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Genetic Evidence for a Morphogenetic Function of the *Saccharomyces cerevisiae* Pho85 Cyclin-Dependent Kinase

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ABSTRACT

The *Saccharomyces cerevisiae PHO85* gene encodes a nonessential cyclin-dependent kinase that associates with 10 cyclin subunits. To survey the functions provided by Pho85, we identified mutants that require *PHO85* for viability. We identified mutations that define seven Pho *E*ighty-*F*ive *R*equiring or Efr loci, six of which are previously identified genes—*BEM2* (*YER155C*), *SPT7* (*YBR081C*), *GCR1* (*YPL075W*), *SRB5* (*YGR104C*), *HFI1* (*YPL254W*), and *BCK1* (*YJL095W*)—with one novel gene (*YMR212C*). We found that mutations in the EFR genes involved in morphogenesis are specifically inviable when the Pho85-associated G1 cyclins encoded by *PCL1* and *PCL2* are absent. *pcl1Δ bem2*, *pcl1Δ pcl2Δ cla4Δ*, and *pcl1Δ pcl2Δ cdc42-1* strains are inviable. *pcl1Δ pcl2Δ mpk1Δ*, *pcl1Δ pcl2Δ bck1*, and *pcl1Δ pcl2Δ cln1Δ cln2Δ* strains are also inviable, but are rescued by osmotic stabilization with 1 m sorbitol. We propose that the G1 cyclins encoded by *PCL1* and *PCL2* positively regulate *CDC42* or another morphogenesis promoting function.

CYCLIN-DEPENDENT kinases (CDKs) are heterodimeric protein kinases found in all eukaryotes. Although most CDKs associate with multiple cyclin subunits, these different cyclin-CDK complexes appear to have similar biological activities. For example, CDK4 associates with four different D-type cyclins that are subject to cell-type specific transcriptional regulation, but these complexes all promote cell-cycle progression through the restriction point (for review see Sherr 1995). The *Saccharomyces cerevisiae* CDK Cdc28 associates with nine different cyclins and these different Cdc28-containing complexes play essential roles in progression through different phases of the cell cycle, suggesting that cyclin binding might also confer some functional specificity on the CDK subunit.

S. cerevisiae has five CDKs. In addition to Cdc28, there are three CDKs (Srb10, Kin28, and Ctk1) that associate with only 1 or 2 cyclins and seem to be involved in regulating RNA Pol II transcription (Liao *et al.* 1995; Sterner *et al.* 1995; Valay *et al.* 1995). The remaining *S. cerevisiae* CDK is Pho85, a nonessential CDK that associates with 10 cyclins that can be divided into two families based on sequence similarity: the Pho80 cyclin family (Pho80, Pcl6, Pcl7, Pcl8, and Pcl10) and the Pcl1,2 family (Pcl1, Pcl2, Clg1, Pcl5, and Pcl9; for review see Andrews and Measday 1998). In association with the cyclin Pho80, Pho85 phosphorylates the transcription factor Pho4, thereby inhibiting the expression of phosphate-starvation induced genes (for review see Lenburg and O'Shea 1996). In association with Pcl8 and Pcl10, Pho85 phosphorylates the Gsy2 glycogen synthase, thereby inhibiting glycogen accumulation during fermentation (Timblin *et al.* 1996; Wilson *et al.* 1999). Pho80-Pho85 is a potent Pho4-directed kinase but phosphorylates Gsy2 very poorly *in vitro*. Pcl10-Pho85 displays the opposite specificity *in vitro* (Wilson *et al.* 1999). These data and the differing phenotypes of strains with mutations in these cyclins indicate that Pho85 complexes containing different cyclin subunits can have distinct functions.

Transcription of *PCL1*, *PCL2*, and *PCL9* peaks in G1, implicating *PHO85* in cell-cycle regulation. The observation that deletion of *PCL1* and *PCL2* is lethal in a strain lacking *CLN1* and *CLN2* (two of the three Cdc28 G1 cyclins), causing growth arrest in G1, supports this hypothesis (Espinoza *et al.* 1994; Measday *et al.* 1994). The cyclins in the Pcl1,2 family may participate in a process that affects cell polarity and morphogenesis. Strains that lack these Pho85-containing kinases display phenotypes such as salt sensitivity, reduced endocytosis, and a random budding pattern (Lee *et al.* 1998), phenotypes that are common to mutants defective in cell polarity and morphogenesis. Pho85-Pcl2 can phosphorylate Rvs167, a protein involved in actin polymerization, providing a possible mechanism by which Pho85 participates in establishing cell polarity during G1 (Lee *et al.* 1998). However, cells must lack all members of the Pcl1,2 family to display these cell-polarity related phenotypes (Lee *et al.* 1998; Tennyson *et al.* 1998). It is possible that the morphogenetic defects of strains lacking these five cyclins are the result of a defect in a single Pho85-regulated process to which all these cyclins contribute or that these phenotypes are a secondary consequence of lesions in several distinct Pho85-regulated processes.

