITC) and CLN3(FITC) in K699 cells, 2.5 h after being switched from glucose to galactose. Gal1p mRNA was detected as one to two large brightly stained foci, with several smaller foci distributed throughout the cytoplasm. CLN3 mRNA was mostly detected in one to two large foci. (D) Two-color FISH of GAL1(TRITC) and CLN3(FITC) in K699 cells, 2.5 h after being switched from glucose to galactose. No overlap between the two transcripts was observed. (E) Quantitation of foci detected in one-color and two-color FISH experiments. These results are averaged from at least three independent hybridizations. (G) Two-color FISH results from experiments in which the detergent wash was omitted to preserve the three-dimensional shape of the cells. GAL1(TRITC) and CLN3(FITC) foci overlapped in WT and ST strains, 2.5 h after being switched from glucose to galactose. Consistent with the live cell imaging of Gal1p-CFP foci, we found that GAL1 mRNA foci were rare when WT cells were grown in a static galactose environment (Fig. S7A). However, the few GAL1 mRNA foci we did detect in these cells were colocalized with CLN3 mRNA. In all cases where GAL1 mRNA was found outside of foci, it was not associated with CLN3 mRNA (Fig. S7A, Lower). The apparent lack of spatial regulation of GAL1 and CLN3 under static conditions suggests that these foci form in response to dynamic conditions to allow cells to rapidly respond to glucose. In support of this, we have found that WT cells do not respond to glucose, either by rapidly degrading GAL1 transcripts or by shortening the length of G1 phase, without prior exposure (Fig. S7D and E). Although our results are not definitive, there is a trend consistent with the dynamic response to glucose arising from the spatial colocalization of GAL1 and CLN3 transcripts.

**Discussion**

**GAL1 Transcripts Are Degraded in Response to Glucose to Optimize Growth in Dynamic Environments.** We began this study with the goal of understanding why glucose-induced GAL1 mRNA degradation leads to a selective advantage in cells growing in environments where glucose is only transiently available. The results of polysome fractionation experiments revealed that persistent GAL1 mRNA can inhibit CLN3 translation, which is normally induced by glucose. The observation of Whi5p-YFP strains showed that the rapid cell cycle response to glucose allowed WT cells to divide more often during bursts of glucose availability. In the absence of this regulation, ST cells missed the opportunity to increase their growth rate in glucose. We also discovered that when glucose was suddenly removed from the galactose-rich environment, cell division was stalled in both strains to allow Gal1p to accumulate again. For WT cells, this decrease in growth rate was balanced by the extra cell divisions in the glucose phase, and these cells wound up breaking even; i.e., their net growth in dynamic conditions was equivalent to their growth in a static galactose environment. Because ST cells did not gain a growth advantage in glucose, the occasional burst of glucose into the galactose-rich environment ultimately inhibited the growth of this strain. We conclude that the glucose-induced decay of metabolic transcripts is what allows cells to maintain optimal growth rates when carbon sources are fluctuating.

**CLN3 Could Scavenge Ribosomes from GAL1 When Glucose Becomes Available.** In this study, we present evidence that the synthesis of Gal1p is spatially regulated under certain conditions. First, concentrated Gal1p-CFP signals were often detected in cells that were inducing GAL1 expression. The percentage of cells in the population that exhibited CFP foci was sensitive to prior growth conditions. We observed the largest number of foci when the culture had recent exposure to both galactose and glucose media. Likewise, we observed few foci in cultures that had been exposed only to galactose. These results suggest that the spatial regulation of Gal1p synthesis is something that occurs in response to dynamic shifts in glucose availability. Consistent with the apparent spatial regulation of Gal1p, GAL1 mRNA was detected in large foci in cells that were expressing high levels of Gal1p. This result, along with the early appearance of foci during Gal1p-CFP induction (Fig. S5), implies that these are sites of protein synthesis rather than aggregates of mature protein, although this hypothesis remains to be formally tested. The results of FISH experiments show that GAL1 and CLN3 transcripts are spatially coregulated when the environment...
is alternated between galactose and glucose. We propose that these transcripts compete for a local pool of translation components. Our hypothesis is supported by the mutual sensitivity of GAL1 and CLN3 translation to reciprocal changes in mRNA levels. When glucose becomes available, GAL1 transcripts are rapidly degraded, freeing up ribosomes (and other translation factors) in the region, which CLN3 transcripts can take advantage of. However, if GAL1 degradation is blocked, CLN3 translation continues to lag and the cell cycle response is delayed, as we saw in the ST strain. In this scenario, the region of concentrated GAL1 transcripts acts like a ribosome bank for CLN3 in the event that glucose is suddenly added to the environment (Fig. S8).

Materials and Methods

Plasmids and Strain Construction. The plasmids used in this study are described in Table S1; strains are listed in Table S2. All cloned genes were amplified by PCR, using primers to incorporate the necessary flanking restriction sites for subsequent ligations. See SI Text for details.

Measurement of mRNA Decay Rates. Total RNA preparations and experiments to determine the half-lives of specific mRNAs were performed as described in ref. 6. Primers used for qRT-PCR are listed in Table S3.

