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Multiple Pathways for Suppression of Mutants Affecting G_1-Specific Transcription in *Saccharomyces cerevisiae*

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ABSTRACT

In the budding yeast, *Saccharomyces cerevisiae*, control of cell proliferation is exerted primarily during G_1 phase. The G_1-specific transcription of several hundred genes, many with roles in early cell cycle events, requires the transcription factors SBF and MBF, each composed of Swi6 and a DNA-binding protein, Swi4 or Mbp1, respectively. Binding of these factors to promoters is essential but insufficient for robust transcription. Timely transcriptional activation requires Cln3/CDK activity. To identify potential targets for Cln3/CDK, we identified multicopy suppressors of the temperature sensitivity of new conditional alleles of *SWI6*. A *bck2*Δ background was used to render *SWI6* essential. Seven multicopy suppressors of *bck2*Δ *swi6-ts* mutants were identified. Three genes, *SWI4*, *RME1*, and *CLN2*, were identified previously in related screens and shown to activate G_1-specific expression of genes independent of *CLN3* and *SWI6*. The other four genes, *FBA1*, *RPL40a/UBI1*, *GIN4*, and *PAB1*, act via apparently unrelated pathways downstream of SBF and MBF. Each depends upon *CLN2*, but not *CLN1*, for its suppressing activity. Together with additional characterization these findings indicate that multiple independent pathways are sufficient for proliferation in the absence of G_1-specific transcriptional activators.

FAITHFUL execution of cell cycle events occurs as a consequence of coordinating the activity of key cell cycle regulators with the factors required for the execution of the events that they regulate. An important component of that regulation involves the coordinate expression of the genes encoding the relevant proteins during specific cell cycle phases. Although cell cycle-dependent transcriptional activation has been recognized for decades, the recent application of genome-wide transcriptional profiling has revealed the extent to which this form of regulation has been implemented (Cho *et al.* 1998; Spellman *et al.* 1998; Iyer *et al.* 2001). Those approaches have revealed a broad array of distinct patterns of cell cycle-dependent transcription and have contributed to the identification and characterization of the transcription factors responsible for those expression patterns.

G_1-specific genes were among the first cell cycle-regulated genes to be recognized and are certainly among the best studied. Those genes are expressed specifically during late G_1 phase preceding the events associated with cell cycle initiation. In the budding yeast, those events include formation of the bud, duplication of the spindle pole body, and DNA replication (Wittenberg and Flick 2003). Among the G_1-specific genes are many encoding elements involved in the regulation and execution of those processes.

Our understanding of G_1-specific transcription comes largely from the study of the expression of the HO gene, which encodes the initiating endonuclease for mating type switching (reviewed by Nasmyth 1993). Those studies led to the identification and characterization of the G_1-specific transcription factor SBF that, together with a second factor MBF, is responsible for expression of this important gene family of genes (reviewed in Breeden 1996). SBF and MBF are both heterodimeric transcription factors composed of a unique DNA-binding component, Swi4 or Mbp1, respectively, and a common component, Swi6. The heterodimeric factors are sufficient for binding to promoter elements known as SCBs and MCBs, respectively. One or both of those elements are generally found in the promoters of G_1-specific genes and the SBF and MBF transcription factors have been shown to bind to the majority of those promoters *in vivo* (Iyer *et al.* 2001).

Although binding of SBF or MBF to a promoter is necessary for transcriptional activation, it is not sufficient. In fact, both transcription factors are known to occupy their binding sites in the promoters of G_1-specific genes during early G_1 phase when those genes are transcriptionally inactive. Transcriptional activation occurs only during late G_1 phase as a consequence of activation of the G_1-specific cyclin-dependent protein kinase Cln3/Cdc28 (Tyers *et al.* 1993; Dirick *et al.* 1995; Stuart

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TABLE 1

Yeast strains used in this study

Yeast strain	Genotype	Source
KFY193	*bck2::ARG4 swi6-4::KAN*	This work
KFY194	*bck2::ARG4 swi6-12::KAN*	This work
TAY477	*bck2::ARG4 swi6::TRP1 HIS2::GAL1:CLN2*	T. Kesti and C. Wittenberg (unpublished data)
TAY461	*bck2::ARG4 cln3::URA3 HIS2::GAL1:CLN2*	T. Kesti and C. Wittenberg (unpublished data)
KFY115	*mbp1::URA3 swi4::LEU2 HIS2::GAL:CLN2*	This work
KFY775	*bck2::ARG4 swi6-4::KAN cln1::URA3*	This work
KFY777	*bck2::ARG4 swi6-4::KAN cln2::URA3*	This work
KFY884	*bck2::ARG4 swi6-4::KAN whi3::HYG*	This work
KFY430	*bck2::ARG4 swi6-4::KAN swe1::LEU2*	This work
KFY 707	*bck2::ARG4 swi6-4::KAN sic1::URA3*	This work

and Wittenberg 1995). Only then does the transcription factor activate expression of the genes, apparently by promoting recruitment of the RNA Pol II to join the mediator complex and complete the formation of the Pol II holoenzyme at the basal promoters (Cosma *et al.* 1999; Bhoite *et al.* 2001; Cosma *et al.* 2001).

Despite the importance of SBF and MBF for the concerted transcriptional activation of G_1-specific promoters, neither transcription factor alone is essential for viability (although *swi4*Δ is lethal in some strains; Ogas *et al.* 1991). However, inactivation of both Swi4 and Mbp1, the DNA-binding components of these factors, results in the failure to proliferate (Koch *et al.* 1993; Nasmyth and Dirick 1991). Although mutations in Swi6, a common component of both factors, cause a severe morphological phenotype, it is not essential for viability unless Bck2 is also inactive (Epstein and Cross 1994; Di Como *et al.* 1995; Wijnen and Futcher 1999). Bck2 is required for the low level of cell cycle-dependent transcriptional activation of G_1-specific genes observed in the absence of Swi6 via a mechanism that remains to be elucidated (Epstein and Cross 1994; Di Como *et al.* 1995).

The products of G_1-specific genes are involved in diverse functions. Many of those are associated with events that occur as a consequence of start, including DNA replication, spindle pole body duplication, septin ring formation, and budding (Wittenberg and Flick 2003). The expression of those genes during G_1 phase facilitates the coordinate execution of those events and reflects an economic use of cellular machinery. However, there is partial functional redundancy among the G_1-specific regulators. For instance, among the SBF and MBF targets are *CLN1*, *CLN2*, *CLB5*, and *CLB6*, important regulators of late G_1 events and DNA replication, which are not required individually for cell proliferation but lead to G_1 arrest when coordinately inactivated (Schwob and Nasmyth 1993). Similarly, *PCL1* and *PCL2*, two other SBF targets are essential in the absence of *CLN1* and *CLN2* (Espinoza *et al.* 1994; Measday *et al.* 1994). Other G_1-specific genes encode essential products, such as DNA polymerases and other replication proteins. Yet, despite the importance of G_1-specific gene products for the execution of a number of critical cell cycle events, inactivation of Swi6 does not lead to cell cycle arrest and, although inactivation along with Bck2 results in arrest, the terminal phenotype is not a characteristic G_1 arrest phenotype (Nasmyth and Dirick 1991). Although poorly characterized, the phenotype of *bck2*Δ *swi6*Δ mutants arrests predominantly as budded cell or multibudded cells with a high degree of cell lysis consistent with defects in cytokinesis and cell wall synthesis (our unpublished observation). Thus, the consequences of a deficiency in SBF and MBF appear to reflect the inefficient execution of post-start events, including but not limited to septin ring formation and cell wall deposition, rather than the failure to progress out of G_1 phase that is characteristic of the complete loss of G_1- and S-phase cyclins.

