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Regulation of Cell Size by Glucose Is Exerted via Repression of the *CLN1* Promoter

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Yeast cells are keenly sensitive to the availability and quality of nutrients. Addition of glucose to cells growing on a poorer carbon source elicits a cell cycle delay during G_1 phase and a concomitant increase in the cell size. The signal is transduced through the *RAS*-cyclic AMP pathway. Using synchronized populations of G_1 cells, we show that the increase in cell size required for budding depends upon *CLN1* but not other G_1 cyclins. This delay in cell cycle initiation is associated specifically with transcriptional repression of *CLN1*. *CLN2* is not repressed. Repression of *CLN1* is not limited to the first cycle following glucose addition but occurs in each cell cycle during growth on glucose. A 106-bp fragment of the *CLN1* promoter containing the three MluI cell cycle box (MCB) core elements responsible for the majority of *CLN1*-associated upstream activation sequence activity is sufficient to confer glucose-induced repression on a heterologous reporter. A mutant *CLN2* promoter that is rendered dependent upon its three MCB core elements due to inactivation of its Swi4-dependent cell cycle box (SCB) elements is also repressed by glucose. The response to glucose is partially suppressed by inactivation of *SWI4*, but not *MBP1*, which is consistent with the dependence of MCB core elements upon the SCB-binding transcription factor (SBF). We suggest that differential regulation of *CLN1* and *CLN2* by glucose results from differences in the capacity of SBF to activate transcription driven by SCB and MCB core elements. Finally, we show that transcriptional repression is sufficient to explain the cell cycle delay that occurs in response to glucose.

The ability to mount adaptive responses to environmental changes is a fundamental property of living systems. This capacity is apparent at both the organismal and the cellular levels and reflects the existence of signaling pathways via which environmental conditions are translated into cellular responses. The stimuli provoking such responses are diverse, as are the mechanisms through which those signals are transduced. Although our understanding of machinery generating and transducing these signals is in many cases limited, it is clear that many of these signals ultimately result in alteration of the pattern of gene expression.

The availability and quality of nutrients comprise one of the environmental conditions which cells must monitor and to which they must respond. This is especially important to free-living microorganisms, such as the budding yeast *Saccharomyces cerevisiae*, which experience frequent dramatic changes in nutrient conditions and as a result require reliable mechanisms for coordinating growth with cell cycle progression. In those cells the decision of whether and when to initiate a new cell cycle occurs during the G_1 interval. When glucose, the favored fermentable carbon source, becomes available to cells growing on poorer carbon sources, they not only change the pattern of expression of many genes involved in carbohydrate metabolism (reviewed in reference 28) but also undergo an increase in cell size (17, 19). That is, upon introduction of glucose the cells transiently delay cell cycle progression during the G_1 phase and so attain a larger size than cells growing on poorer carbon sources. This increase has been attributed to a resetting of the minimal cell size required for budding (19).

Upon addition of glucose to the growth medium cells experience a transient spike in intracellular cyclic AMP (cAMP) levels followed by a resetting of basal cAMP to a higher level (4, 29, 35, 36). This increase in cAMP requires activation of adenylate cyclase, which in yeast results from activation by Ras-GTP (reviewed in reference 34). It is not clear whether the spike, the increased basal level, or both are important for the physiological responses to glucose. The only known target for cAMP in yeast cells is cAMP-dependent protein kinase. A number of observations support the view that the adjustment of cell size in response to glucose is induced via this pathway. First, mutants with reduced Ras pathway activity or with reduced but unregulated cAMP-dependent protein kinase activity bud at a smaller cell volume on poor carbon sources and fail to increase their size in response to glucose (1, 3, 36). Next, cells expressing constitutively activated Ras2, which in yeast is an activator of adenylate cyclase, display a large cell size when growing on nonglucose carbon sources and fail to further increase that size in response to glucose (1). Finally, artificially elevating the level of intracellular cAMP (by applying high levels of cAMP exogenously to cells compromised for cAMP turnover) results in an increase in the minimal budding size of cells growing on glucose (3, 36). It has not been possible to evaluate whether cAMP can mimic the effect of glucose on the size of cells growing on other carbon sources because exogenous cAMP is lethal to cells under those conditions (29). Nevertheless, these observations support a role for cAMP and, as a consequence, for cAMP-dependent protein kinase as mediators of the glucose-induced increase in the minimum budding size.

Genetic analysis suggests that the G_1 cyclins are critical targets for the glucose–cAMP-induced increase in cell size (3, 36). This is consistent with the observation that cell volume at

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TABLE 1. Yeast strains used in this study

Strain	Relevant genotype[a]	Source or reference
15Daub	*MAT***a** *ade1 his2 leu2 trp1 ura3Δns bar1Δ*	38
CWY228	*MAT***a** *cln1::TRP1*	38
CWY229	*MAT***a** *cln2::LEU2*	38
DSY560	*MAT***a** *cln3Δ ade1 his2 leu2 trp1 ura3Δns*	This study
CWY227	*MAT***a** *cln1::TRP1 cln3::URA3*	This study
DSY790	*MAT***a** *cln2x/s cln3Δ LEU2::GAL1-CLN3*	This study
DSY12	*MAT***a** *swi4::LEU2*	32
DSY910	*MAT***a** *mbp1::URA3*	33
CWY762	*MAT***a** *cln2::LEU2 TRP1::CLN2p-CLN1*	This study
CWY765	*MAT***a** *cln2x/s cln3Δ LEU2::GAL1-CLN3 TRP1::CLN2p-CLN1*	This study
CWY780	*MAT***a** *cln1::TRP1 URA3::CLN1p-CLN2*	This study
CWY785	*MAT***a** *cln1Δ cln3Δ LEU2::GAL1-CLN3 URA3::CLN1p-CLN2*	This study

[a] All strains are derivatives of 15Daub and have the same genotype except as indicated.

budding is largely a reflection of the activity of G_1 forms of the cyclin-dependent protein kinase Cdc28 and, consequently, of the abundance of the G_1 cyclins (8, 15, 21). Of the three G_1 cyclins, Cln1 and Cln2 appear to be largely responsible for promoting cell cycle progression, whereas Cln3 acts primarily to promote transcription of *CLN1* and *CLN2* and other G_1-specific genes (12, 33). Although all three G_1 cyclins are important in establishing the size at which cells initiate a new cell cycle, *CLN1* and, to a lesser extent, *CLN2* appear to be targets of the cAMP signal (3, 36). Consistent with the results of that genetic analysis, exogenously added cAMP strongly represses expression of both *CLN1* and *CLN2.*

Glucose is the physiologically relevant inducer of this response which resets the cell volume required for budding. Using synchronized populations of G_1 daughter cells, we confirm that *CLN1* is the primary target for the glucose-induced size increase. We show that the glucose-induced increase in cell size is exerted, largely, through repression of *CLN1* expression, that this repression occurs during each cell cycle when cells are growing on glucose, and that repression occurs at the level of the promoter. Swi4-dependent cell cycle box (SCB)-binding transcription factor (SBF)-dependent upstream activation sequence (UAS) elements of *CLN1* are probable targets for this repression. In contrast, neither the SBF-dependent gene *CLN2* nor the MluI cell cycle box (MCB)-binding transcription factor (MBF)-dependent gene *RNR1* is transcriptionally repressed in response to glucose. Both are strongly repressed by exogenously added cAMP. Our data support the view that differential regulation of *CLN1* and *CLN2* results from differences in their *cis*-acting binding sites for SBF. Finally, we show that regulation of the *CLN1* promoter is sufficient and, at least in part, necessary for modulation of cell size in response to glucose. Thus, although *CLN1* and *CLN2* are coordinately regulated under many conditions (40), the present study provides one condition in which differential transcriptional regulation of these genes leads to a physiological response.

MATERIALS AND METHODS

Strains and plasmids. The yeast strains used in this study were all congenic with 15DaubΔ, a *bar1Δ ura3Δns* derivative of BF264-15D (*MAT***a** *ade1 his2 leu2-3,112 trp1-1*[a]) (27). The relevant genotypes of individual strains used in this study are provided in Table 1.

The episomal *CEN4 CYC1-lacZ* reporter gene plasmids described in this report are all derivatives of pCZΔ (6). Constructions of plasmids p*CLN2-lacZ* (pΔ5′728) and p*CLN2Δscb-lacZ* (pΔ*scb*) were as described previously (32). For the plasmid p*CLN1p-lacZ*, the *Xho*I/*Afl*II fragment (−683 to −267) containing the *CLN1* promoter was ligated to *Xho*I and *Xba*I linkers, respectively, digested with the appropriate enzyme, and cloned into a *Xho*I/*Xba*I-digested pCZΔ plasmid. The plasmid p*CLN1*(UAS)-*lacZ* contains a 106-bp fragment of the *CLN1* promoter (−633 to −527) driving *CYC1-lacZ*. This fragment was obtained by annealing four oligonucleotides containing this sequence and *Eco*RI and *Sal*I sites at the 3′ and 5′ ends, respectively. The fragment was cloned into the *Eco*RI and *Sal*I site of pCZΔ.