Microfluidics. Microfluidic devices were prepared as described in ref. 6 and Fig. S2. Medium containing glucose was labeled by trace amounts of a fluorescent dye, sulforhodamine 101 (0.01 mg/mL). Image acquisition was performed on a Nikon Eclipse Ti epifluorescent inverted microscope outfitted with fluorescence filter cubes optimized for CFP, YFP, and rhodamine imaging and a phase-contrast–based autofocus algorithm. Images were acquired using a Photometrics CoolSNAP HQ2 cooled CCD camera, controlled by Nikon Elements software. The cell chamber was photographed every 5 min in three channels to record cell morphology (40× phase), Gal1p-CFP (434 nm) or WhIs-YFP (520 nm), and glucose (560 nm). Single cell trajectories were generated using custom cell-tracking software developed in our laboratory (18), and CFP or YFP vs. time plots was created using Matlab or Microsoft Excel.

Flow Cytometry. Cells were processed for flow cytometry, as follows: Cells were briefly pelleted and resuspended in 2 mL 70% ethanol (EtOH) and stored at −20 °C overnight. The next day, the EtOH-fixed cells were rehydrated in 5 mL dH2O, sonicated for 10 s at 40% power, and washed twice in 50 mM Tris, pH 8.0. Cell pellets were then resuspended in RNaseA (2 mg/mL) and incubated overnight at 37 °C. Cells were pelleted again, treated with pepsin (5 mg/mL) for 30 min at 37 °C, and then stained with propidium iodide (50 μg/mL) and run through a FACScan flow cytometer (Becton Dickinson). Flow cytometry data were analyzed using Gatinglogic software. The size of the G1 fraction in each sample was estimated by measuring the percentage of cells in the 1N peak; the position of the G1 gate was kept constant for all samples. The reported results are averages of three independent experiments run in parallel. See SI Text for detailed protocol.

Gene Expression Measurements. GAL1, CLN2, and CLN3 mRNA levels were measured by quantitative RT-PCR, as described for Fig. 1. Each experiment was performed in triplicate. Gene expression is displayed relative to results from WT. Measurements of Gal1p levels were derived from Gal1p-CFP signals in single cells at t = 150 min in the microfluidics experiments and are represented in the graph relative to the WT sample. See SI Text for detailed protocol.

Polysome Fractionation. Yeast polysomes were prepared and fractionated according to the protocol at http://openwetware.org/images/4/47/PlyrRNA.pdf. Polysomes were fractionated on 36-mL 10–47% sucrose gradients by ultracentrifugation (27 K, 4 h, 4 °C) in an SW28 Beckman rotor. Fractions (1 mL) were collected from the flow-through into an equal volume of cold 100% EtOH in 2-mL microfuge tubes, stored overnight at −20 °C, and pelleted by centrifugation (14 K, 30 min, 4 °C), and the RNA was purified using the RNeasy kit (QiAGEN) following the manufacturer’s protocol for RNA cleanup. Equal volumes of purified RNA (usually 10 μL) were used for qRT-PCR, as described above, to measure the relative amounts of GAL1 and CLN3 in the fractions of that region of the gradient containing ribosomes (usually fractions 17–32). See SI Text for detailed protocol; the primer sequences are listed in Table S2.

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Supporting Information

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SI Text

Culture Conditions. Yeast cells adapt to growth on galactose more rapidly if they had prior exposure to galactose (1). Likewise, we have observed that cells respond to glucose more readily if they have been grown in glucose before, although the mechanism behind this observation is unknown. Because past growth conditions influence the dynamics of the response to changes in carbon source, in all of our experiments the initial cultures were started from single colonies inoculated into synthetic medium containing a mixture of galactose and glucose (1%/1%). As expected, we found that cells growing in this mixed medium exhibited diauxie (i.e., they metabolized the glucose in the medium before using the galactose). After an overnight incubation, the starter culture was diluted 1:100 into fresh 1%/1% medium. We determined that the glucose of the fresh culture became depleted, and GAL1 expression was concomitantly induced, within 4 h of dilution. Using this culturing scheme, we were able to ensure that the cells used in our experiments had experienced both glucose and galactose in their recent past and could respond with maximum efficiency to either sugar.

Microfluidics Device. The microfluidic chip used in this study is an improved version of the device described in Bennett et al. (2) and is designed for the study of Saccharomyces cerevisiae cells in a defined microenvironment. This new device features a superior mixing system that leads to more reproducible output profiles of inducer compared with the previous design (Fig. S2) and methods to change the mixing ratio in time, generating reproducible concentration waves of inducer. Furthermore, the chip includes a yeast trap, which allows for long duration imaging and tracking of yeast cells, while reducing clogging from excess growth. In addition, this new device is less complex, reducing the number of ports from eight to five. It was manufactured using standard photolithography methods, with the layout of the device drawn in Autocad (AutoDesk) and the masks printed by CAD/Art Services.

A salient feature of this microfluidic device is the inclusion of staggered herringbone mixers (SHM), designed to cause corkscrew effects in convective fluid flow, which increases the surface area present for diffusive mixing. Increased surface area leads to reduced mixing times compared with simple diffusion across the interface of two laminar fluid streams. We maximized the flow velocity through this region, which limits the delay time for an input signal to reach the cell chamber while still ensuring adequate mixing. In contrast, the cell chamber region was designed to have a lower flow velocity by greatly increasing the channel width in this area. This lower velocity prevents cells from being forcibly removed from the cell trap region, while still allowing clearing of excess growth (Fig. S2D).