In the interest of identifying genes that act upstream or in concert with Swi6 to activate G_1-specific transcription, we performed a screen for dosage suppressors of a *bck2*Δ *swi6-ts* mutant. That screen resulted in the identification of a number of genes that when present in increased dosage in cells can relieve or reduce the temperature sensitivity of the screening strain. Each of the genes identified in this manner had been previously studied. On the basis of our current understanding they exhibit little apparent commonality of function. Our analysis of these suppressors has revealed that most act independently of *SWI6* and *BCK2*. They are competent to bypass a null mutation of Swi6, suggesting that they either act downstream of Swi6 or facilitate a parallel pathway sufficient to bypass the deficiency. This study suggests that the roles of Swi6 targets are disparate in

TABLE 2

Plasmids used in this study

Plasmid	Parent and insert	Source
YEp24	YEp24	Struhl *et al.* (1979)
YEplac181	YEplac181	Gietz and Sugino (1988)
YEplac195	YEplac195	Gietz and Sugino (1988)
pRS416	pRS416	Sikorski and Hieter (1989)
SS6-78	YEp24-genomic DNA	This study
SS6-395	YEp24-genomic DNA	This study
SS6-396	YEp24-genomic DNA	This study
SS6-406	YEp24-genomic DNA	This study
pKF162	YEplac195-*FBA1*	This study
pKF142	YEplac195-*PAB1*	This study
pKF160	YEplac195-*GIN4*	This study
pKF158	YEplac195-*RPL40a*	This study
pKF181	YEplac181-*PAB1-1*	This study
pKF182	YEplac181-*pab1*Δ*RRM1*	This study
pKF183	YEplac181-*pab1*Δ*RRM2*	This study
pKF184	YEplac181-*pab1*Δ*RRM3*	This study
pKF185	YEplac181-*pab1*Δ*RRM4*	This study
pKF186	YEplac181-*pab1*Δ*C*	This study
pKF187	YEplac181-*pab1-180*	This study
pBD1378	pRS316-*SWI6*	Sidorova *et al.* (1995)
pKF163	YEplac181-*GIN4*	This study
YEp/*gin4*^{K48M}	YEplac181-*gin4*^{K48M}	Longtine *et al.* (1998)
pRS316-*CDC3*-GFP	pRS316-*CDC3*-GFP	Caviston *et al.* (2003)
pUB221	pRS-*CUP1-UBI*-6xHis-Myc	Willems *et al.* (1996)

nature and that multiple independent defects contribute to the lethality resulting from the inactivation of G_1-specific transcription.

MATERIALS AND METHODS

Yeast strains, cultures, and plasmids: All yeast strains were isogenic to an *arg4*Δ derivative of BF264-15D (*MAT***a** *ura3*Δ*ns ade1 his2 leu2-3, 112, trp1-1*^{a}; Richardson *et al.* 1989). The relevant genotypes of strains used in this study are provided in Table 1. All strains were grown in standard culture medium and standard yeast genetic methods were used.

To test temperature sensitivity and suppression cells were grown to log phase, counted, and diluted to ~1000 cells per microliter. Tenfold serial dilutions were prepared and 5 μl of each dilution were spotted onto plates selective for the plasmid or on YEPD or YEPG plates. Plates were incubated 2–3 days at the temperatures indicated. Two or three independent transformants were used for each strain. The data presented are representative from at least two independent experiments.

Standard molecular biology techniques were used for all cloning and PCR procedures. Plasmids used in this study are listed in Table 2. Details on primers, plasmids, and cloning strategies are available on request.

Cell size analysis: Cell size analysis was performed using a Coulter Z2 particle cell analyzer (Beckman-Coulter). Cell size distribution was analyzed using the Z2 AccuComp software (Beckman-Coulter).

Microscopy: For visualization of Cdc3-GFP, cells were fixed in methanol at −70°. Differential interference microscopy (DIC) and fluorescence microscopy were performed using an Eclipse E800 microscope (Nikon, Melville, NY) with a ×100 objective. Cell images were captured with a Quantix CCD (Photometrics, Tucson, AZ) camera using IPLab Spectrum software (Signal Analytics, Vienna, VA). Phase-contrast microscopy was performed on an Axiostar plus (Zeiss) with a ×40 objective. Images were captured with a Coolpix 4500 (Fuji) camera.

RNA and protein analyses: Cells were grown to log phase at permissive temperature. The culture was split and half of the cells remained at permissive temperature whereas the other half was shifted to 37° for 6 hr. Cell pellets collected for RNA and protein were frozen and stored at −80°.

Total RNA isolation and Northern blotting were performed as described previously (Stuart and Wittenberg 1994). The membrane was hybridized with radiolabeled probes (Hi-Prime) in buffer H (100 mm sodium phosphate buffer, pH 7.0, 400 mm NaCl, 5 mm EDTA, 1% SDS, 10% dextran sulfate, and 0.1 mg/ml denatured salmon sperm DNA). Northern blots were analyzed by Phospho-Imaging and ImageQuant software.

Protein extracts were prepared in RIPA buffer [1% doxycholic acid, 1% Triton-X100, 0.1% SDS, 250 mm NaCl, 50 mm Tris, pH 7.5, 10 mm sodium pyrophosphate, phosphatase inhibitors (5 mm EDTA, 5 mm EGTA, 50 mm NaF, and 0.1 mm orthovanadate), and protease inhibitors (1 mm PMSF, 2 μg/ml aproteine, leupeptin, and pepstatin)]. Cells were broken with glass beads three times for 40 sec in a FastPrep FP120 (Qbiogene, Carlsbad, CA). Proteins were separated on 10% SDS-PAGE, blotted onto a PVDF membrane, and detected by polyclonal rabbit anti-Swi6 (generous gift from Linda Breeden) or anti-Cdc28 antibody.

Construction of temperature-sensitive *swi6* mutants: Temperature-sensitive *swi6* alleles were isolated by a modification

of the technique described by Muhlrad *et al.* (1992). SWI6 was amplified from YIplac204-*SWI6* with primers homologous to the MCS of pUC19 under mutagenic conditions (1 mm dCTP, dTTP, 0.2 mm dGTP, dATP, 30 pmol of each primer, 7 mm $MgCl_2$, 0.5 mm $MnCl_2$, 20 mm Tris-HCl, pH 8.4, 50 mm KCl, and 0.5 units of Taq DNA polymerase in a total volume of 100 μl). The PCR product was cotransformed with *Bam*HI-digested YCplac22 into a *bck*Δ *swi6*Δ strain kept alive by *GAL-CLN2*. *In vivo* gap repair resulted in YCplac22 plasmids containing mutagenized *SWI6*. Transformants were grown on glucose-containing plates at 25° and then replica plated and colonies unable to grow on 37° were selected. Plasmids containing temperature-sensitive alleles of *SWI6* were rescued and subcloned to replace wild-type *SWI6* in a *bck2*Δ strain.

Multicopy suppressor screen: Strains *bck2*Δ *swi6-4* and *bck2*Δ *swi6-12* were transformed with a genomic Yep24-based library (Carlson and Botstein 1982). We screened a total of 144,000 transformants for growth at 1° higher than maximal permissive temperature (34°–35°). A total of 529 colonies were isolated that were able to grow at the higher temperature. These clones were tested for loss of suppression on FOA plates. The remaining clones were classified according to the extent of suppression into three groups, 1°, 2°, or 3° above restrictive temperature. In total 258 clones were analyzed, 53 of the 1° suppressors, 222 of the 2° suppressors, and 18 of the 3° suppressors. Analysis was first done by restriction digest and clones appearing to be different as judged from three different digests (*Eco*RV, *Hin*dIII, and *Xba*I) were sequenced to identify the genomic inserts. The suppressing gene was then identified by subcloning and retesting of suppression. Once identified, genes were amplified by PCR using Vent polymerase (New England Biolabs, Beverly, MA) and cloned into YEplac195 or YEplac181. In the fully suppressing class (3°) all but 2 clones (containing *SWI4*) contained plasmids with the *SWI6* gene. Most of the 2° suppressors contained *SWI4* or *SWI6* but *RME1* (1 clone), *CLN2* (4 clones), *BCK2* (1 clone), *PAB1* (5 clones), and *GIN4* (10 clones) were also found in that class. *RPL40a* (3 clones) and *FBA1* (1 clone) were found among the 1° suppressors. No other genes were confirmed as suppressors after rescreening.