To generate p*CLN2p-CLN1*, the *Sal*I/*Bam*HI fragment of pΔ5′728 was cloned into YIplac204. The resulting plasmid, YIplac204-*CLN2p*, was then digested with *Xho*I, blunted with T4 polymerase, ligated to a *Kpn*I linker, and digested with *Sac*I and *Kpn*I. The *Kpn*I/*Sac*I fragment of *CLN1* (−333 to 456) was ligated into *Sac*I/*Kpn*I-digested YIplac204-*CLN2p*. The resulting plasmid, YIplac204-*CLN2p-CLN1*, was linearized with *Spe*I and transformed into yeast. Transformants having the appropriate genomic structure were identified by PCR.

To generate p*CLN1p-CLN2*, the *Fsp*I/*Spe*I fragment of *CLN2* (−341 to 489) was ligated to *Kpn*I linkers, digested with *Kpn*I, and ligated into *Kpn*I/*Xba*I-digested YIplac211, resulting in YIplac211-*CLN2*. A *Kpn*I fragment of *CLN1* (−673 to −334) was ligated into the *Kpn*I-digested plasmid YIplac211-*CLN2*. The resulting plasmid, YIplac211-*CLN1p-CLN2*, was linearized with *Xho*I and transformed into yeast. Transformants with the appropriate genomic structure were identified by PCR.

Culture conditions, cell synchronization, and cell size analysis. All strains were grown in standard culture media: yeast extract-peptone-dextrose, minimal complete medium, or minimal medium lacking uracil. The carbon source was were either 2% glucose or 2% glycerol–1% ethanol.

Synchronous populations of small G_1 cells were obtained by centrifugal elutriation. Cells were inoculated into 2 liters of minimal medium containing glycerol-ethanol as a carbon source and grown overnight at 30°C to an optical density at 600 nm of 0.5. Centrifugal elutriation was performed as described by Stuart and Wittenberg (33). The elutriated population of G_1 cells was concentrated by centrifugation and divided into two aliquots which were resuspended in 200 ml of prewarmed minimal medium containing either glucose or glycerol-ethanol. Samples were taken for cell size analysis, determination of budding index, and RNA analysis at 20-min intervals for a period of 3 to 9 h.

Cell size analysis was performed with a Coulter Channelyzer. Statistical analysis was performed with software developed by Stefan Lanker and C.W. Approximately 1.5×10^5 cells were diluted into 20 ml of physiological saline, and the mean cell volume in femtoliters was determined.

For determination of budding index, 200 formaldehyde-fixed cells were counted and the proportion of budded cells was determined for each time point.

For RNA analysis from asynchronous cultures, cells were grown at 30°C to an optical density at 600 nm of 0.2 on minimal complete medium containing either 2% glucose or 2% glycerol–1% ethanol. Cells were harvested, and RNA was isolated as described below.

RNA analysis. Total RNA was obtained from frozen cell pellets as described previously (32). RNase protection analysis was performed on 10 μg of total RNA with *CLN2*, *ACT1*, and *lacZ* riboprobe as described previously (32). The template for the *CLN1* riboprobe consisted of a 190-bp fragment from the 5′ end of the *CLN1* gene starting with the start codon. This fragment was cloned into pSP72 (Promega), and antisense riboprobes were synthesized from the RNA polymerase T7 promoter.

Northern blot analysis of 10 μg of total RNA was performed according to standard protocols (30). Probes were prepared from 1.6-kb *CLN1* and *CLN2* open-reading-frame, 1.6-kb *ACT1* (*Bam*HI/*Hin*dIII) and 2.6-kb *RNR1* (*Eco*RI) fragments by random priming (Random Primed DNA Labeling Kit; Boehringer Mannheim).

Quantitation of the RNase protection and Northern blot data was performed with a PhosphorImager (Molecular Dynamics).

RESULTS

Glucose induces an increase in the minimum budding size of cells growing on poorer carbon sources. Yeast cells undergo an increase in the minimal volume required for budding when

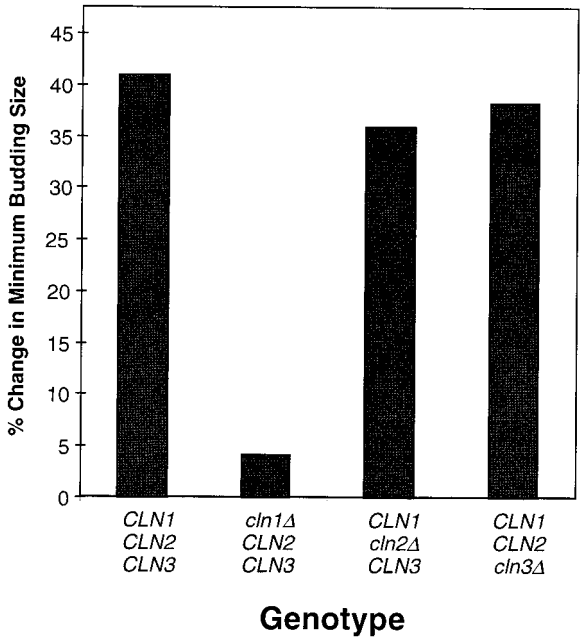

FIG. 1. The percent change in minimum budding size of wild-type and *CLN*-deficient strains following a shift from glycerol-ethanol to glucose-containing medium. Synchronous populations of unbudded G_1 daughter cells prepared by centrifugal elutriation from cells growing on glycerol-ethanol-containing medium were either reinoculated into fresh glycerol-ethanol-containing medium or inoculated into medium containing glucose. Samples were then taken at 20-min intervals and analyzed for cell size and budding index. Minimum budding size (MBS) is defined for the purpose of this study as the cell volume at which the budding index of a synchronous population of G_1 daughter cells achieves 50% of its maximal level. A representative experiment is presented for each strain. The percent change is defined as follows: % change in MBS = [(MBS on glucose − MBS on glycerol-ethanol)/MBS on glycerol-ethanol] × 100.

shifted to glucose from a poorer carbon source (17, 19). This is best observed by evaluating the mean cell volume at budding in synchronous populations of small G_1 daughter cells obtained by centrifugal elutriation. A population of small G_1 cells was prepared from cells growing on a nonfermentable carbon source (2% glycerol–1% ethanol) and reinoculated either into the same medium or into 2% glucose; samples were then collected at 20-min intervals for analysis of cell volume and of the proportion of budded cells as an indicator of cell cycle initiation. An increase of ~40% in the cell volume required for budding was observed in the glucose-grown cultures relative to those maintained on glycerol-ethanol medium (Fig. 1 and Fig. 2A, top panel). A similar adaptation has been shown to occur upon shift into glucose from a number of other carbon sources (19, 31). It should be noted that although the growth rate of the cells inoculated into glucose greatly exceeds that of cells reinoculated into glycerol-ethanol medium, the glucose-grown cells initiate budding at a later time than the glycerol-ethanol-grown cells. Thus, the size increase is not simply due to the increase in growth rate but results, at least in part, from a delay in the capacity of cells to initiate a new cell cycle. The terms cell size increase and cell cycle delay are used interchangeably throughout this study.

The glucose-induced G_1 delay is mediated primarily through *CLN1*. To investigate the importance of specific G_1 cyclins in effecting the delay we have carried out the same analysis shown in Fig. 2A using a series of isogenic yeast strains carrying single and double mutations in the *CLN1*, *CLN2*, and *CLN3* genes. The change in minimum budding size of each of these strains following a shift from glycerol-ethanol to glucose-containing media (Fig. 1) demonstrates that *CLN1* is of primary importance in effecting the cell cycle delay. Cells lacking functional *CLN1* did not undergo an increase in minimum budding size. Furthermore, cells with *CLN1* as their only functional *CLN* gene (see Fig. 5B) reached a cell volume prior to budding that was almost twice that of cells growing in glycerol-ethanol-containing medium. This finding is in agreement with previous studies with glucose or exogenous cAMP (2, 36). This more extensive analysis establishes that the capacity of glucose to induce a G_1-phase delay is unaffected by the characteristic cell size of the strain. That is, the extent to which cells are affected by glucose appears not to be related to the relative capacity of their functional cyclins to promote cell cycle progression but instead depends upon whether the specific cyclin that remains functional is a target for that regulation. For example, *CLN3*-deficient cells, which are substantially larger than wild-type cells when growing on either carbon source, undergo a comparable delay upon shift into glucose. In contrast, cells deficient in *CLN1* are comparable in size to wild-type cells but fail to undergo a significant size increase in response to glucose. Finally, cells ectopically expressing *CLN2* during early G_1 phase (under control of the *CLN3* promoter) bud at an exceedingly small cell size (33, 38) but still undergo a substantial delay in response to glucose (7). The delay in those cells remains dependent upon a functional *CLN1* gene.