Another significant improvement in this unique design is in the dial-a-wave (DAW) junction region (Fig. S2C). The function of the DAW junction is to interface the fluid streams from the two input reservoirs with a minimum of diffusive mixing, because diffusion can lead to nonlinear changes in mixing ratios when the input pressures are altered. These nonlinearities are especially pronounced for unbalanced mixing ratios. Although the chip used in Bennett et al. (2) had a shunt network, the flow system was uneven with far more flow directed to the DAW junction outlet than the shunt network. Our current system is designed so that flow through each shunt and the DAW outlet is fairly constant under all mixing ratios. Moreover, the shunt architecture has been made more symmetrical, preventing gross changes in the fluid interface when mixing ratios are changed. The results of these changes can be seen in Fig. S2E, which demonstrates a linear system response as the applied pressure is increased.

In addition to the DAW junction, we have improved how external pressure is applied to the input syringes. The previous device made use of pneumatic valves to regulate this pressure; however, the valves were difficult to control reproducibly at the low working pressures (1–2 psi or 700–1,400 mm of water) required by this system. Furthermore, the pneumatic system was incapable of generating reproducible pressure steps of <1 mm of water. To simplify the application of external pressure, we have adopted a linear actuator system (RoboCylinder; Intelligent Actuator) to physically move the input syringes up and down, using gravity to apply different levels of hydrostatic pressure. We have found that hydrostatic pressure provides a more uniform and stable pressure source for microfluidics than most pneumatic systems can deliver. Moreover, the linear actuator system has a minimum step size of 10 μm, which is far superior to the previous pneumatic system and is at least one order of magnitude greater than the sensitivity of the device.

The competitive growth experiments described in Fig. 1 C and D were performed in our monster DAW chip, which has eight independent growth chambers of the type described in Fig. S2 in a single device. The other microfluidic experiments were done using the device diagrammed in Fig. S2.

Cell Cycle Analysis in Synchronous Cultures. To increase the dynamic range of the cell cycle experiments shown in Figs. 2B and 3C, which were done in asynchronous cultures, we repeated the analysis in synchronous cultures and monitored bud emergence as a marker for cell cycle entry. In these experiments, the culture was grown to midlog phase in galactose medium, arrested with nocardazole at the G2/M transition, and released into fresh galactose medium. The culture was allowed to synchronously move through mitosis while in galactose. We monitored mitotic progression by DAPI staining. Once ~80% of the population had reached G1, glucose was added and bud emergence was monitored by phase-contrast microscopy over time. As expected, in the WT strain the rate of bud emergence increased in cultures after glucose addition relative to those that continued in galactose medium. However, the stable (ST) strain had the same rate of bud emergence with and without glucose addition. These data support the results obtained from asynchronous cultures and are presented in Figs. S3B and S5A.

Characterization of the GAL1 ST Effect on Cell Cycle Dynamics. To identify the relevant aspects of GAL1 repression, we assayed the cell cycle response in cells expressing a variety of tet-transactivator (tTA)-driven GAL1 alleles. We found that cells responded most readily to glucose if both forms of inhibition were used (addition of doxycycline to repress transcription and inclusion of the 5′-UTR for mRNA decay). However, in the absence of the 5′-UTR, transcriptional repression did not have an effect on the cell cycle response to glucose (Fig. S5C). This result indicated that the degradation of GAL1 mRNA has a stronger influence over the cell cycle response than transcriptional repression. The strength of the G1 delay caused by GAL1 mRNA was related to the level of GAL1 expression. A gal1Δ strain expressing the tTA-WT plasmid had a normal cell cycle response to glucose; however, this same plasmid caused a G1 delay in a strain that retained the endogenous GAL1 gene (Fig. S3D). This effect was even stronger when tTA-ST was used in place of tTA-WT. Therefore, both ST and tTA-WT produced a dominant negative G1 exit phenotype under these conditions. We next altered the ability of the overexpressed GAL1 to absorb translational resources (Fig. S3 E and F). First, we deleted the sequence between two NdeI sites in the coding sequence of tTA-driven GAL1 plasmid, which reduced the length of the
mRNA by about half (805 bp deleted). The ST version of this allele was a weaker inhibitor of the cell cycle response to glucose than the full-length ST allele, but still led to a significant G1 delay phenotype. To determine whether it was the length of the mRNA or simply the length of the ORF that was important, we inserted a frameshift mutation into the full-length ST allele to truncate the ORF at about the same position as the NdeI deletion. The phenotype caused by expression of this allele was similar to what we saw with the NdeI deletion mutant. These results suggest that it is the length of the ORF, but not necessarily the total length of the message, that is important for the phenotype. Because both truncated alleles lack the Gal1p kinase domain, these results also indicate that increased Gal1p activity is not the source of the cell cycle defect.

**Whi5p-YFP Trajectories.** The results of the Whi5p-YFP experiments indicate that there is a considerable difference between WT and ST strains with regard to G1 regulation (Fig. S4), which seems odd given the comparatively mild differences we measured by flow cytometry. However, it should be mentioned that tagging Whi5p with YFP appears to have made the cells overly sensitive to the expression of GAL1. First, these cells grew poorly in galactose relative to untagged strains, particularly under dynamic conditions. Furthermore, when we attempted to ectopically express GAL1 from the tTA promoter, these cells arrested in G1 even when in glucose. Interpreted in the context of our hypothesis, this result suggests that the YFP tag interferes with the nuclear export of Whi5p and makes the cells sensitive to genetic changes that inhibit cell cycle entry. This caveat also suggests that the differences observed between WT and ST cells in this assay are likely exaggerated.