RESULTS

Screen for high-copy suppressors of *bck2*Δ *swi6-4* and *bck2*Δ *swi6-12* mutants: Temperature-sensitive *swi6* mutants were constructed *in vitro* by PCR mutagenesis of *SWI6* and screened for their ability to support growth of a *bck2*Δ *swi6*Δ mutant at 25° but not at 37°. The in-

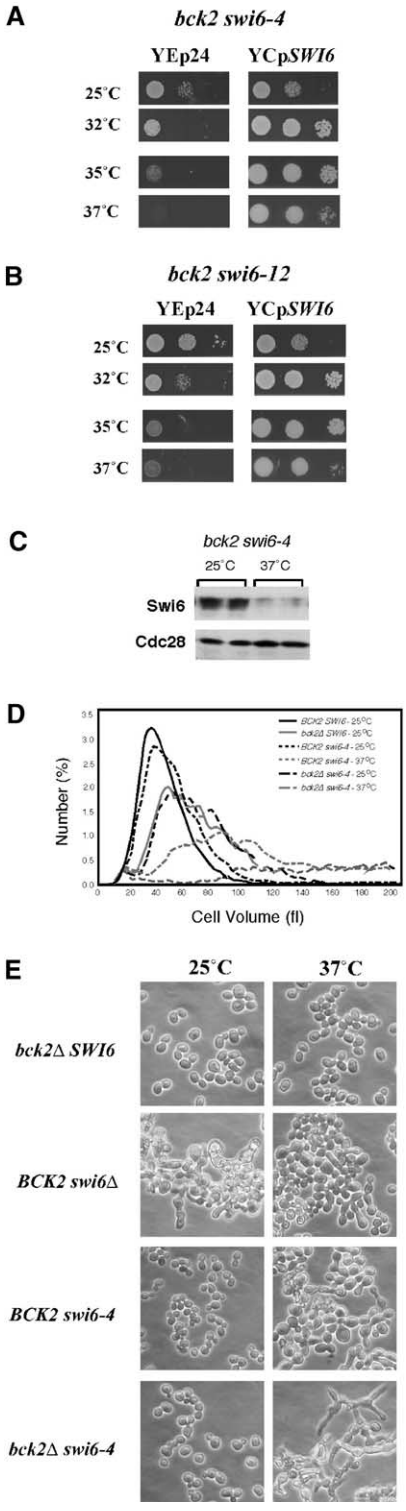


Figure 1.—Characterization of *bck2*Δ *swi6-ts* mutants. (A and B) Thermosensitivity of *bck2*Δ *swi6-4* and *bck2*Δ *swi6-12* mutants. *bck2 swi6-4* (A) or *bck2 swi6-12* (B) with either control plasmid (Yep24) or YCp *SWI6* plasmid were grown to log phase in −URA medium at 25°. Cells were counted and 10-fold serial dilutions with a starting dilution of ~5000 cells were spotted onto −URA plates and incubated at the temperatures indicated for 2–3 days. (C) Swi6 protein is thermolabile in *swi6-ts* mutant. Cells were grown to log phase and then split and either grown at permissive temperature or shifted to 37° for 6 hr. After separation on a 10% SDS gel and Western blotting, Swi6 was detected with a polyclonal anti-Swi6 antibody. Cdc28 is shown as loading control. (D) Cell size of *bck2* and *swi6* mutants. Strains carrying the indicated *SWI6* and *BCK2* alleles were grown at 25° in YEPD and then either left untreated or shifted to 37° for 3 hr. Approximately 1.5×10^5 cells were sonicated and diluted into 20 ml of physiological saline solution and the mean cell volume in femtoliters was determined with a Coulter Z2 Channelyzer. Cell number in each size class is presented as a percentage of the total population. (E) Morphology of *bck2* and *swi6* mutants. Cells carrying the indicated *BCK2* and *SWI6* alleles were grown to log phase at 25° in YEPD and then either left untreated or shifted to 37° for 3 hr. Cells were imaged by phase-contrast microscopy on an Axiostar plus microscope (Zeiss) with a ×40 objective.

activation of *BCK2* is required to render cells lacking Swi6 function inviable. Two such mutant alleles, *swi6-4* and *swi6-12* in a *bck2*Δ background, were chosen for the genetic screen on the basis of both their apparent lack of mutant phenotype at 25° and their inviability at 37°. These alleles were introduced at single genomic copy into a *bck2*Δ strain to yield *bck2*Δ *swi6-4* and *bck2*Δ *swi6-12*. These strains have restrictive temperature of 34°–35° (Figure 1, A and B). As a measure of the functionality of these alleles, we analyzed cell size and morphology at both the permissive and restrictive temperature (Figure 1, D and E; data not shown). The *swi6-4* allele has only a modest cell size phenotype and no morphological phenotype at the permissive temperature. This is in contrast to either the same allele at the restrictive temperature or the *swi6*Δ mutant, which exhibits a similar morphological defect (Figure 1E). Although inactivation of *BCK2* results in an increase in cell size, it causes no morphological defect and combining *bck2*Δ with either allele results in no additional size or morphological phenotype. Nevertheless, it dramatically enhances the temperature-sensitive phenotype. The presence of *SWI6* on a plasmid complements both the temperature sensitivity and morphological phenotypes of both the *swi6-4* and *swi6-12* mutant alone or in combination with *bck2*Δ. Finally, analysis of the Swi6 protein in the *swi6-4* temperature-sensitive mutant demonstrates that the protein is thermolabile (Figure 1C). These observations suggest that these alleles of *swi6* are largely wild type at permissive temperature but inactive at the restrictive temperature. Yet, strikingly, analysis of the accumulation of the transcripts of two Swi6-dependent genes, *CLN1* and *CLN2*, revealed that are both significantly diminished at the permissive temperature and only slightly further affected at the restrictive temperature (Figure 5).

After transformation with a YEp24-based yeast genomic library, we screened ~1.4×10^5 transformants for growth at 1° above the maximum permissive temperature. We have analyzed the majority of the suppressing plasmids from the 529 suppressed strains (see MATERIALS AND METHODS for details of the screen). Most of those clones carried plasmids containing *SWI4*. The next most frequent isolated gene was *SWI6*. In addition, plasmids containing *BCK2*, *CLN2*, and *RME1*, genes previously shown to suppress mutations in *swi6*, were isolated (EPSTEIN and CROSS 1994; DI COMO *et al.* 1995; TOONE *et al.* 1995). Two genes, *MSN1* or *NHP6A*, which had previously been shown to suppress the defect in *ho::LacZ* expression caused by the temperature-sensitive *swi6-405* and *swi6-406* alleles bearing mutations in the ankyrin repeat region of *SWI6* (SIDOROVA and BREEDEN 1999), were not among those identified in this screen. In addition, we have isolated plasmids containing four genes not previously shown or predicted to suppress a *bck2 swi6*^{ts} mutant (Figure 2A) each of which is discussed below.