Glucose differentially regulates *CLN1* and *CLN2* transcription. The relatively greater importance of *CLN1* over *CLN2* in mediating the cell cycle delay in response to glucose is consistent with the effect of glucose on accumulation of the *CLN1* and *CLN2* transcripts. The abundance of those transcripts in RNA prepared from cells responding to a shift from glycerol-ethanol into glucose was analyzed by an RNase protection assay (Fig. 2A). As suggested by previous analysis with asynchronous populations (36), the accumulation of *CLN1* transcripts in small G_1 daughter cells is strongly repressed in response to glucose relative to its level in cells maintained on glycerol-ethanol-containing medium. In contrast, the accumulation of a number of G_1-specific transcripts, including *CLN2*, *RNR1* (Fig. 2A), and *SWI4* (14), is not repressed. Despite the differences in their expression level, all of these G_1-specific transcripts, including *CLN1*, are delayed in response to glucose. The differential effect of glucose contrasts with the strong repression of all of these genes that is observed in response to exogenously applied cAMP (2, 36).

Since cells growing continuously on glucose are larger and have a larger minimum budding size than cells grown on poorer carbon sources, it seemed possible that repression of *CLN1* expression occurred during each cell cycle in glucose-grown cells. We have examined that hypothesis in two ways. First, small G_1 cells were prepared by centrifugal elutriation from an asynchronous population growing on glucose. The G_1 population was then reinoculated into glucose and subjected to the same analysis as performed previously. To assess the relative level of expression, the *CLN1* and *CLN2* transcripts were analyzed with the same preparation of RNA probe as used for the analysis presented in Fig. 2A. The level of the *CLN1* and *CLN2* transcripts was then normalized to the maximal level accumulated on glycerol-ethanol medium (Fig. 2B). This experiment clearly shows that the level of the *CLN1* transcript is reduced in each cell cycle during growth on glucose relative to the level in glycerol-ethanol-grown cells, whereas the level of the *CLN2* transcript is unaffected. That observation predicts that *CLN1* expression should be reduced in asynchronous populations of cells growing continuously on glucose compared to those growing on glycerol-ethanol. To test that prediction we

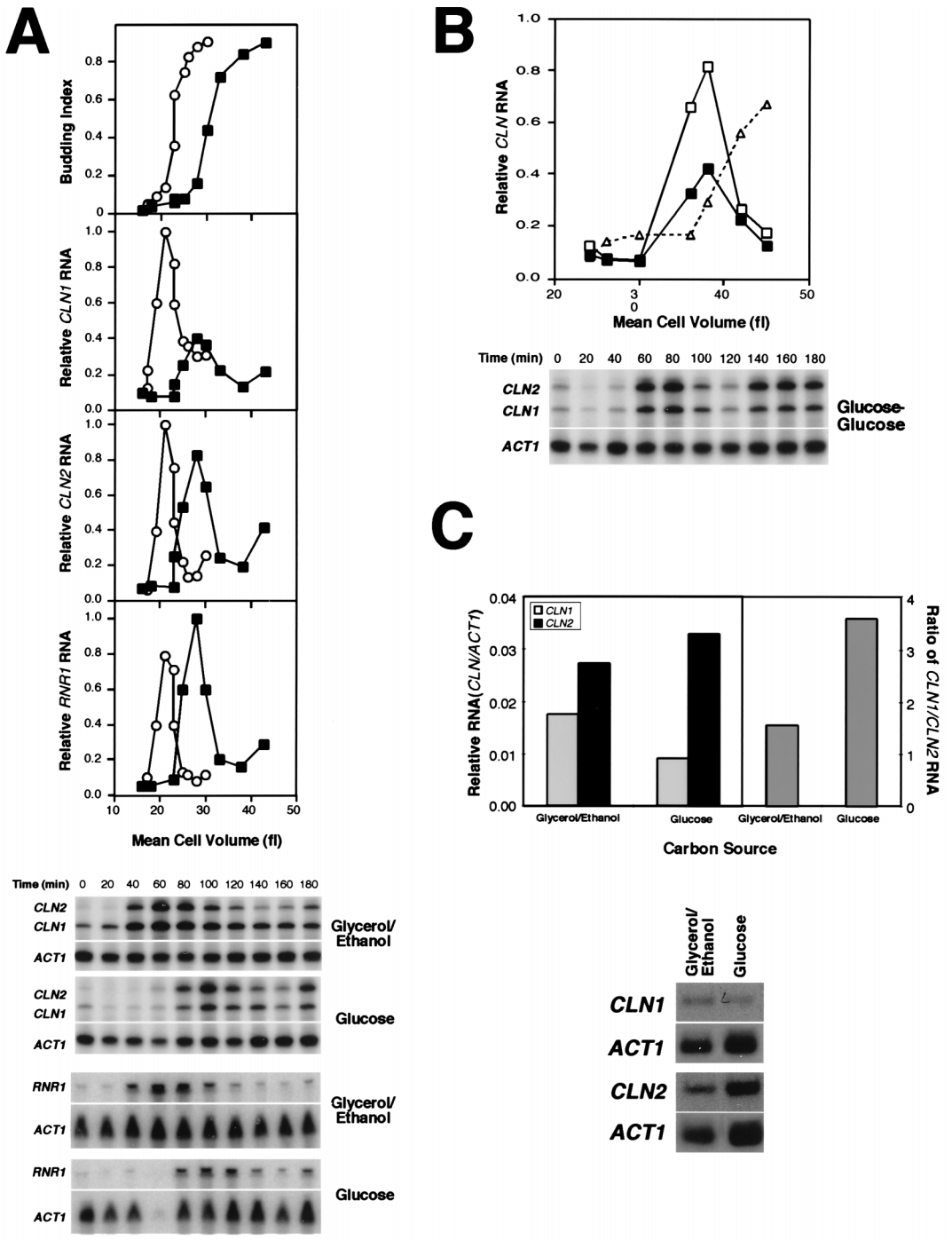

FIG. 2. (A) Glucose differentially regulates the accumulation of *CLN1* and *CLN2* transcripts. A synchronous culture of small G_1 cells (15Daub) pregrown on minimal complete medium containing glycerol-ethanol was prepared by centrifugal elutriation and inoculated into fresh medium containing either glycerol-ethanol (○) or glucose (■) as a carbon source. Samples were taken at 20-min intervals over a period of 3 h and evaluated for cell volume, budding index, and *CLN1*, *CLN2*, and *RNR1* transcript levels. The budding index is plotted relative to cell volume (top graph). *CLN1*, *CLN2*, and *RNR1* transcript levels (normalized to *ACT1* transcript level and then presented as the proportion of the maximal level of that transcript achieved on glycerol-ethanol-containing medium) were plotted as a function of cell volume (lower three graphs). Primary data from RNase protection assays for *CLN1*, *CLN2*, and *ACT1* and Northern blots for *RNR1* and *ACT1* are presented in the lower part of panel A. (B) *CLN1* transcription is repressed in cells maintained on glucose during each cell cycle. A synchronous culture of small G_1 cells (15Daub) was pregrown on glucose, elutriated, and then reinoculated into glucose. *CLN1* (■) and *CLN2* (□) transcripts were analyzed as described for panel A with the same preparations of probe. The transcript levels are presented as values relative to the maximal level achieved on glycerol-ethanol medium. The budding index is plotted relative to cell volume (△) in the same graph. Primary data from RNase protection assays for *CLN1*, *CLN2*, and *ACT1* are presented in the lower part of panel B. (C) Comparison of *CLN1* and *CLN2* levels in asynchronous cultures during continuous growth on glycerol-ethanol or glucose. Cells were grown to log phase on either glycerol-ethanol- or glucose-containing medium, and the level of *CLN1* (gray bars) and *CLN2* (black bars) transcript was evaluated relative to that of *ACT1* by Northern blot analysis (left panel). The ratio of *CLN1* to *CLN2* transcript level under each condition is also presented (right panel). Primary data from the Northern blot analysis are presented in the lower part of panel C.

analyzed the level of *CLN1* transcript accumulating in unperturbed asynchronous populations growing on each carbon source (Fig. 2C). Both the absolute level of the *CLN1* and the *CLN2* transcripts and the ratio of the *CLN1* transcript to the *CLN2* transcript under each condition are presented. The ratio of *CLN1* to *CLN2* is utilized to eliminate the potential contribution of differences in cell cycle distribution between the two populations, which are doubling at different rates. Again, even in populations containing both mother and daughter cells, the repression of *CLN1* by glucose is easily detectable. Thus, it is likely that repression of *CLN1* occurs during the G_1 phase of each cell cycle and contributes to the larger size of glucose-grown cells.