**Polysome Distributions of GAL1 and CLN3.** Yeast cells growing on an alternative carbon source respond to glucose by increasing global levels of protein synthesis. This global increase was detected in our polysome fractionation experiments. As illustrated in Fig. S5C, the amount of polysomes detected in lysates derived from galactose cultures increased after the addition of glucose. The polysome profiles of WT and ST cultures were comparable after glucose addition (Fig. S5D). When we compared the polysome distributions of GAL1 mRNA in cells before and after glucose addition (Fig. S5E), we found the distribution shifted toward the right in both WT and ST strains. As expected, the level of GAL1 mRNA was also decreased in WT strains in response to glucose, whereas it remained stable in ST cells. These results indicate that GAL1 transcripts have access to the additional ribosomes that are made available by the addition of glucose. CLN3 polysome distributions also shifted to the right with the addition of glucose in both WT and ST strains (Fig. S5F). However, the degree of change was reduced in ST cells relative to WT cells (Fig. 3). We can explain this observation in the context of the ribosome bank model illustrated in Fig. S8, which predicts that CLN3 mRNA does not acquire as many ribosomes in ST cells because it is still competing with the stable GAL1 transcripts.

**Gal1p-CFP Accumulation Is Not Subject to Cell Cycle Position.** Whereas overexpression of CLN3 was able to hasten the response of ST cells to glucose, it had the unexpected consequence of slowing the accumulation of Gal1p-CFP in cells during growth in galactose. In the first 150 min in galactose, ST + CLN3 cells growing in a microfluidic device under dynamic conditions accumulated only 70% the amount of Gal1p-CFP compared to the ST or WT strains (Fig. S6A). qRT-PCR results showed that the level of GAL1 mRNA was not decreased in this strain, suggesting that the ability to efficiently produce Cin3p came at the expense of GAL1 translation, not transcription. The ST + A315T-CLN3 cells were even more defective in GAL1 accumulation; many of them never displayed Gal1p-CFP expression, and those that did accumulated on average only 50% of the WT level by 150 min. These results were surprising given the small increase in CLN3 mRNA that these cells had and considering that GAL1 mRNA is present at much higher concentrations than CLN3 when cells are in galactose. It appeared that the relationship of GAL1 and CLN3 was reciprocal and that excess CLN3 transcripts could interfere with the translation of GAL1 mRNA when cells were switched from glucose to galactose. We assayed the effect that increased CLN3 had on the growth of cells when they were first exposed to galactose and found that, whereas the ST + CLN3 strain grew normally, the ST + A315T-CLN3 strain was impaired (Fig. S6B). The level of Gal1p produced by ST + CLN3 (70%) appears to be sufficient for growth in galactose, whereas the amount produced by ST + A315T-CLN3 (50%) is not. This result suggests that there is a threshold level of Gal1p expression that is required for growth and that unregulated CLN3 translation inhibits the accumulation of Gal1p to sufficient levels.

We considered the possibility that GAL1 translation may be subject to cell cycle position and that, by decreasing the G1 fraction of the cell population, increased CLN3 expression may have indirectly prevented Gal1p-CFP accumulation. However, the single cell trajectories do not support this hypothesis. WT cells began accumulating Gal1p-CFP at the same rate whether or not they were in G1 at the start of the galactose phase. In fact, in both WT and WT + CLN3, the cell cycle was responsive to Gal1p levels rather than the other way around. Upon being shifted to galactose medium, the cells paused wherever they were in the division cycle until Gal1p-CFP began to accumulate, at which point they resumed cell division (Fig. S6C). This point was reached within ~30 min for WT cells, but not until ~150 min in WT + CLN3 cells. These observations are consistent with there being a threshold level of Gal1p needed for cell division in galactose medium, rather than Gal1p synthesis being affected by cell cycle position. To confirm that CLN3 expression was not affecting Gal1p accumulation through its effect on cell cycle position, we replaced the CLN3 plasmid with one encoding a nonfunctional protein, Cin3p-ΔNLS (3). We found that Gal1p-CFP accumulated with dynamics and steady-state levels similar to what we observed in WT + CLN3 cells (Fig. S6D). These results support our model that it is the translation of CLN3 and not the activity of the protein product that prevents GAL1 translation.

**WT Cells Growing in Static Galactose Do Not Respond Quickly to Glucose.** In all of our dynamic growth experiments, we found that cells responded to glucose most readily if they had been exposed to glucose before. This observation suggests that there is some type of metabolic memory that allows cells to recognize or even to anticipate glucose if it has been available in the recent past. In fact, WT and ST cells usually responded to glucose with similar dynamics if the cultures were completely naive to glucose. This similarity was especially striking in the GAL1 mRNA decay curves, where both WT and ST transcripts were quite stable in cells that had never before experienced glucose in the medium (Fig. S7A). Consistent with this, WT Whi5p-YFP localization dynamics did not change in response to glucose when the cells had not been exposed to glucose before (Fig. S7C). These results indicate that cells increase control of GAL1 mRNA levels and G1 regulation in response to dynamic growth conditions.