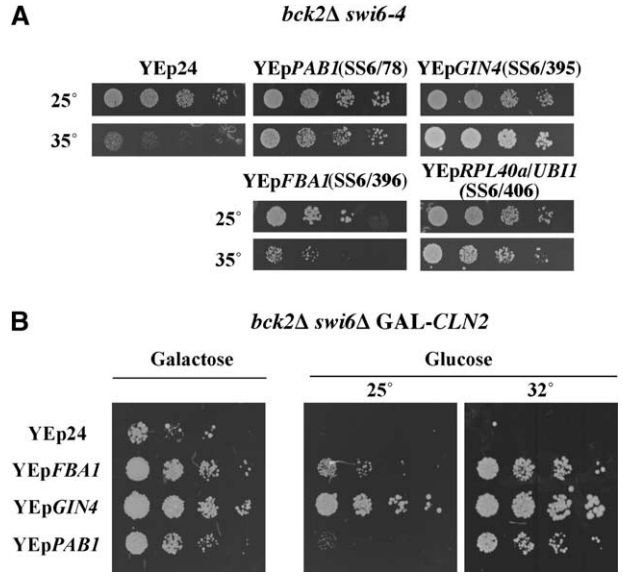


FIGURE 2.—Overview of *bck2*Δ *swi6-ts* suppressors. (A) Dosage suppressors of *bck2*Δ *swi6-ts* mutants. After screening ~1×10^5 transformants at 1° higher than maximal restrictive temperature, plasmids SS6/78, SS6/395, SS6/396, and SS6/406 were isolated as dosage suppressors of *bck2 swi6-ts*. *bck2 swi6-4* cells were retransformed with these plasmids, grown to log phase at 25° in −URA medium and spotted as described for Figure 1. The suppressing genes were identified by subcloning as *PAB1, GIN4, FBA1*, and *RPL40a/UBI1*, respectively. (B) Bypass of *bck2*Δ *swi6*Δ. *FBA1, GIN4*, and *PAB1* not only are able to suppress the temperature sensitivity of *bck2 swi6-ts*, but also can bypass a *swi6* deletion. A *bck2*Δ *swi6*Δ strain kept alive by *GAL-CLN2* was transformed with the dosage suppressors shown in A. Cells were grown to log phase in −URA medium containing galactose, spotting was done as described above onto plates containing galactose or glucose, and plates were incubated at the indicated temperatures.

Enhanced expression of aldolase can bypass the requirement for Swi6: Subcloning of plasmid SS6/396 revealed that the suppressing gene on that plasmid is *FBA1*, which encodes fructose-bisphosphate aldolase II, the glycolytic enzyme catalyzing the conversion of fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phoshophate. Glycolytic flux has been implicated in post-transcriptional regulation of *MCM1* (CHEN and TYE 1995). *SWI4* transcription is dependent on *MCM1* (MCINERNY *et al.* 1997). We reasoned that perhaps increasing *FBA1* expression increased glycolytic flux, thereby increasing the expression of *SWI4*, which was the most abundant suppressor isolated in the same screen. To evaluate that possibility, we analyzed *SWI4* transcripts in both *bck2 swi6-ts* and wild-type cells that were overexpressing *FBA1*. No significant difference in the level of *SWI4* mRNA was observed in those strains compared to cells expressing a control plasmid (data not shown).

Surprisingly, *FBA1* not only is able to suppress a *bck2 swi6-ts* strain, but also can bypass the requirement for

TABLE 3

Properties of multicopy suppressors of *swi6-ts*

Yeast strain	*FBA1*	*RPL40a/UBI1*	*GIN4*	*PAB1*
*bck2*Δ *swi6-4*	+	+	+	+
*bck2*Δ *swi6*Δ *GAL1:CLN2*[a]	+	−	+	(above 30°)
*bck2*Δ *cln3*Δ *GAL:CLN2*[a]	−	+	−	+
*mbp1*Δ *swi4*Δ *GAL:CLN2*[a]	+	−	−	−
*bck2*Δ *swi6-4 cln1*Δ	+	+	+	+
*bck2*Δ *swi6-4 cln2*Δ	−	−	−	−

Suppression (+) of the double deletion strains by the designated multicopy plasmid indicates a capacity of those cells to form colonies on YEPD plates at 30° unless otherwise indicated. Suppression (+) of the strains carrying the temperature-sensitive *swi6-4* allele indicates a capacity to form colonies on YEPD plates at 32°.

[a] These strains carry *GAL-CLN2* integrated at the *HIS2* locus. *CLN2* at its genomic locus is wild type.

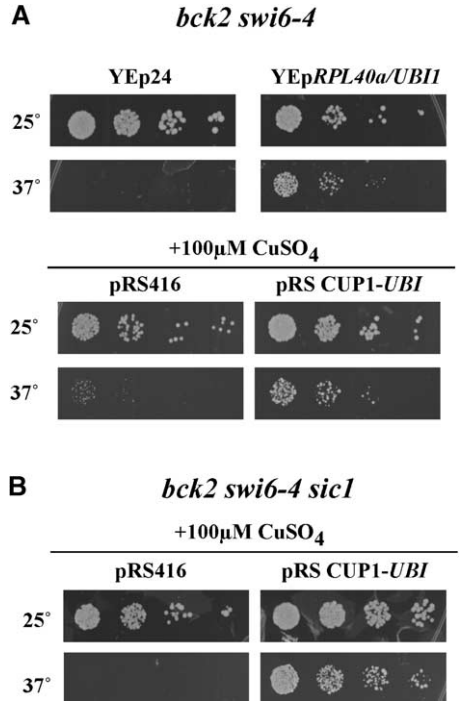


FIGURE 3.—The ubiquitin moiety of *RPL40a* suppresses *bck2*Δ *swi6-ts*. (A) *UBI1* is the suppressing moiety of *RPL40a*. *bck2 swi6-4* cells containing either control plasmid (YEp24) or YEp*RPL40a/UBI1* were grown and spotted as described in Figure 1. *bck2 swi6-4* cells were transformed with control plasmid (pRS416) or a plasmid expressing *UBI1* from the inducible *CUP1* promoter. Cells were then grown to log phase at permissive temperature in −TRP medium, spotted onto −TRP plates containing 100 μM $CuSO_4$, and incubated at the indicated temperatures for 3 days. (B) Suppression by *UBI1* is not dependent upon *SIC1*. *bck2 swi6-4 sic1* cells were transformed with control plasmid (pRS416) or a plasmid expressing *UBI1* from the inducible *CUP1* promoter. The spotting assay was performed as described in A.

Swi6 as shown by its ability to enable a *bck2*Δ *swi6*Δ *GAL-CLN2* strain to grow on glucose (Figure 2B). Although the suppression by *FBA1* appears to be dependent upon a functional *CLN3* and/or *BCK2* gene, it does not depend upon SBF or MBF (Table 3). This is surprising since the role of *CLN3* is thought to be exerted through Swi6 (WIJNEN *et al.* 2002). This remains to be investigated further.

Overexpression of ubiquitin suppresses *bck2*Δ *swi6-ts*: The suppressing gene on clone SS6/406 (Figure 3A) was identified as *RPL40a/UBI1*, a naturally occurring fusion protein composed of ribosomal protein L40 (C-terminal half) and ubiquitin (N-terminal half). To determine whether the ribosomal protein L40 or ubiquitin is responsible for suppression, an open reading frame consisting solely of ubiquitin coding sequences was expressed from the *CUP1* promoter on a 2μ plasmid. As shown in Figure 3A, ubiquitin is able to suppress *bck2 swi6-ts* to an extent similar to that of the full-length *RPL40a/UBI1* gene.