Glucose-induced repression of *CLN1* transcription is exerted through the major *CLN1* UAS. Experiments were next undertaken to establish whether the specific repression of *CLN1* observed in the previous experiments occurred at the level of transcription initiation. To address this issue a UAS-deficient *CYC1* promoter fused to the *lacZ* open reading frame (6) was placed under control of either the *CLN1* promoter or the *CLN2* promoter and introduced into a wild-type yeast strain. Expression of *lacZ* from that construct was analyzed by RNase protection in a synchronous culture of G_1 daughter cells undergoing a shift from glycerol-ethanol to glucose according to the same experimental regimen described previously (Fig. 3). The behavior of the *lacZ* transcript expressed from these promoters was found to be identical to the behavior of the endogenous *CLN1* and *CLN2* transcripts. The *CLN1* promoter was repressed in response to glucose, whereas the maximal activity of the *CLN2* promoter was essentially unchanged. Thus, the *CLN1* promoter is sufficient to confer glucose-induced repression upon the *CYC1-lacZ* reporter. We conclude that regulation of *CLN1* by glucose is exerted at the level of transcription initiation as opposed to transcript stability.

The coordinate expression of *CLN1* and *CLN2* during the cell cycle has been presumed to be a consequence of the presence of SCB elements (9, 22, 23, 32) in both promoters. SCB elements are target sites for SBF (reviewed in reference 5), one of the primary mediators of G_1-specific transcription. However, recent findings of Partridge et al. (24) demonstrate that the major UAS activity associated with the *CLN1* promoter is confined to a 106-bp fragment which lacks recognizable SCB elements and that the SCB elements which can be recognized have no associated UAS activity. Instead, the major *CLN1* UAS has multiple sequences related to MCB elements, the target site for MBF (reviewed in reference 5) (Fig. 4A), a second G_1-specific transcriptional activator. That study further showed that these MCB core elements are responsible for approximately 75% of G_1-specific expression of *CLN1* and that they act as binding sites for SBF in vitro. We therefore considered the possibility that these sequences were the targets for negative regulation of *CLN1* transcription by glucose.

To evaluate the involvement of the major *CLN1* UAS in glucose-induced repression of *CLN1* transcription, a 106-bp fragment of the *CLN1* promoter containing the MCB core sequences was placed upstream of the *CYC1-lacZ* fusion in pCZΔ and introduced into a wild-type yeast strain. This strain was then subjected to the same experimental regimen as that utilized in the previous experiments, and the accumulation of *lacZ* transcripts was analyzed (Fig. 4B). As in the previous experiment in which the intact *CLN1* promoter was used, the accumulation of *lacZ* transcripts was repressed when cells were transferred from glycerol-ethanol to glucose. This repression correlated with the G_1 delay induced by glucose. Thus, the

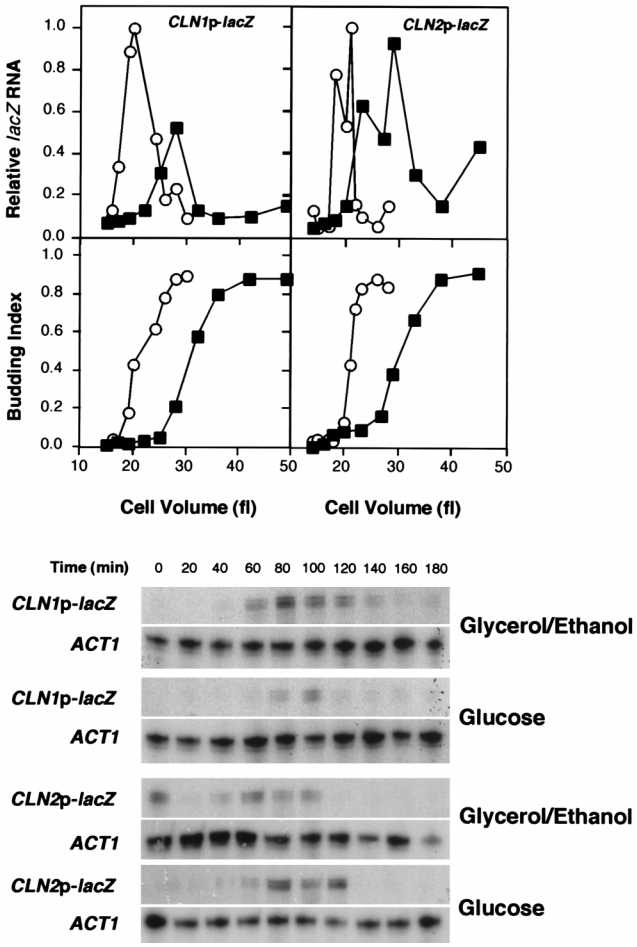

FIG. 3. Glucose-induced repression of *CLN1* occurs at the level of the promoter. Wild-type cells (15Daub) carrying either *CLN1p-CYC1-lacZ* or *CLN2p-CYC1-lacZ* in the centromeric plasmid pCZΔ (6) were subjected to the same regimen as described for Fig. 2A. The *lacZ* and *ACT1* transcripts were quantitated by RNase protection assay, and the values from cells growing in glycerol-ethanol (○)- or glucose (■)-containing medium were plotted relative to the maximal level achieved on glycerol-ethanol-containing medium (top graph). The budding index is plotted versus the cell volume (bottom graph). Primary data from the RNase protection assays for *lacZ* and *ACT1* are presented below. Low points in *CLN2p-lacZ* samples at 80 min on glycerol-ethanol and at 100 min on glucose resulted from degradation of high-molecular-weight RNA in those samples.

106-bp fragment carrying the major *CLN1* UAS was sufficient to confer G_1-specific repression on *CYC1-lacZ*.

Since this small fragment of the *CLN1* promoter, which contains three MCB core elements, was sufficient to explain glucose repression of *CLN1* expression, we considered the possibility that repression was exerted through those elements. The presence of active MCB core elements in the *CLN2* promoter allowed us to test this hypothesis (22, 23, 32) (Fig. 4A). In contrast to their primary role in the *CLN1* promoter, the MCB core elements of the *CLN2* promoter contribute less than half of its UAS activity (32). Thus, mutational inactivation of all three SCB elements yielded a promoter (*CLN2Δscb*) that drives cell cycle-dependent transcription to approximately 30% of the wild-type level, the majority of which can be attributed to the MCB core elements (32). Cells carrying the *CLN2Δscb* promoter fused to *CYC1-lacZ* in pCZΔ were subjected to the same synchrony and carbon source shift regimen as in the previous experiments, and the accumulation of the *lacZ* re-