**SI Materials and Methods**

**Plasmid and Strain Construction.** The plasmids used in this study are described in Table S1. All cloned genes were amplified by PCR using primers to incorporate the necessary flanking restriction sites for subsequent ligations. The PCR reactions were carried out in a 50-μL volume containing 10 μL 5× Buffer HF, 1 μL 10 mM dNTP mix, 4 μL 10-μM primers (total), 25–50 ng DNA template, and 0.5 μL Phusion (NEB). PCR products were cloned into pCR-Blunt II TOPO vector (Invitrogen), according to the manufacturer’s instructions. Amplified genes were then subcloned into appropriately...
ate vectors, as follows: Construction of pBB14 was described in Bennett et al. (2). For pBB21, the GAL1 coding region, starting with the first ATG, was amplified from pBB14 and subcloned into BamHI/NotI-digested pCM185. For pBB31, the genomic region upstream of GAL1 (~300 to −1), including the 5′-UTR, was fused to the YFP gene using nested PCR; the fused product was subcloned into BamHI/NotI-digested pCM185. pBB35 is identical to pBB31, except that it lacks the GAL1 sequence. To create pBB35, a fragment of GAL1 (~1,000 to +450) was amplified by PCR from genomic DNA and subcloned into BamHI/XhoI of pBluscriptII-KS+. The LEU2 marker was then ligated into the GAL1 sequence upstream of the promoter between the EcoRI and Scal sites. To create a randomized 5′-UTRGal1 sequence, the first 100 bp up-stream of GAL1 was subjected to three rounds of randomization resulting in the following sequence:

5′ TAAAAATGCTAGTTATATCATAGAATTCAAA TGGTTCATTCAAGTTCGTTATGATITTTATACCGA TCTAACCAATAACACTAATAGTTCTAA 3′.

To make pBB36, the GAL1 (1 + 1 to +450) fragment was fused to the randomized 5′-UTRGal1 sequence by nested PCR. The upstream GAL1 (~1,000 to −100) fragment was then fused to GAL1 (random to +450) in a second round of nested PCR. The GAL1 (~1,000 to random to +450) fragment was then subcloned into BamHI/Xhol of pBluscriptII-KS+. The TRP1 marker was ligated into the GAL1 sequence upstream of the promoter between the EcoRI and Scal sites. For integration, pBB35 and pBB36 were digested with Dral to release the GAL1 fragment. pBB40 was made by amplifying Gal10 including 700 bp of upstream sequence and ligating the PCR product into SpeI/Sacl of pF6a-hKANMX6. To make pBB49, the CLN3 coding region plus 900 bp of upstream sequence was amplified from genomic DNA and subcloned into SalI/NotI of pRS315. For pBB50, A31ST-CLN3 was amplified from A31ST-pMT10 (generously provided by M. Polymenis) using the same primer pair and ligated into SalI/NotI of pRS315. pBB21-dNdeI (ITa-ST short) was made by digesting pBB21 with NdeI to release an 805-bp fragment and religating the plasmid. pBB21-s851C (ITa-ST short) was made by site-directed mutagenesis using pBB21 as a template.

 Yeast strains used in this study are described in Table S2. All yeast strains were made using standard methods for transformation and selection. The construction of K699-1c2y has been described previously. yBB114 and yBB115 were derived from K699-1c2y by integrating the Dral fragments released from pBB35 and pBB36, respectively. This integration resulted in separating GAL10 from the Gal1′-10 bidirectional promoter. To restore the Gal10 promoter, pBB40 was linearized with StuI and integrated at the Gal10 locus. yBB122 was derived from yBB115 by deleting CLN3 with hphNT1 (hygromycin B resistance), using standard one-step gene replacement.

Measurement of the 5′-UTR of GAL1 mRNA. To amplify the 5′-UTR of GAL1 mRNA, total RNA was prepared from ~3 × 10⁷ K699 cells grown to log phase in synthetic medium with 2% galactose. The RLM-RACE kit (Qiagen) was then used according to the manufacturer’s protocol for 5′ RACE. The gene-specific primers used were GAL1-EcoRI 5′ RACE 1 (5′ GAA TTC GGCAGCAAGCATATAAATCATATGAC 3′) and GAL1-EcoRI 5′ RACE 2 (5′ GAA TTC GGCAGCTACACAAATTCACTGG 3′). Reaction products were separated on a 2% Tris-acetate EDTA agarose gel and visualized by ethidium bromide staining (Fig. S1).

Flow Cytometry. To approximate the growth conditions of the microfluidics experiments, single colonies were inoculated into synthetic medium containing a mixture of glucose and galactose (1%/1%), grown to saturation overnight at 30 °C, diluted 1:100 in 20 mL fresh medium (1%/1%), and grown for 4 h. The cells were then pelleted and resuspended in 20 mL of medium containing 1% galactose and returned to growth at 30 °C for 4 h to induce the galactose network. At t = 0 min, 30 mL fresh medium containing 1.66% glucose was added directly to the galactose culture, to a final glucose concentration of 1%. Samples (5 mL) were collected every 5 min and processed for flow cytometry, as follows: Cells were briefly pelleted and resuspended in 2 mL 70% ethanol (EtOH) and stored at −20 °C overnight. The next day, the EtOH-fixed cells were rehydrated in 5 mL dH₂O, sonicated for 10 s at 40% power, and washed twice in 50 mM Tris, pH 8.0. Cell pellets were then resuspended in RNaseA (2 mg/mL) and incubated overnight at 37 °C. Cells were pelleted again, treated with pepsin (5 mg/mL) for 30 min at 37 °C, and then stained with propidium iodide (50 µg/mL) and run through a FACScan flow cytometer (Becton Dickinson). Flow cytometry data were analyzed using GataEC software. At any given time the culture consists of cells with one copy of the genome (1N) and those that have undergone DNA replication and possess two copies of the genome (2N). The size of the G1 fraction in each sample was estimated by measuring the percentage of cells in the 1N peak; the position of the G1 gate was kept constant for all samples. The reported results are averages of three independent experiments run in parallel.