Assessment of the ability of *RPL40a/UBI1* to suppress various multiple gene mutants revealed that it is unable to bypass a complete deletion of *SWI6* in the *bck2*Δ background and is dependent on both *CLN3* and *CLN2* for suppression of the temperature-sensitive allele (Table 3). This suggests that increased accumulation of ubiquitin suppresses by enhancing the effectiveness of Swi6-dependent transcriptional activation or of one or more Swi6 targets rather than by simply bypassing the requirement for Swi6.

It has been shown that overexpression of ubiquitin can suppress the temperature sensitivity of a mutant of *CDC34*, which encodes an E2 ubiquitin ligase required for cell cycle progression (PRENDERGAST *et al.* 1995). Degradation of Sic1, a critical target of Cdc34, is required for induction of the G_1/S transition. We considered the possibility that overexpression of ubiquitin would suppress the inviability of *bck2 swi6* mutants by reducing Sic1 levels, thereby reducing the requirement for Cln1 and Cln2. To evaluate that possibility, *SIC1* was deleted in a *bck2 swi6-ts* strain and growth in the presence and absence of overexpressed *UBI1* was evaluated under nonpermissive conditions for the *bck2 swi6-ts* mutation (Figure 3B). Those experiments revealed that deletion of *SIC1* fails to rescue a *bck2 swi6-ts* strain and, furthermore, that overexpression of ubiquitin is still able to suppress the *bck2 swi6-ts* mutant in the absence of *SIC1*. We conclude that suppression of Swi6 by ubiquitin is likely to be a consequence of perturbation of the ubiquitin-proteasome system caused by the increased abundance of ubiquitin.

The G_1-specific gene *GIN4* can bypass the requirement for *SWI6*: The suppressing gene on clone SS6/395 (Figure 2A) was identified as *GIN4*, one of a family of Nim1-like protein kinases of *Saccharomyces cerevisiae*. *GIN4* was isolated as a mutant that causes synthetic lethality with *cln1*Δ *cln2*Δ (CVRCKOVA *et al.* 1995; BENTON *et al.* 1997)

and later was shown to be a member of the G_1-specific gene family (Cho *et al.* 1998; Spellman *et al.* 1998). *GIN4* is able to not only suppress *bck2*Δ *swi6-ts* when expressed from a multicopy plasmid, but also bypass the requirement for *SWI6* in a *bck2*Δ background (Figure 2B). That suppression is strictly dependent on *CLN2* (Table 3). Furthermore, deletion of *GIN4* in a *bck2 swi6-ts* severely enhances the temperature sensitivity (data not shown). Together, these findings underscore the importance of coordinate *SWI6*-dependent regulation of *GIN4*, *CLN1*, and *CLN2*.

The Wee1-related kinase Swe1 specifically inhibits the mitotic form of Cdc28 (Booher *et al.* 1993) and delays entry into mitosis in response to defects in septin ring formation (Barral *et al.* 1999) and bud emergence (Sia *et al.* 1996). *SWE1*, like *GIN4* and the other genes encoding Nim1-like protein kinases, *KCC4* and *HSL1*, are *SWI6*-dependent G_1-specific genes (Spellman *et al.* 1998; Iyer *et al.* 2001). It has been suggested that *GIN4* may act redundantly with *HSL1* and *KCC4* to negatively regulate *SWE1* and thereby induce entry into mitosis (Barral *et al.* 1999). Because we found severe septin ring defects in *bck2 swi6-ts* mutants that are at least partially suppressed by *GIN4* on a multicopy plasmid (Figure 4A), we evaluated whether the inactivation of *SWE1* abolishes the suppressing effect of *GIN4*. As shown in Figure 4B deletion of *SWE1* fails to rescue a *bck2*Δ *swi6ts* mutant. Furthermore, suppression by *GIN4* is independent of *SWE1* (Figure 4B).

To evaluate the mechanism by which *GIN4* might suppress defects arising in the *bck2*Δ *swi6-ts* mutant, we asked whether rescue of the mutation was dependent upon Gin4 protein kinase activity. It has been previously established that $gin4^{K48M}$, a *gin4* mutant in which an invariant lysine in the kinase domain is altered to alanine (Longtine *et al.* 1998; Figure 4C), is able to localize to septin rings but unable to suppress either the temperature sensitivity of septin mutant *cdc12-6* or the synthetic lethality arising from combined mutations in *cdc12* and *gin4*. Surprisingly, $gin4^{K48M}$, when present on a multicopy plasmid, retains the capacity to suppress the *bck2 swi6-ts* to an extent similar to that of the wild-type gene. Although it is unclear whether the capacity of Gin4 to suppress *bck2*Δ *swi6*Δ mutants and normalize their septin ring structure (Figure 4A) is dependent upon localization of Gin4 to septin rings, its protein kinase activity is dispensable for this function.

Poly(A)-binding protein *PAB1* can bypass the requirement for *SWI6* at high temperature: The suppressing gene on clone SS6/78 (Figure 2A) was shown by subcloning to be *PAB1*, which encodes poly(A)-binding protein. *PAB1* has several functions. First, it plays roles in polyadenylation, poly(A) length control, and mRNA turnover via the deadenylation/decapping pathway (Morrissey *et al.* 1999; Otero *et al.* 1999) and, second, it plays a role in poly(A)-dependent translation (Sachs *et al.* 1997). The latter function is mediated by the ability

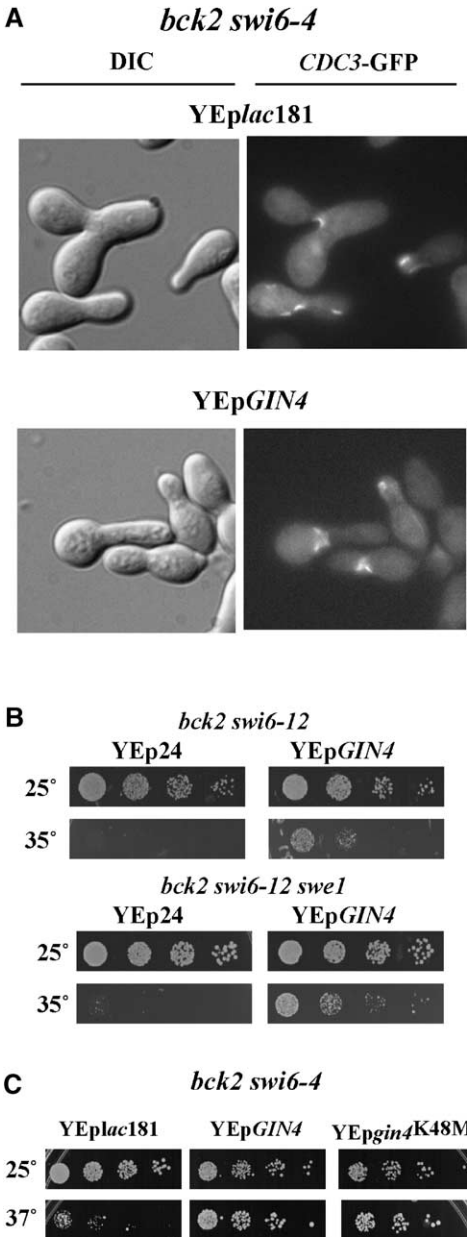


Figure 4.—Suppression of *bck2*Δ *swi6-ts* by *GIN4*. (A) Morphogenetic and septin ring defects in *bck2*Δ *swi6-ts* mutants with and without multicopy *GIN4*. *bck2 swi6-4* strains carrying either control plasmid (YEplac181) or YEp*GIN4* were transformed with YCp*CDC3*-GFP. Cells were grown to log phase at permissive temperature and then shifted to 35° for a time course; the 10-hr point is shown here. For microscopic analysis, cells were fixed in methanol at −70°. DIC and fluorescence microscopy were performed using an Eclipse E800 microscope with a ×100 objective. (B) Suppression by *GIN4* is not dependent upon *SWE1*. *bck2 swi6-12* cells (top) and *bck2 swi6-12 swe1* (bottom) were transformed with a control plasmid (Yep24) or YEp*GIN4*. Spotting assays were done as described in Figure 1. (C) Suppression by *GIN4* is not dependent upon a functional protein kinase domain. *bck2 swi6-4* cells were transformed with a control plasmid (YEplac181), YEp*GIN4*, or YEp-$gin4^{K48M}$. YEp$gin4^{K48M}$ harbors a mutation that renders *GIN4* kinase inactive (Longtine *et al.* 1998). Cell growth and spotting assays were performed as described above.