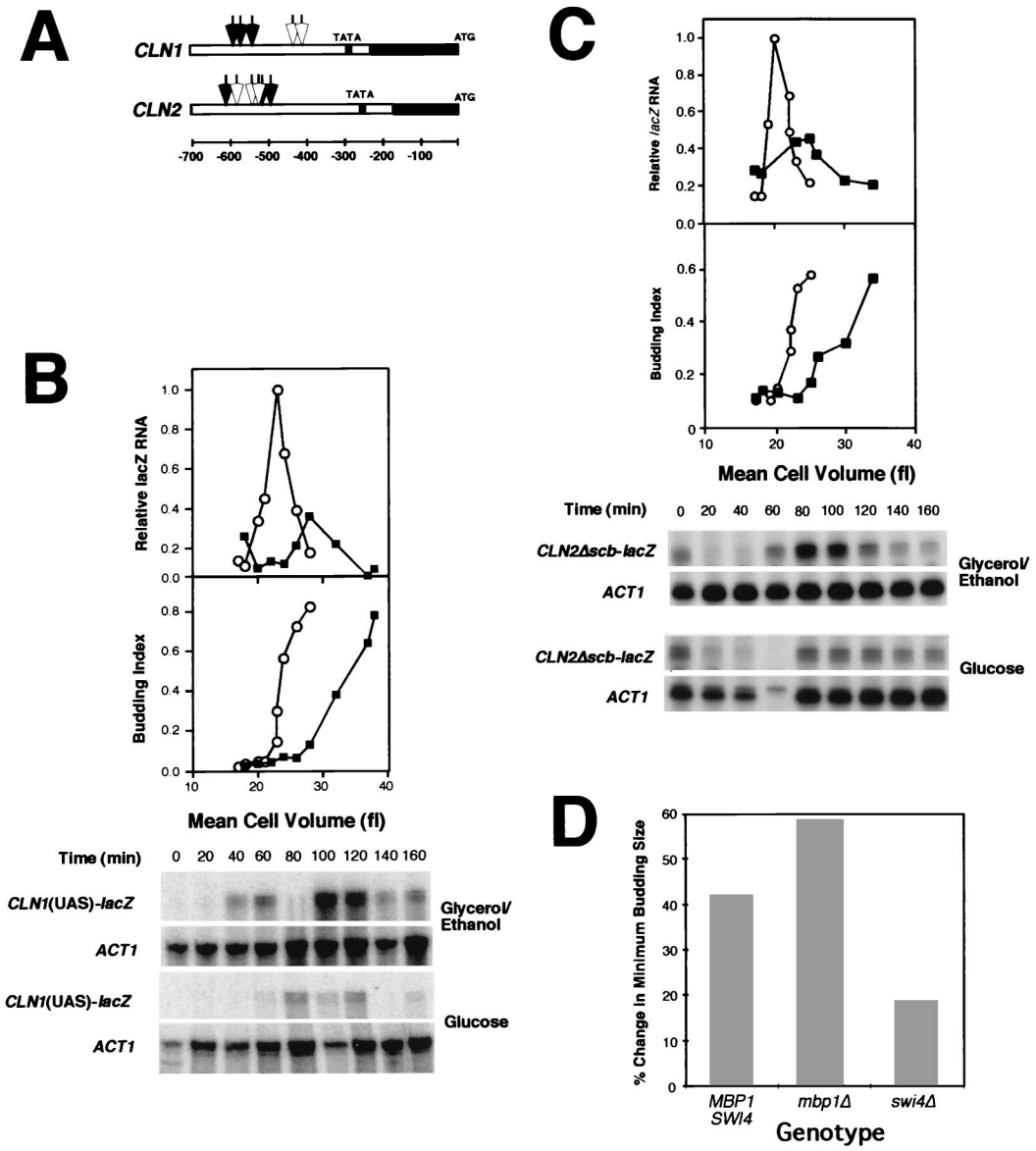

FIG. 4. (A) Diagram of relevant features of the *CLN1* and *CLN2* promoters. Open arrows indicate the position of SCB elements. Closed arrows indicate the position of MCB core elements. The solid black bars represent the transcribed regions, and the open bars represent the nontranscribed promoter sequences. The gray regions represent the sequences surrounding the TATA element that are conserved between the *CLN1* and *CLN2* promoters. The scale is designated below in base pairs. (See references 9, 23, 24, and 32.) (B) Glucose-induced transcriptional repression of *CLN1* is conferred by the major *CLN1* UAS. The effect of glucose upon the expression of the *CYC1-lacZ* reporter of pCZΔ driven by a 106-bp fragment containing the major *CLN1* UAS (24) was analyzed in synchronized G_1 cells and plotted relative to cell volume as described in the legend to Fig. 3 (top graph). The budding index is plotted as a function of cell volume (bottom graph). Cells were grown in medium containing either glycerol-ethanol (○) or glucose (■) as a carbon source. Primary data from RNase protection assays for *lacZ* and *ACT1* are presented in the lower part of panel B. The 80-min sample from the glycerol-ethanol-grown culture is not plotted because in that sample there was significant degradation of high-molecular-weight RNA (see lower panel). (C) The effect of glucose upon the expression the *CYC1-lacZ* reporter in pCZΔ driven by a *CLN2* promoter deficient in SCB elements, p*CLN2*Δ*scb* (32), was analyzed in synchronized G_1 cells and plotted relative to cell volume as described in the legend to Fig. 3 (top graph). The budding index is plotted versus cell volume (bottom graph). Cells were grown in medium containing either glycerol-ethanol (○) or glucose (■) as a carbon source. Primary data from RNase protection assays for *lacZ* and *ACT1* are presented below. Data from 20 through 160 min are represented on the graph. (D) Effect of *swi4*Δ and *mbp1*Δ mutations on the glucose-induced increase in minimum budding size. Cell volume at budding was determined in synchronous cultures of *swi4*Δ and *mbp1*Δ cells either growing on glycerol-ethanol medium or shifted to glucose medium. The percent change in minimum budding size is presented. A representative experiment is presented for each strain. All values were determined as described for Fig. 1 and in Materials and Methods.

porter transcript was then analyzed (Fig. 4C). In striking contrast to the wild-type *CLN2* promoter, the *CLN2*Δ*scb* promoter is strongly repressed in response to glucose. The extent of repression is similar to that observed with the *CLN1* promoter driving the same reporter. Although this experiment does not directly assign that repression to the MCB core elements, these results are consistent with regulation exerted either directly through those elements or through elements that depend upon MCB core elements for their repressive activity. The failure of the wild-type promoter to be significantly repressed under the same conditions (Fig. 3) demonstrates that SCB elements are not targets for this regulation.

Since MCB core elements, as well as SCB elements, are targets for SBF (reviewed in reference 5), we would predict

that the inactivation of SBF but not MBF would suppress the effect of glucose on cell size. To test this hypothesis we evaluated the effect of mutational inactivation of *SWI4* and *MBP1*, the DNA binding components of SBF and MBF, respectively, on the ability of glucose to induce an increase in the minimal cell volume at budding. Strains containing disrupted alleles of either *SWI4* or *MBP1* were subjected to the carbon source shift regimen previously described and the cell volume at budding was determined (Fig. 4D). As predicted, whereas *mbp1*Δ mutants exhibited a substantial increase in cell size in response to glucose, that response was largely eliminated in *swi4*Δ mutants. Thus, SBF is required to achieve a glucose-induced delay in cell cycle progression equal to that of the wild type.

Transcriptional repression is sufficient and, at least in part, necessary for the glucose-induced increase in the size at budding. The previous experiments demonstrate that the MCB core elements of the *CLN1* promoter are sufficient to explain the behavior of the *CLN1* transcript in response to glucose. To address the importance of transcriptional repression by glucose in delaying cell cycle initiation, the *CLN1* gene was placed under control of the *CLN2* promoter, which is not repressed in response to glucose. This was accomplished by fusing the *CLN2* promoter to the *CLN1* gene just upstream of their highly conserved TATA elements (Fig. 4A). The *CLN1* gene in this construct was truncated within the open reading frame. When integrated at the *CLN1* locus by homologous recombination, this construct results in a hybrid gene (*CLN2p-CLN1*) that has all of the known UAS activities of the *CLN2* promoter yet retains the transcriptional start site and the transcribed sequences of the *CLN1* gene. The integrated fusion gene was shown to be functional based upon its ability to complement a deficiency of all three *CLN* genes (data not shown). Another useful consequence of this integration was that the native *CLN1* promoter was left driving a nonfunctional truncated gene, producing an RNA transcript (*cln1^{tr}*) that was detectable with the *CLN1* probe.

The *CLN2p-CLN1* construct was introduced into both a *cln2*Δ strain and a *cln2*Δ *cln3*Δ strain to evaluate its effect on the extent of the glucose-induced delay in cell cycle initiation. The *cln2*Δ strain carrying this construct was also analyzed to evaluate the effect of glucose on the expression of *CLN1* transcripts. Northern blot analysis with the full-length *CLN1* open reading frame as a probe enabled us to detect transcripts expressed from both the native *CLN1* promoter (*cln1^{tr}*) and the *CLN2* promoter (*CLN2p-CLN1*) (Fig. 5A). Two important observations can be made from this experiment. First, *CLN2p-CLN1* is not repressed in response to glucose, whereas the truncated *CLN1* transcript continues to be repressed. This demonstrates that regulation at the level of the promoter is not only sufficient but also necessary for glucose repression of *CLN1* transcript accumulation. Second, failure to repress the *CLN1* transcript results in partial suppression of the glucose-induced increase in budding size. This is most easily observed by comparing the strains carrying the *CLN2p-CLN1* construct to equivalent strains having either a wild-type *CLN1* or a wild-type *CLN2* gene (Fig. 5B). That comparison reveals that expression of *CLN1* under control of the *CLN2* promoter eliminates a portion of the increase in cell size that is observed in the comparable strains having wild-type *CLN1*. Thus, transcriptional regulation of *CLN1* accounts for approximately one-half of the increase in budding size observed in response to glucose. Posttranscriptional mechanisms are likely to account for the remainder of the delay.