To look at G1 progression in synchronous cultures (Figs. S3B and S5A), starting cultures were prepared in rich medium containing a mixture of glucose and galactose, diluted 1:100 in the same medium containing 2% galactose, and grown for 20 h. No- codazole (15 µM final) was then added and the culture was incubated for another 2 h until >80% were arrested in G2/M. To release the cells, the nocodazole was removed with three 50-mL washes in sterile dH₂O. The cells were released into fresh medium containing 2% galactose. After 60 min, glucose was added to 2%. Samples were collected every 15 min after release and fixed in 70% EtOH. Fixed samples were sonicated and then stained with DAPI (5 ng/mL) in PBS. The mitotic fraction in each sample was determined by observing the shape of DAPI-stained nuclei. The budding and unbudded fractions were determined by phase-contrast microscopy.

**Growth Rate Measurements in Dynamic Conditions.** For Fig. S6B, single colonies were inoculated into synthetic medium containing a mixture of glucose and galactose (1%/1%), grown to saturation overnight at 30 °C, diluted 1:100 in the same medium, and grown for another 4 h. The density of each culture was then monitored and recorded as OD₆₀₀ vs. time (hours). The graphs represent the growth curves of each strain normalized to 1.0 at time t = 0.

**Polysome Fractionation.** Single colonies were inoculated into synthetic medium containing a mixture of glucose and galactose (1%/1%), grown to saturation overnight at 30 °C, diluted 1:100 in 100 mL fresh medium (1%/1%), and grown for another 4 h. The cells were then pelleted and resuspended in 20 mL of the same medium containing 1% galactose and returned to growth at 30 °C. The density of each culture was then monitored and recorded as OD₆₀₀ vs. time (hours). The graphs represent the growth curves of each strain normalized to 1.0 at time t = 0.
that anneals inside the coding sequence. The diagram represents the position of the two primers in GAL1. The product of the PCR contains the 5′ curves of each strain normalized to 1 at time (1 mL) were collected from the detector (ISCO) equipped with a digital chart recorder. Fractions were then fixed and hybridized according to the protocol at (http://web.mit.edu/biophysics/data.html). Image acquisition was performed on a Nikon Eclipse Ti epifluorescent inverted microscope outfitted with fluorescence filter cubes for DAPI, TRITC, and FITC. Images were acquired using a Photometrics CoolSNAP HQ2 cooled CCD camera controlled by Nikon Elements software and processed in ImageJ. For experiments shown in Fig. 4F, a modified version of the FISH protocol for hybridization of yeast in solution found at http://web.mit.edu/biophysics/data.html was used. The following exceptions were made: We used the same fluorescent probes for GAL1 and CLN3 described above instead of the oligo probes recommended in the published protocol. We also included an extra high-stringency wash step in 2× SSC/40% formamide for 30 min at 37 °C to reduce background. Images were acquired and processed as described above. Quantitation represents results from 100–300 individual cells per hybridization.