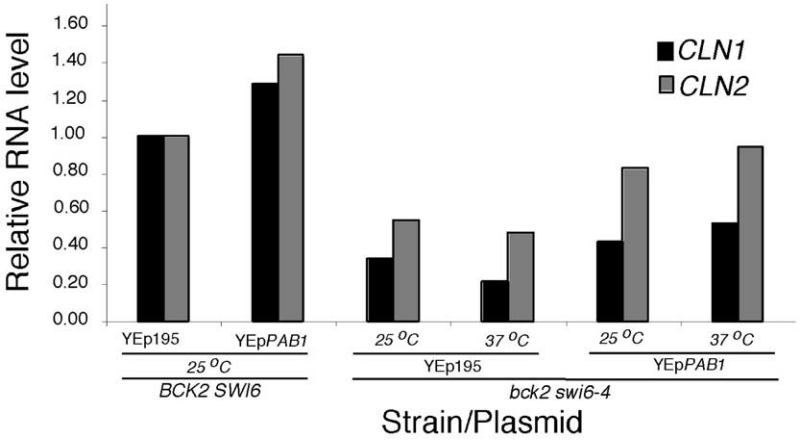


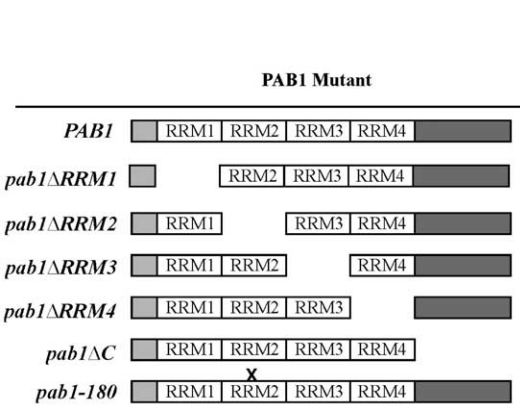
FIGURE 5.—Analysis of G_1 cyclin transcripts and protein in *bck2*Δ *swi6-4* mutants containing multicopy *PAB1*. (A) Overexpression *PAB1* leads to an increase in *CLN1* and *CLN2* transcript. Wild-type and *bck2 swi6-4* cells were transformed with either control plasmid or plasmid overexpressing *PAB1*. Cells were grown to log phase in −Ura medium. The cells were then split and either grown at permissive temperature or shifted to 37° for 6 hr. *CLN1*, *CLN2*, and ACT1 transcript levels were determined by Northern blotting. *CLN1* and *CLN2* RNA levels were normalized to *ACT1* RNA and are presented as the proportion of the RNA level in wild-type cells at the permissive temperature.

of *PAB1* to bind to the 5′-cap-binding protein eIF4G and thereby stimulate the recruitment of the 40S ribosomal subunit (TARUN and SACHS 1995).

To evaluate whether overexpression of *PAB1* affects *CLN1* and *CLN2 RNA* we analyzed the abundance of those transcripts in cells carrying *PAB1* on a multicopy plasmid (Figure 5). Whereas both *CLN1* and *CLN2* RNA levels are reduced in the *bck2*Δ *swi6-4* mutant relative to wild-type cells at both permissive and restrictive temperatures, the levels of both transcripts are increased by multicopy *PAB1* with *CLN2* increasing to nearly the wild-type level. The increase in expression of those genes in cells carrying multicopy PAB1 was approximately twofold at 37° and approached wild-type levels. A similar effect of *PAB1* is seen in wild-type cells (Figure 5). An increase in expression of *SWI4* and *GIN4* was also observed. These findings suggest that overexpression of *PAB1* suppresses *bck2 swi6-ts* through a mechanism similar to that of G_1 cyclins. However, in synchronized populations of the same strains, no increase of *CLN2* transcripts could be demonstrated (data not shown). This suggests that the increased levels in *CLN2* mRNA observed in asynchronous cells is due to an effect on cell cycle distribution (data not shown) and not a more direct effect of *PAB1* on gene expression.

Suppression of *bck2 swi6-ts* by *PAB1* is very efficient, enabling cells to grow well at 37° and enhancing the growth of *bck2 swi6-ts* cells even at permissive temperature when grown on solid medium. A similar effect on growth rate was not detectable when the same cells were grown at the permissive temperature on liquid medium (data not shown). Multicopy *PAB1* is able to not only suppress the conditional lethality of the *bck2 swi6-ts* strain, but also bypass a *bck2*Δ *swi6*Δ mutant (Table 3). However, that suppression is effective only at elevated temperatures (32°), not at lower temperatures (18° and 25°; Figure 2B and data not shown). The relevance of this requirement for increased temperature is unclear.

The Pab1 protein contains four N-terminal RNA recognition motifs (RRMs; Figure 6). RRMs are found in a number of different RNA-binding proteins and have been associated with the capacity to interact with RNA. RRM2 is the most important RRM for Pab1 function and has been shown to participate in both poly(A) binding and the interaction of Pab1 with eIF4G (DEARDORFF and SACHS 1997; KESSLER and SACHS 1998). A point mutation in RRM2, *pab1-180* has been shown to compromise the ability of Pab1 to bind to eIF4G. In contrast, RRM4 plays a role in the interaction of Pab1 with nonpolyadenyalated RNAs. *In vitro* data suggest that RRM1,



PAB1 Mutant	Relative Translational Efficiency (fold) (Kessler 1998)	Restrictive Temperature
PAB1	1.0	37°C
pab1ΔRRM1	2.9	≥37°C
pab1ΔRRM2	0.2	34°C
pab1ΔRRM3	1.9	37°C
pab1ΔRRM4	0.3	35°C
pab1ΔC	2.0	34°C
pab1-180	0.2	≥37°C

FIGURE 6.—Suppression of *bck2*Δ *swi6-4* by RNA-binding motif mutants of *PAB1*. *bck2 swi6-4* cells were transformed with control plasmid or with plasmids containing mutant forms of *PAB1* depicted on the left side of the table. The relative translational efficiency observed in Pab1-depleted *in vitro* translation extracts supplemented with the indicated Pab1 mutant proteins is derived from KESSLER and SACHS (1998). Cells were spotted as described and the minimum restrictive temperature of the strain is shown.