The experiment described above shows that posttranscriptional mechanisms can contribute to the repression of *CLN1* in response to glucose. However, a similar analysis of *CLN2* expressed under control of the *CLN1* promoter (*CLN1p-CLN2*) suggests that such mechanisms are not necessary. The *CLN1p-CLN2* fusion was constructed essentially as described above for *CLN2p-CLN1* and integrated into either *cln1*Δ or *cln1*Δ *cln3*Δ strains. These strains, in which the *CLN1p-CLN2* construct replaces *CLN2*, were analyzed by the same regimen described previously, and the cell volume at budding was established (Fig. 5B). Unlike the *CLN2p-CLN1* strains, these strains exhibited an increase in budding size in response to glucose that was either similar to or more dramatic than the equivalent strain expressing *CLN1* under control of its own promoter. In contrast, when *CLN2* is expressed under control of its own promoter, little or no size increase is observed. We conclude that transcriptional repression of the *CLN1* promoter is sufficient to account for the cell cycle delay observed in response to glucose.

DISCUSSION

The G_1 cyclins Cln1 and Cln2 in conjunction with the Cdc28 cyclin-dependent protein kinase act to promote cell cycle initiation (reviewed in reference 26). Their activity during the cell cycle is regulated primarily at the level of protein abundance, which is a consequence of periodic transcriptional activity (reviewed in reference 5) coupled with regulated protein instability (11, 18, 39). The expression of these critical cell cycle regulators has been shown to be coordinately regulated both during the cell cycle and in response to environmental regulators of cell proliferation, including mating pheromone and nutrient limitation (40). Coordinate expression is predicted by their common mode of transcriptional regulation. Both genes are targets of SBF and are dependent upon *CLN3* and *CDC28* for activation (reviewed in reference 5).

However, genetic analysis of *CLN* mutants has suggested that these genes are, in some circumstances, independently regulated. Inactivation of *CLN1*, but not of other G_1 cyclin genes, suppresses the glucose-induced increase in minimum size required for entry into a new cell cycle, suggesting that *CLN1* is a specific target for negative regulation by glucose (36). In this study we have used synchronized populations of G_1 daughter cells to confirm that *CLN1* is required for glucose to effect an increase in the minimum size for cell cycle initiation. We extend previous studies by establishing here that *CLN1* transcription is specifically repressed in glucose-grown cells. Neither *CLN2* nor other G_1-specific transcripts are repressed in response to glucose. *CLN1* repression is not a transient response to a shift to glucose from poorer carbon sources, since it occurs during each cell cycle when cells are growing continuously on glucose. This suggests that repression of *CLN1* transcription is a consequence of the higher basal level of cAMP observed in cells maintained on glucose (4, 29) and is not solely a response to the glucose-induced spike of cAMP.

In contrast to the rather specific effect of glucose on *CLN1* expression, treatment of cells with exogenous cAMP, the presumed mediator of the glucose signal, results in dramatic repression of *CLN2*, *RNR1*, and a number of other G_1-specific genes. Thus, although the effect of added cAMP is in many ways similar to the effect of glucose, there are significant differences. Whether this is simply due to a distinction between the intracellular level of cAMP attained in response to glucose and that attained by treatment of cells with cAMP or whether it is instead a reflection of a qualitative difference between the signals generated by these two stimuli is unclear. It is known that elements of the RAS-cAMP pathway are required for the glucose-induced increase in minimum budding size, and cAMP treatment appears to mimic effects of hyperactivation of that pathway.

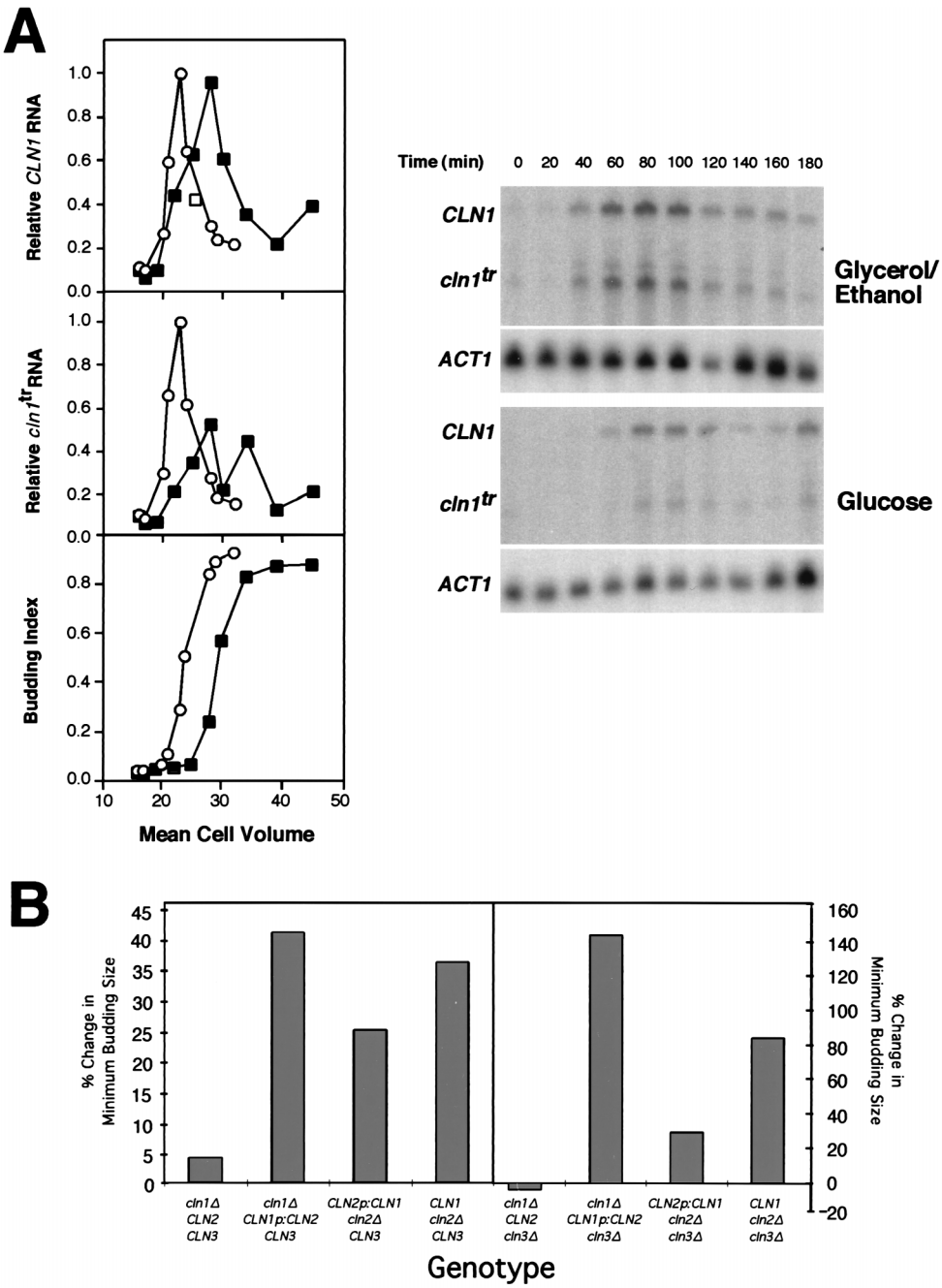

FIG. 5. Transcriptional repression of *CLN1* is required for full induction of the glucose-induced increase in minimum budding size. (A) The effect of glucose upon the *CLN1* transcripts expressed from a *CLN2p-CLN1* fusion (*CLN1*) and from a truncated *CLN1* gene (*cln1*^{tr}) under control of its own promoter was analyzed in synchronized G_1 cells and plotted relative to cell volume as described in the legend to Fig. 3. Both forms of the *CLN1* transcript were detected by Northern blot analysis with the *CLN1* open reading frame as a probe. The budding index is plotted versus cell volume (bottom graph). Cells were grown in medium containing either glycerol-ethanol (○) or glucose (■) as a carbon source. Primary data from Northern blot analysis of *CLN1*, *cln1*^{tr}, and *ACT1* are presented at right. (B) Exchange of the *CLN1* and *CLN2* promoters reveals that transcriptional regulation is sufficient and, at least in part, necessary for the glucose-induced increase in budding size. Cell volume at budding was determined in strains carrying a single integrated copy of *CLN2p-CLN1* or *CLN1p-CLN2* at the *CLN1* or *CLN2* locus, respectively. Cells were either grown continuously on glycerol-ethanol or shifted to glucose. A representative experiment is presented for each strain. Data for the relevant *CLN*-deficient strains were either reproduced from Fig. 1 or determined as described for Fig. 1 and in Materials and Methods. The percent change in minimum budding size is presented.