Fig. 51. (A) Decay of the tTA-driven 5′-UTRGAL1-YFP fusion transcript in cells growing in glucose (blue) and galactose (red). The 5′-UTRGAL1 destabilized the YFP transcript in glucose, but not galactose. (B) Results of RLM-RACE for GAL1 mRNA. In this experiment, a short RNA linker of known sequence was ligated to the 5′ end of mRNAs. After reverse transcription, the 5′ end of the gene of interest was amplified using a primer that anneals to the linker and a reverse primer that anneals inside the coding sequence. The diagram represents the position of the two primers in GAL1. The product of the PCR contains the 5′-UTR plus 152 bp of GAL1 sequence. (C) Growth rates of WT (blue) and ST (red) in 2% glucose (solid line) and 2% galactose (dashed line). The graph represents the growth curves of each strain normalized to 1 at time t = 0.
Fig. S2.  (A) Schematic of the dial-a-wave (DAW) device described in the text. Ports 1 and 2 are the inputs for the DAW system. Port 3 is an outlet for the shunt network. Port 4 serves as an alternate waste port for the DAW system during the experiment. The device's primary flow network (dark blue) is 10 μm in height and the cell trap (red) is 3.5 μm in height. The region between the dial-a-wave junction and the cell chamber contains staggered herringbone mixers (green), which are 2 μm higher than the channel height. These mixers introduce a corkscrew effect into the fluid flow, which improves mixing. (B) Fluorescence micrograph of WT cells growing in the yeast trap of the DAW device. Cells are constricted to grow in a monolayer and are easily distinguished. To create single cell trajectories of Gal1p-CFP expression, individual cells are selected in ImageJ (yellow circle) and the pixel density inside the circle is measured. The frame is then advanced, the circle position is adjusted so that it remains directly over the same cell, and the process repeated. (C) Dial-a-wave junction region at different mixing ratios, as simulated using the program Comsol. The two inlets (from ports 1 and 2) are on the right side, and the outlet leading to the cell chamber is in the left center. (D) Graphic representing the cell chamber region during cell loading (Upper) and while the experiment is running (Lower). (E) Mixing ratio as the input pressure is increased on port 1. The red line represents the ideal linear response, which the system closely approximates. (F) Sample output of the DAW junction as measured near the cell trap. This is a square wave with a 4-h period and a 22-h duration.
Fig. S3.  (A) Diagram of experimental design for measuring the cell cycle response to glucose in synchronous populations. Cells were grown overnight in YEP medium with mixed glucose and galactose (1%/1%) and then diluted into fresh medium containing 2% galactose and grown to log phase. Nocodazole was then added to arrest cells at the G2/M transition. Arrested cells were released into fresh medium containing 2% galactose and allowed to synchronously pass through mitosis into G1. Mitotic progression was monitored by DAPI staining. When >80% of the cells were in G1, glucose was added to the medium. The transition of cells from G1 into S phase was monitored by bud counting. (B) Results of G1 progression after glucose addition for synchronous WT and ST cultures. The size of the G1 fraction over time after glucose addition is plotted. The WT cells (blue line) responded to glucose by increasing the rate of cell cycle entry. The ST cells (red line) did not respond to the addition of glucose and entered the cell cycle at the same rate as they did in galactose medium (black dashed line). (C) G1 fractions of asynchronous cultures plotted against time after glucose addition. The endogenous GAL1 gene has been deleted in these strains and replaced with a tTA-driven plasmid encoding GAL1 with (WT) or without (ST) its 5'-UTR. Transcription of GAL1 was repressed by addition of doxycycline (dox) to the medium. Cells responded to glucose most rapidly when both levels of repression were used (tTA-WT plus dox). In the absence of glucose-mediated mRNA degradation (ST), transcriptional repression had little effect on the rate of the cell cycle response to glucose (tTA-ST plus dox vs. tTA-ST minus dox). (D) Cell cycle response to glucose plus dox in cells expressing tTA-GAL1 in addition to the endogenous GAL1 gene. The excess WT-GAL1 expression caused a delay in the cell cycle response (blue line). Ectopic expression of ST-GAL1 in this background had a stronger effect (red line) than tTA-WT. The gal1Δ/tTA-WT curve from C is included for comparison. (E and F) Cell cycle response to glucose plus dox in cells expressing tTA-ST plasmids with truncated ORFs. The tTA-ST short allele has an 805-bp deletion from between two NdeI sites in the coding sequence. The tTA-ST shift allele has a frameshift mutation at position 851. Both types of mutations partially suppress the cell cycle delay phenotype of the tTA-ST allele. All graphs are representative results from at least two independent experiments run in triplicate.
Fig. S4. (A) Cell cycle profiles of WT and ST cultures in galactose and galactose plus glucose, measured by flow cytometry. The cell cycle profiles of both WT and ST cultures indicate that each strain spent less time in G1 phase (represented by the indicated peak) when glucose was available. (B) Time course of cell cycle profiles for WT and ST cells after glucose addition. Batch cultures were grown to log phase in medium containing 2% galactose. At time $t = 0$ min, glucose was added to the culture to 2%. Cells were collected every 5 min, fixed, and processed for flow cytometry. (C) Increased global levels of protein synthesis in response to glucose leads to the production of Cln3p, the first cyclin in the cascade that drives the cell cycle. Cln3p enters the nucleus and causes Whi5p, an inhibitor of S phase, to be expelled from the nucleus. Cln3p also induces the expression of a number of cell cycle genes, such as CLN2, that promote S phase and reinforce the cytoplasmic retention of Whi5p. This feedback loop makes the entry into S phase irreversible and commits the cell to division. In this experiment, cells expressing Whi5p-YFP were grown in a microfluidic chemostat and imaged every 5 min using time-lapse fluorescence microscopy. Whi5p-YFP is easily detected in the nuclei of G1 cells, but the signal is weak when Whi5p is dispersed in the cytoplasm upon cell cycle entry. The heat map key shows the measured change in the level of maximum Whi5p-YFP signal for the indicated cell (white arrow) as it progressed through a division cycle. (D) Trajectories of Whi5p-YFP signal in single WT (Upper) and ST (Lower) cells growing in constant galactose expressed as heat maps. (E) Complete set of trajectories of Whi5p-YFP signal in single WT (Upper) and ST (Lower) cells growing in constant galactose with 4-h pulses of glucose every 4 h, expressed as heat maps. The dashed lines indicate periods during which glucose was present.
Fig. S5.  (A) G1 fraction of synchronous cultures of WT and ST (from Fig. S3A) and ST + CLN3 cultures over time after glucose addition, measured as for Fig. S3A. (B) Schematic of polysome fractionation of WT and ST cells 30 s after glucose addition. Fractions were collected and analyzed by spectrophotometry (A_{254}) to detect ribosome complexes. Total RNA was prepared from each fraction and the relative level of CLN3 mRNA was measured by qRT-PCR. (C) Polysome profiles (A_{254}) of a WT culture growing in galactose for 3 h and the same culture 30 s after the addition of glucose. (D) Overlay of A_{254} traces for WT and ST cultures 30 s after glucose addition. (E) GAL1 polysome distribution in galactose and 30 s after glucose addition. After glucose addition, GAL1 mRNA shifts to the right for both WT and ST, indicating an increase in polysome association, consistent with the global rise in translation. As expected, the level of GAL1 mRNA decreases in the WT culture, but not in the ST culture. (F) CLN3 polysome distribution in galactose and 30 s after glucose addition. CLN3 mRNA shifts to the right in response to glucose; however, the induction of CLN3 translation in response to glucose is greater for WT than for ST cultures. In both E and F the galactose distributions shown are from the ST strain; there were no significant differences between WT and ST polysome distributions in galactose cultures.
Increased translation of CLN3 mRNA inhibits GAL1 translation. (A) Comparison of relative GAL1 mRNA levels and Gal1p levels in various strains, 2.5 h after cells were switched to growth in galactose. mRNA levels were measured in batch cultures by qRT-PCR; Gal1p levels are averaged from single-cell Gal1p-CFP trajectories derived from time-lapse microscopy experiments; error bars indicate ±1 SD. Increased expression of CLN3 led to a slower accumulation of Gal1p in ST cells, which could not be attributed to lower GAL1 mRNA levels. Increased CLN3 translation (A315T-CLN3) exacerbated this effect, indicating that the increased translation of CLN3 occurred at the expense of GAL1 translation. (B) Growth of various strains immediately after a switch to galactose in batch cultures. ST + A315T-CLN3 cells exhibited a growth defect when switched to growth on galactose. (C) Phase-contrast and fluorescence micrographs of representative WT and WT + CLN3 cells during the first 200 min of galactose induction. Upon being switched to growth in galactose, cells of both genotypes stalled wherever they were in their division cycle until Gal1p had accumulated. The contrasts of the fluorescence images are scaled independently for each individual trajectory. (D) Average Gal1p-CFP accumulation in WT (Top), WT + CLN3[A315T] (Middle), and WT + cln3−ΔNLS cells (Bottom), which overexpress a nonfunctional Cln3 protein, growing in a microfluidic chemostat; error bars are ±1 SD. (E) Time-lapse images of Gal1p-CFP and Gal2p-YFP induction in WT and WT + CLN3 cells. As shown in D, Gal1p-CFP accumulation is inhibited in WT + CLN3. In contrast, Gal2p-YFP accumulates at the same rate in both strains and is not affected by increased CLN3 expression.
Fig. S7. (A) GAL1/CLN3 foci were rarely detected by FISH in WT cells that had been grown in galactose for 24 h. Cells with diffuse GAL1 mRNA staining (Lower) did not show colocalization with CLN3 mRNA. (B) Quantitation of GAL1 mRNA foci detected in WT cells grown under dynamic conditions or in constant galactose: combined results of two independent hybridization experiments. (C) GAL1 mRNA decay curves for WT and ST strains grown in dynamic conditions after glucose addition (from Fig. 1C). (D) GAL1 mRNA decay curves for WT and ST strains grown in static galactose conditions after glucose addition. Cells were grown overnight in medium containing 2% galactose and then diluted into the same medium 4 h before glucose was added. Following glucose addition, samples were collected every 5 min and processed for qRT-PCR, as described in Fig. 1C. (E) Whi5p-YFP trajectories of single WT cells grown in static galactose conditions. Cells were grown overnight in medium containing 2% galactose and then diluted into the same medium 4 h before being loaded into the microfluidic chip. Whi5p-YFP localization was recorded over time by time-lapse microscopy, as described in Fig. 3. The dashed black line indicates the introduction of glucose into the growth medium. In contrast to what was observed in dynamic conditions, no change in G1 duration occurred after glucose addition. These results show that cells that are naive to glucose do not respond rapidly to the addition of glucose to the growth medium.
Ribosome Bank Model