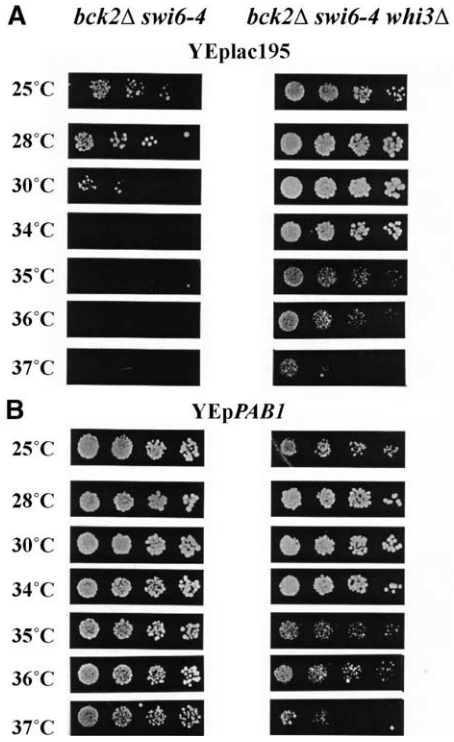


FIGURE 7.—Role of *WHI3* in suppression of *bck2*Δ *swi6-4*. (A) Inactivation of *WHI3* suppresses *bck2*Δ *swi6-4*. *bck2 swi6-4* (left) and *bck2 swi6-4 whi3* (right) cells carrying a control plasmid were spotted onto −URA plates as described and incubated for 2 days at the temperature indicated. (B) Multicopy *PAB1* depends upon *WHI3* for suppression of *bck2*Δ *swi6-4*. *bck2 swi6-4* (left) and *bck2 swi6-4 whi3* (right) cells carrying YEp*PAB1* were analyzed as described in A.

RRM4, and the C terminus of Pab1 mediate the translation function associated with the Pab1-poly(A) tail interaction. No specific function has been attributed to RRM3 (OTERO *et al.* 1999).

To test which domain of *PAB1* is responsible for suppression of a *bck2 swi6-ts* we have cloned *PAB1* mutants (KESSLER and SACHS 1998; OTERO *et al.* 1999) into multicopy plasmids and evaluated their capacity to suppress the *bck2 swi6-ts* mutation (Figure 6). Whereas YEp-*pab1*Δ*RRM2*, YEp-*pab1*Δ*RRM4*, and YEp-*pab1*Δ*C* have lost the ability to suppress *bck2 swi6-ts*, YEp-*pab1*Δ*RRM1*, YEp-*pab1*Δ*RRM3*, and YEp-*pab1-180* suppress to an extent similar to that of wild type *PAB1* (YEp-*PAB1-1*). On the basis of these results we conclude that the ability of *PAB1* to suppress *bck2 swi6-ts*, while dependent upon specific domains, is not dependent upon domains associated with a specific Pab1-associated function. Instead, it appears to be dependent upon the overall efficiency of translation supported by each of the *pab1* mutations, which, on the basis of their capacity to complement the defect in *in vitro* translation observed in Pab1-depleted extracts, is reduced in the *pab1*Δ*RRM2*, *pab1*Δ*RRM4*, and *pab1*Δ*C* mutants (KESSLER and SACHS 1998; Figure 6).

Whi3, another RNA-binding protein with RRM motifs, has been shown to delay G_1-specific transcription and progression out of G_1 phase (NASH *et al.* 2001). Although it has been proposed to act by directly binding to the *CLN3* mRNA, *CLN2* mRNA also appears to be a target (GARI *et al.* 2001). Because *PAB1* suppresses a defect in a G_1-specific transcriptional activator, we reasoned that one of its roles might be to antagonize the G_1-specific function of Whi3. To evaluate that possibility we first asked whether inactivation of *WHI3*, like overexpression of *PAB1*, suppressed the thermosensitivity of a *bck2 swi6-4* mutant. That analysis revealed that a *bck2*Δ *swi6-4 whi3*Δ strain grew at substantially higher temperatures than the control strain without *whi3*Δ (Figure 7A). However, that suppression was not nearly as effective as that conferred by multicopy *PAB1* (Figure 7B, left). Furthermore, it did not suppress the lethality associated with *bck2*Δ *swi6*Δ (data not shown). Because the strain carrying *whi3*Δ grew significantly less well than one carrying multicopy *PAB1* at temperatures above 35°, we could ask whether the suppression by *PAB1* was dependent upon *WHI3*. To do so, *YEplac195-PAB* was introduced into the *bck2*Δ *swi6-4 whi3* strain and the thermolability of that strain was compared to the thermolability of the strain lacking the plasmid. Strikingly, *PAB1* conferred no suppression of *bck2*Δ *swi6-4* additional to that conferred by *whi3*Δ (Figure 7B, right), suggesting that it depends upon *WHI3* to suppress *swi6-ts* mutants. Although it is difficult to establish without further analysis, this finding suggests that Pab1 might act to directly antagonize the effect of Whi3 on specific mRNA including, but not limited to, that encoding G_1 cyclins.

DISCUSSION

A screen for multicopy suppressors of temperature-sensitive alleles of *SWI6* was performed under conditions in which those mutations were rendered conditionally lethal by the inactivation of *BCK2*. Although the intention of the screen was to identify elements of the G_1-specific transcriptional machinery, from the outset several mechanisms were envisioned by which the inviability of the *bck2*Δ *swi6-ts* strain might be suppressed. First, overexpression of a gene might stabilize the defective Swi6 or otherwise enhance the activity of either MBF or SBF, or both. Second, genes that are dependent upon *SWI6* for expression might be expressed sufficiently when present in multiple copies to bypass that dependence. Third, overexpression of genes that can activate G_1-specific transcriptional targets independent of SBF and MBF might lead to suppression, as had been previously described for *RME1* (TOONE *et al.* 1995). Fourth, suppression might result from overexpression of genes that bypass the requirement for *SWI6* by bypassing the specific requirement for an essential target of Swi6-dependent transcription. Consequently, in addition to identifying genes encoding proteins that become

rate limiting when *SWI6* is inactivated, this screen provides insight into processes that become essential in the absence of *SWI6*. Finally, because *bck2*Δ is required for the lethality of the *swi6-ts* mutants, suppressors of *bck2*Δ could also be isolated.

In addition to *SWI6* and *BCK2*, suppressors that fall into each of these categories on the basis of prior analysis were identified in the course of the screen. As expected we isolated *SWI4*, an element of the SBF transcription factor that is able to act independent of *SWI6* when sufficiently expressed (Andrews and Moore 1992; Primig *et al.* 1992; Sidorova and Breeden 1993) and, therefore, that falls into both the first and third classes. Unlike *SWI4*, another suppressor, *RME1*, falls into the third class because it activates *CLN2* transcription independent of SBF, MBF, or their promoter binding sites. *CLN2*, a target of SBF that is known to be sufficient to bypass the requirement for G_1-specific transcription, belongs to the second class. All four remaining genes isolated in the screen appear to suppress downstream of *SWI6*. However, with the exception of *GIN4*, they are not targets of SBF or MBF and, therefore, fall into one of the last two categories.

Whether any of these suppressors act specifically by suppressing the deficiency in *bck2*Δ is very difficult to address. None of the mutants efficiently suppresses the morphological phenotype that results from the *swi6* mutation. However, neither does *CLN2* or *RME1*, both of which clearly act by overcoming the defect in *CLN2* expression. Next, *GIN4*, as a target of *SWI6*, is likely suppressing by overcoming the defect in *GIN4* expression. Finally, because *BCK2* affects the same targets as *SWI6* (Wijnen and Futcher 1999), it will be suppressed downstream by the same genes that suppress *swi6*.