CLN1 is the only gene among those examined in this study which undergoes substantial transcriptional repression in response to glucose. In contrast, glucose induces a delay in the accumulation of all G_1-specific transcripts, including *CLN1*. The source of this delay is not understood. One hypothesis to explain this pattern of gene expression is that all G_1-specific transcription is initially repressed in response to glucose and that cells are initially delayed during the G_1 phase. However, under these conditions the *CLN2* transcript continues to accumulate until it reaches a level equivalent to that observed in cells growing on a poorer carbon source, thereby generating sufficient cyclin-dependent kinase (CDK) activity to promote cell cycle progression. This scenario holds that *CLN1* is more effectively repressed than other G_1-specific genes and, unlike

those genes, never achieves the levels observed in cells maintained in medium without glucose. Several observations appear to be at odds with this hypothesis. First, this hypothesis predicts that, like the *CLN2* transcript in wild-type cells, the *CLN1* transcript in *CLN2*-deficient cells would be delayed but would ultimately reach the same level as that achieved in medium lacking glucose. Yet, the kinetics of *CLN1* transcript accumulation in *cln2*Δ mutants is similar to that observed in wild-type cells in being both delayed and repressed (7). Next, where it has been examined the duration of the delay in G_1-specific transcripts appears to be equivalent to the duration of the delay in budding (7). Since the timing of budding is a reflection of the timing of expression of G_1 cyclins, the lack of a cell cycle delay in *CLN1*-deficient cells suggests that in those cells there is no delay in the program of G_1-specific transcription. This leaves us with the hypothesis that under the conditions of the glucose-induced delay, *CLN1* not only influences the timing of cell cycle initiation but also affects the timing of activation of G_1-specific transcription. Although that proposal appears inconsistent with evidence that the activation of G_1-specific transcription is independent of the activity of *CLN1* and *CLN2* (12, 33), these genes have been shown to be capable of promoting their own expression under some conditions (10, 13, 33). Resolution of this issue awaits further analysis.

To understand the basis for the differential regulation of the *CLN1* and *CLN2* promoters by glucose, we have attempted to localize the *cis*-acting targets required for repression. We have shown that a 106-bp fragment encompassing the major *CLN1* UAS is sufficient to confer glucose-induced repression upon a UAS-deficient reporter (Fig. 4C). The UAS activity associated with this fragment is derived from three MCB core elements (24). Related MCB core elements in the *CLN2* promoter also appear to be repressed by growth on glucose-containing medium. However, this is only observed when the SCB elements, the primary *cis*-acting elements of the *CLN2* promoter, are eliminated (32). Unexpectedly, the MCB core elements of both the *CLN1* and *CLN2* promoters depend primarily upon *SWI4*, the DNA binding component of SBF, and not upon *MBP1*, the DNA binding component of MBF, for maximal expression (24, 31, 32). Partridge and coworkers have shown that SBF binds effectively to the MCB core elements from *CLN1* in vitro (24). Consistent with the importance of SBF for activation of those elements, inactivation of *SWI4* but not *MBP1* partially suppresses the extent of the glucose-induced increase in budding size.

We speculate that the differential effect of glucose upon these two genes is explained by the difference in their *cis*-acting targets for SBF. For instance, it is possible that cells respond to glucose by altering the capacity of SBF to activate expression of MCB core elements without affecting its activity toward SCB elements. Alternatively, a lower affinity for MCB core elements than for SCB elements may be an inherent property of SBF, and glucose may simply act to lower its overall activity. It must also be true that the activity of MBF is not similarly affected since *RNR1* and other MBF-dependent genes are not repressed by glucose. At present we cannot exclude the possibility that a *cis*-acting element distinct from the MCB core elements is responsible for the modulation of activity we observed in response to glucose since the minimal *CLN1* promoter fragment used in this study includes other sequences. However, if such elements are present in the *CLN1* promoter, then similar elements must also exist in the *CLN2* promoter since the SCB-deficient promoter is repressed by glucose. The recently described Xbp1 transcriptional repressor (20) is clearly not responsible for *CLN1* repression since the Xbp1 binding site is absent both from the glucose-responsive 106-bp fragment of the *CLN1* promoter and from the *CLN2* promoter.

Analysis of cells carrying *CLN1* expressed under control of the wild-type *CLN2* promoter, which is not repressed by glucose, shows that posttranscriptional mechanisms can contribute to the cell cycle delay caused by glucose. Nevertheless, cells carrying a complementary construct with *CLN2* under the control of the *CLN1* promoter are very sensitive to glucose, suggesting that transcriptional repression alone is sufficient. Since the *CLN1* and *CLN2* promoters drive transcription at comparable levels and with similar cell cycle timing, this experiment eliminates several caveats associated with previous experiments that used constitutive hyperexpression of *CLN1* (2, 36). The high level of expression used in those experiments resulted in full suppression of the cAMP-induced delay.

Our results suggest that posttranscriptional mechanisms can contribute to this response. Although a number of regulatory mechanisms are possible, the involvement of cAMP-dependent protein kinase in this response (2, 3, 36) suggests a role for protein phosphorylation. Phosphorylation of G_1 cyclins, including Cln1, has been well documented (18, 37, 40). At least some of those modifications are involved in inducing protein turnover (18, 39, 41). However, analysis of the Cln1 protein expressed from the *CLN2* promoter failed to reveal a decrease in abundance in response to glucose (14). Furthermore, there is still no evidence for direct phosphorylation of G_1 cyclins by cAMP-dependent protein kinase. Alternatively, protein kinase A may affect the activity of the Cln1-CDK complex. This issue has yet to be investigated.

In contrast to the regulation of *CLN1* expression described in this study, other cyclins appear to be induced on glucose. *CLN3* is one example. The addition of glucose to a culture growing on a carbon-poor source increases the level of *CLN3* expression as much as 10-fold (16, 31). Conversely, the number of *CLN3* transcripts decreases as cells deplete glucose and undergo the diauxic shift (16). Surprisingly, this dramatic change in *CLN3* expression which occurs upon addition of glucose fails to have a significant effect on the expression of *CLN1* and *CLN2*. It is unclear whether this results from a failure of the increase in *CLN3* transcripts to result in a commensurate increase in Cln3 protein due to translational regulation (25) and/or Cln3-associated CDK activity or whether it instead results from a greater requirement for Cln3 to activate *CLN1* and *CLN2* transcription on glucose. Yet, it is clear that a deficiency in *CLN3* has a dramatic effect on both the timing and the extent of *CLN1* and *CLN2* gene expression on both glucose and poorer carbon sources (12, 33, 37). Therefore, glucose and *CLN3* must act independently to affect *CLN1* gene expression and thereby the timing of cell cycle initiation.

The physiological relevance for the glucose-induced delay in cell cycle initiation remains an enigma. It is likely to reflect an important adaptation to the change in growth rate. For example, it seems reasonable to suggest that cells growing on nonfermentable carbon sources benefit from an increase in the surface/volume ratio that is not necessary when cells are growing on fermentable carbon sources. However, this interpretation appears to be incorrect since the effect on cell size is restricted to glucose and a few other carbon sources that support rapid growth. Other fermentable sugars, such as sucrose and raffinose, fail to induce a cell size increase.

An explanation that is perhaps more trivial is that the increase in cell size results solely from the inability of generation time to keep pace with rapid growth. This hypothesis is supported by the observation that cells growing continuously in a chemostat with glucose as a carbon source initiate budding at a cell size characteristic of the age of the mother (number of bud scars) when glucose is limiting (17). However, as the concentration of glucose increases and cells approach the mini-

mum generation time their size at budding increases. The magnitude of that increase is comparable to the increase observed when cells were shifted to glucose in the present study. This effect on cell size is related to growth rate and not glucose per se since nitrogen limitation results in an increase in cell cycle time and an accompanying decrease in cell volume at budding (19). This explanation would be consistent with the failure of the fermentable carbon sources that are incapable of supporting rapid growth to cause a size increase.

Although the latter explanation is consistent with most experimental observations it is not without its inconsistencies. Notably, Baroni et al. (3) have shown that in glucose-grown cells a G_1 delay and an associated increase in cell size at budding occur in response to exogenously added cAMP. Yet cAMP fails to increase the biosynthetic capacity of those cells. Furthermore, the apparent increase in the minimum size required for budding resulting from cAMP treatment can occur in cells which are restricted from growth by starvation for nitrogen (2). Thus, it appears that cAMP treatment can effectively separate the effect of glucose on the growth rate from its effect on the minimum size required for budding, suggesting that the explanation for this finding is more complex. Furthermore, the observation that the response is associated with alterations in gene expression that are sufficient to explain the behavior indicates that it is unlikely to simply reflect a passive effect of the relationship between growth rate and cell cycle progression. We conclude that the ability of cells to increase in size in response to glucose is either physiologically important or a manifestation of a regulatory pathway that plays some other physiologically important role.