Protein synthesis

mRNA for reproduction

mRNA for growth

cell components and division machinery

AND

ATP and building blocks

GAL1 mRNA

CLN3 mRNA (with uORF)

ribosome

B Translational Bottleneck Model

galactose

Cln3p

mRNA for growth

Protein synthesis

cell division

Fig. 58. (A) GAL1 and CLN3 transcripts are spatially coregulated when the environment dynamically switches between galactose and glucose. This observation indicates that these transcripts may compete for a local pool of translation components, explaining their mutual sensitivity to reciprocal changes in mRNA levels. The uORF on CLN3 messages (depicted as a black bead) makes GAL1 more competitive for translation when both types of mRNA are present. Therefore, in galactose GAL1 is strongly expressed and cell division is slowed through G1, allowing Gal1p to accumulate to high levels. When glucose becomes available, GAL1 transcripts are rapidly degraded, freeing up the ribosomes in the region, which CLN3 transcripts can take advantage of. This process leads to a concomitant rise in CLN3 translation and the induction of cell division. Therefore, the region of concentrated GAL1 transcripts acts like a ribosome bank for CLN3 in the event that glucose is suddenly added to the environment. (B) Model for how energy balance is achieved in different carbon sources. The spatial regulation of GAL1 and CLN3 mRNAs creates a translation bottleneck. Because both proteins that derive energy from carbon metabolism and those that assemble cell cycle components are required for cell division, a balance must be struck in the translation of mRNAs for each function. If the translation of cell cycle machinery increases, it does so at the expense of GAL gene translation, and cell division is delayed presumably because the cell cannot extract enough energy from galactose. On the other hand, if GAL1 mRNA persists in the cytoplasm after glucose becomes available, the translation of cell division components (CLN3) is hampered, and G1 is prolonged despite the availability of energy from the breakdown of glucose. Because glucose metabolism requires only the basal level of gene expression, this model may explain why it supports the fastest growth rate of yeast cells and may illustrate the biological basis of diauxic growth.

Table S1. Plasmids used in this study

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Table S2. Strains used in this study

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**Table S3. qPCR primers used in this study**

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