Although the isolation of *SWI4* would appear to support the argument that interactors or upstream components of SBF can suppress a *swi6-ts* mutant by direct interaction, it has been established that overexpression of *SWI4* alone is sufficient to induce SBF-dependent gene expression in the absence of *BCK2* and *SWI6* (Andrews and Moore 1992; Primig *et al.* 1992; Sidorova and Breeden 1993). *BCK2* has been shown to be sufficient in the absence of *SWI6* to promote low-level expression of G_1-specific genes, although the mechanism by which it affects transcription of SBF-dependent genes is unknown (Epstein and Cross 1994; Di Como *et al.* 1995; Wijnen and Futcher 1999). Thus, these observations are not sufficient to confirm the efficacy of the screen. Finally, it has also been established that either increasing the copy number of *CLN2*, a target of SBF, or inducing its expression via increasing the copy number of *RME1*, a transcriptional activator that acts independently of SBF, is sufficient to suppress a *bck2*Δ *swi6*Δ. Nevertheless, our failure to isolate genes having a clearly identifiable role in transcriptional activation argues that such positively acting molecules might not exist or, perhaps more likely, may not be accessible via this screen. One likely explanation for the failure to identify novel targets of Cln3/CDK in this screen is that the relevant targets for activation of Swi6-dependent transcription act to negatively regulate that process. We have recently identified one such target as an SBF-associated transcriptional inhibitor (de Bruin *et al.* 2004).

More surprising than the finding that this screen did not identify genes encoding factors that behaved as transcriptional activators was the precise nature of the genes that were isolated. Perhaps aldolase, a highly abundant glycolytic enzyme, is the most surprising suppressor. Although it is possible that the suppression by *FBA1* is a nonspecific effect on osmoregulation or cell integrity due solely to its abundance, other abundant cytosolic proteins were not isolated. It is more likely to be associated with the enzymatic function of *FBA1*. Overproduction of this enzyme might induce a signal of a nutrition-rich environment. *CLN3* expression is regulated by nutrition availability (Gallego *et al.* 1997; Polymenis and Schmidt 1997; Wu *et al.* 1999; Newcomb *et al.* 2003) although it is unclear how *CLN3* would act in the absence of the transcription factor. The potential for such overlap in function has been documented (Miller and Cross 2000; Edgington and Futcher 2001). Alternatively, aldolase could have a direct and heretofore unrecognized role in transcriptional activation, as has been recently reported for glyceraldehyde 3-phosphate dehydrogenase (encoded by the *TDH1-TDH3* genes in yeast; Zheng *et al.* 2003).

PAB1, like *FBA1*, is involved in a general metabolic role in cells: namely, global regulation of translation. However, a number of observations led us to believe that suppression of *bck2*Δ *swi6-ts* by *PAB1* occurs via a mechanism more directly related to G_1-specific gene expression. First, human *PAB1* can overcome the G_1 arrest occurring in response to mating pheromone (Edwards *et al.* 1997). In that circumstance the effect of human *PAB1* was shown to be dependent on *CLN1*. In contrast, we found the suppression of *bck2 swi6-ts* by multicopy *PAB1* to be strictly dependent upon *CLN2* (Table 1). However, it is likely that this distinction is not due to differences in the source of *PAB1* but rather to the nature of the screen. Another observation that appears to link translation efficiency specifically to G_1-specific transcription is the identification of *SWI4* as synthetic lethal with *eIF4G-DN300*, a mutant of the translation initiation factor eIF4G unable to bind to *PAB1* (A. Sachs, personal communication). Finally, we isolated *EAP1*, a gene recently described as a binding partner of eIF4E (Chial *et al.* 2000) in a screen for genes that, like *CLN3* and *SWI6*, are synthetic lethal with *BCK2* (T. Kesti and C. Wittenberg, unpublished observation). All of these observations are consistent with a specific effect of *PAB1* on G_1-phase progression or gene expression.

Despite these connections, we found little direct evidence for a specific effect on the cell cycle machinery. Nevertheless it remains possible that a more general

effect of *PAB1* on translation rate manifests itself as a specific effect on the cell cycle via an effect on a rate-limiting G_1-specific transcriptional target. The finding that suppression of *bck2 swi6-ts* by multicopy *PAB1* depends upon *WHI3* suggests that Pab1 can act, at least in part, to antagonize Whi3 function, perhaps by abrogating its effect on *CLN2* or *CLN3* mRNA.

Ubiquitin is also involved in a process of general importance to the cell. Yet, suppression of the Swi6 deficiency by overexpression of ubiquitin appears consistent with the critical role for G_1-specific transcription products in directing the degradation of proteins that restrict proliferation. We hypothesized that enhanced degradation of Sic1 might explain the suppressive effect of increased ubiquitin on *bck2*Δ *swi6-ts* but found that inactivation of Sic1 is not sufficient to suppress *bck2*Δ *swi6-ts* or to interfere with the capacity of ubiquitin to suppress that mutant. Thus, if increased ubiquitin leads to enhanced degradation of a natural target of the G_1-specific cell cycle machinery, it does so via one or more proteins other than Sic1. Alternatively, overexpression of ubiquitin may interfere with other functions responsible for the lethality of these mutants.

Expression of *CLN2* from a heterologous promoter is sufficient to bypass the lethality of *bck2*Δ *cln3*Δ, *bck2*Δ *swi6*Δ, or *cln1*Δ *cln2*Δ *cln3*Δ mutants. In contrast, inactivation of Sic1 bypasses the lethality caused by a *CLN* deficiency, but not that caused by either of the other mutants. Because *CLN2* is sufficient to induce phosphorylation-dependent degradation of Sic1, we must assume that it plays additional essential roles in the context of *bck2*Δ *cln3*Δ and *bck2*Δ *swi6*Δ mutants. In the absence of Cln3, *GAL*-expressed *CLN2* is an effective activator of G_1-specific transcription (Cross and Tinkelenberg 1991; Dirick and Nasmyth 1991), a function that is likely to be important in the suppression of *bck2*Δ *cln3*Δ mutants, which are severely compromised in that regard. However, *CLN2* must play a quite different role in the *bck2*Δ *swi6*Δ strain where neither SBF nor MBF is functional and, as a consequence, the targets of Cln/CDK required for transcriptional activation cannot act. In this context it is likely that Cln2 suppresses via its capacity to directly promote cell cycle progression through either normal or ectopic mechanisms. Thus, there are clearly multiple distinct pathways via which Cln2 can act to suppress a deficiency in G_1-specific functions.

The nature of suppression by *CLN2* must be taken into account when considering the mechanisms utilized by the multicopy suppressors because suppression by each depends upon *CLN2* (Table 3). The *bck2*Δ *swi6-ts* strain must remain competent to express *CLN2* and its expression or function may be facilitated by the overexpression of the suppressing gene. Although it is possible that *PAB1* acts by facilitating Cln2 translation, its effect is not noticeable in terms of the level of Cln2 protein in an asynchronous culture. Alternatively, the suppressing gene might act by facilitating a pathway that is sufficient in the presence of *CLN2* but not in its absence. *GIN4*, which functions in the pathway that is required for viability in the absence of *CLN2*, may suppress by such a mechanism. We have established that, as targets of the G_1-specific transcriptional machinery, both *GIN4* and *CLN2* are poorly expressed in a *bck2*Δ *swi6-ts* mutant (Figure 5 and data not shown). Although increasing the copy number of *GIN4* is sufficient to support viability, that suppression is dependent upon Cln2. We suggest that either the level of *GIN4* expression remains sufficiently low in the *swi6* mutant to require *CLN2* or the poor expression of other Swi6-dependent gene products creates a situation in which both *GIN4* and *CLN2* become essential.

Together, these data suggest that defects in the G_1-specific transcriptional apparatus can be suppressed via diverse pathways. Although this is consistent with the involvement of G_1-specific genes in many functions, it appears that a relatively limited subset of those pathways is sufficient for viability. Whereas some of the suppressors (*e.g.*, *PAB1* and *UBI1*) are associated with rather general functions, others (*e.g.*, *CLN2* and *GIN4*) appear to be more specific. Although the specific mechanisms of suppression are unclear, it may be that *CLN2* and *GIN4* define the G_1-specific pathways that are essential for viability whereas *PAB1* and *UBI1* act via more general mechanisms to facilitate deficiencies in those pathways.

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