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The first two authors contributed equally to this work.

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REFERENCES

1. **Baroni, M. D., E. Martegani, P. Monti, and L. Alberghina.** 1989. Cell size modulation by *CDC25* and *RAS2* genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**2715–2723.
2. **Baroni, M. D., P. Monti, and L. Alberghina.** 1994. Repression of growth-regulated G1 cyclin expression by cyclic AMP in budding yeast. Nature **371:**339–342.
3. **Baroni, M. D., P. Monti, G. Marconi, and L. Alberghina.** 1992. cAMP-mediated increase in the critical cell size required for the G1 to S transition in *Saccharomyces cerevisiae*. Exp. Cell Res. **201:**299–306.
4. **Beullens, M., K. Mbonyi, L. Geerts, D. Gladines, K. Detremerie, A. W. H. Jans, and J. M. Thevelein.** 1988. Studies on the mechanism of the glucose-induced cAMP signal in glycolysis and glucose repression mutants of the yeast *Saccharomyces cerevisiae*. Eur. J. Biochem. **172:**227–231.
5. **Breeden, L.** 1996. Start specific transcription in yeast. Curr. Top. Microbiol. Immunol. **208:**95–127.
6. **Buchman, A. R., and R. D. Kornberg.** 1990. A yeast *ars*-binding protein activates transcription synergistically in combination with other weak activating factors. Mol. Cell. Biol. **10:**887–897.
7. **Chapman-Shimshoni, D.** Unpublished data.
8. **Cross, F.** 1988. *DAF1*, a mutant gene affecting size control, pheromone arrest, and cell cycle kinetics of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**4675–4684.
9. **Cross, F. R., M. Hoek, J. D. McKinney, and A. H. Tinkelenberg.** 1994. Role of Swi4 in cell cycle regulation of CLN2 expression. Mol. Cell. Biol. **14:**4779–4787.
10. **Cross, F. R., and A. H. Tinkelenberg.** 1991. A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. Cell **65:**875–883.
11. **Deshaies, R. J., V. Chau, and M. Kirschner.** 1995. Ubiquitination of the G1 cyclin Cln2p by a Cdc34p-dependent pathway. EMBO J. **14:**303–312.
12. **Dirick, L., T. Böhm, and K. Nasmyth.** 1995. Roles and regulation of Cln-Cdc28 kinases at the start of the cell cycle of *Saccharomyces cerevisiae*. EMBO J. **14:**4803–4813.
13. **Dirick, L., and K. Nasmyth.** 1991. Positive feedback in the activation of G1 cyclins in yeast. Nature **351:**754–757.
14. **Flick, K., and C. Wittenberg.** Unpublished data.
15. **Hadwiger, J. A., C. Wittenberg, M. A. de Barros Lopes, H. E. Richardson, and S. I. Reed.** 1989. A family of cyclin homologs that control G1 phase in yeast. Proc. Natl. Acad. Sci. USA **86:**6255–6259.
16. **Heideman, W.** Personal communication.
17. **Johnston, G. C., C. W. Ehrhardt, A. Lörincz, and B. L. A. Carter.** 1979. Regulation of cell size in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **137:**1–5.
18. **Lanker, S., M. H. Valdivieso, and C. Wittenberg.** 1996. Rapid degradation of the G1 cyclin, Cln2, induced by CDK-dependent phosphorylation. Science **271:**1597–1601.
19. **Lörincz, A. T., and B. L. A. Carter.** 1979. Control of cell size at bud initiation in *Saccharomyces cerevisiae*. J. Gen. Microbiol. **113:**287–295.
20. **Mai, B., and L. Breeden.** 1997. Xbp1, a stress-induced transcriptional repressor of the *Saccharomyces cerevisiae* Swi4/Mbp1 family. Mol. Cell. Biol. **17:**6491–6501.
21. **Nash, R., G. Tokiwa, S. Anand, K. Erickson, and A. B. Futcher.** 1988. The *WHI1*$^+$ gene of *S. cerevisiae* tethers cell division to cell size and is a cyclin homolog. EMBO J. **7:**4335–4346.
22. **Nasmyth, K., and L. Dirick.** 1991. The role of *SWI4* and *SWI6* in the activity of the G1 cyclins in yeast. Cell **66:**995–1013.
23. **Ogas, J., B. Andrews, and I. Herskowitz.** 1991. Transcriptional activation of *CLN1*, *CLN2*, and a putative new G1 cyclin (*HCS26*) by *SWI4*, a positive regulator of G1-specific transcription. Cell **66:**1015–1026.
24. **Partridge, J. F., G. E. Mikesell, and L. L. Breeden.** 1997. Cell cycle-dependent transcription of *CLN1* involves Swi4 binding to MCB-like elements. J. Biol. Chem. **272:**9071–9077.
25. **Polymenis, M., and E. V. Schmidt.** 1997. Coupling of cell division to cell growth by translational control of the G1 cyclin CLN3 in yeast. Genes Dev. **11:**2522–2531.
26. **Reed, S.** 1995. START and the G1-S phase transition in budding yeast, p. 40–62. *In* B. D. Barnes and D. M. Glover (ed.), Cell cycle control. IRL Press, Oxford, England.
27. **Richardson, H. E., C. Wittenberg, F. R. Cross, and S. I. Reed.** 1989. An essential G1 function for cyclin-like proteins in yeast. Cell **59:**1127–1133.
28. **Ronne, H.** 1995. Glucose repression in fungi. Trends Genet. **11:**12–17.
29. **Russell, M., J. Bradshaw-Rouse, D. Markwardt, and W. Heideman.** 1993. Changes in gene expression in the Ras/adenylate cyclase system of *Saccharomyces cerevisiae*: correlation with cAMP levels and growth arrest. Mol. Biol. Cell **4:**757–765.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Stuart, D.** Unpublished data.
32. **Stuart, D., and C. Wittenberg.** 1994. Cell cycle-dependent transcription of *CLN2* is conferred by multiple distinct *cis*-acting regulatory elements. Mol. Cell. Biol. **14:**4788–4801.
33. **Stuart, D., and C. Wittenberg.** 1995. *CLN3*, not positive feedback, determines the timing of *CLN2* gene expression in cycling cells. Genes Dev. **9:**2780–2794.
34. **Thevelein, J. M.** 1992. The RAS-adenylate cyclase pathway and cell cycle control in Saccharomyces cerevisiae. Antonie Leeuwenhoek **62:**109–130.
35. **Thevelein, J. M., and M. Beullens.** 1985. Cyclic AMP and the stimulation of trehalase activity in the yeast *Saccharomyces cerevisiae* by carbon sources, nitrogen sources and inhibitors of protein synthesis. J. Gen. Microbiol. **131:**3199–3209.
36. **Tokiwa, G., M. Tyers, T. Volpe, and B. Futcher.** 1994. Inhibition of G1 cyclin activity by the Ras/cAMP pathway in yeast. Nature **371:**342–345.
37. **Tyers, M., G. Tokiwa, and B. Futcher.** 1993. Comparison of the *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. EMBO J. **12:**1955–1968.
38. **Valdivieso, M. H., K. Sugimoto, K.-Y. Jahng, P. M. B. Fernandes, and C. Wittenberg.** 1993. *FAR1* is required for posttranscriptional regulation of *CLN2* gene expression in response to mating pheromone. Mol. Cell. Biol. **13:**1013–1022.
39. **Willems, A. R., S. Lanker, E. E. Patton, K. L. Craig, T. F. Nasson, N. Mathias, R. Kobayashi, C. Wittenberg, and M. Tyers.** 1996. Cdc53 targets phosphorylated G1 cyclins for degradation by the ubiquitin proteolytic pathway. Cell **86:**453–463.
40. **Wittenberg, C., K. Sugimoto, and S. I. Reed.** 1990. G1-specific cyclins of *S. cerevisiae*: cell cycle periodicity, regulation by mating pheromone, and association with the p34^{CDC28} protein kinase. Cell **62:**225–237.
41. **Yaglom, J., M. H. Linskens, S. Sadis, D. M. Rubin, B. Futcher, and D. Finley.** 1995. p34Cdc28-mediated control of Cln3 cyclin degradation. Mol. Cell. Biol. **15:**731–741.