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As such, *PHO85* provides a model system for understanding the situations in which cyclin binding confers functional specificity on the CDK subunit and the mechanisms by which this specificity is achieved.

In this study, we performed a screen for Pho *E*ighty-*F*ive *R*equiring (Efr) mutants—mutants that require *PHO85* for viability. If the functional specificity of Pho85 is achieved by cyclin association, we reasoned that we might be able to phenocopy the inviability of different *efr pho85* strains by deleting the cyclins that associate with Pho85 to perform the particular function required for viability. By classifying different Efr mutants according to which Pho85-associated cyclins they require, we hoped to discover functional differences among the cyclins and something about the functions they perform. Our screen identified seven genes that when mutated make *PHO85* essential for viability. Characterization of these mutants and directed tests for synthetic lethality between *PHO85* and several other genes provides us with clues about the functions provided by the G1-specific Pho85-associated kinases.

MATERIALS AND METHODS

Yeast manipulation: Yeast were cultured and manipulated according to standard laboratory procedures, which have been described previously (Guthrie and Fink 1991). Strains and plasmids used in this article can be found in Tables 1 and 2, respectively. Details of the construction of original plasmids are available on request.

The *GAL1-10::PHO85* allele was generated by transforming MY0192 with plasmid MP136 linearized with *Bgl*II. The vast majority of transformants displayed a Pho$^+$ phenotype on galactose-containing media and a constitutive *PHO5* expression, or Pho^c, phenotype on dextrose-containing media. One of these transformants was plated on 5-fluoroorotic acid-containing plates. A Ura$^-$ colony that displayed a galactose-dependent Pho85$^+$ phenotype was identified and the presence of the *GAL1-10* promoter replacing the *PHO85* promoter was confirmed by PCR. This strain was named MY0246.

The transposon-insertion-containing DNA fragments we used as a mutagen in our screen for Efr mutants were derived from eight different pools of a yeast genomic library that had been mutagenized with a Tn3 transposon (Burns *et al.* 1994). Four alleles of *EFR1* were obtained from yeast transformed with fragments from two separate pools. Two alleles of *EFR2* were obtained from yeast transformed with fragments from one pool of the library.

To generate new alleles of *PHO85*, *PHO85* was amplified by error-prone PCR using *Taq* DNA polymerase at standard conditions except the concentration of $MgCl_2$ was lowered from 1.5 to 1.34 mm and $MnCl_2$ was added to 160 μm. We screened for temperature-sensitive (ts) alleles of *PHO85* by cotransforming a *cln1*Δ *cln2*Δ *GAL1-10::CLN1 pho85*Δ strain (MY0126) with these PCR products and MP056 digested with *Eco*RI. Transformations were plated on SD at room temperature. Temperature-sensitive alleles of *PHO85* were identified by replica plating the transformation plates and screening for colonies that were unable to grow at 37°. Positives from this screen were then tested for temperature-sensitive constitutive Pho5 activity (a ts-Pho^c phenotype) by an acid-phosphatase activity plate assay performed on strains growing in SG medium at room temperature or 37°. We recovered the *PHO85* plasmid from three strains with a strong temperature-dependent Pho85 phenotype, and the *PHO85* allele from each was used to replace the wild-type *PHO85* locus. The allele that we named *pho85-9* displays the most dramatic temperature-sensitive phenotype and was used in all experiments. *pho85-9* strains display a variety of Pho85 phenotypes at elevated temperature such as an inability to grow in glycerol medium (Gilliquet and Berben 1993) and supersensitivity to hydroxyurea (our unpublished observations) though we found that the nonpermissive temperature for different Pho85 phenotypes varied from 30° to 37°. We could not test whether the *pho85-9* allele results in a glycogen hyperaccumulation phenotype (Huang *et al.* 1996; Timblin *et al.* 1996) because we cannot detect this phenotype in our *pho85*Δ strains.

High-copy plasmids carrying each of the Pho85-associated cyclins were made as follows. Genomic clones of *PCL7*, *PCL8*, *PCL9*, and *PCL10* were identified in a YEp13 genomic library (Nasmyth and Tatchell 1980) by colony hybridization using fragments isolated from pBA949, pBA946a, pBA950, and pBA945a, respectively. Fragments containing the cyclin locus from these plasmids were then subcloned into pRS426 using naturally occurring restriction enzyme sites. Plasmids containing other Pho85-associated cyclins were generated by subcloning fragments containing *PCL1* (from pFHE27), *PCL2* (from pBA619), *PHO80* (from pAC800), *PCL5* (from pBA906), and *PCL6* (from pRS425-*PCL6*, a generous gift of Anita Sil) into pRS426. pRS426-*CLG1* (pBA904) and the other pBA plasmids were the generous gift of Brenda Andrews.

Strains containing deletions of various Pho85-associated cyclins were constructed as follows. Deletion of *PCL1* was performed by transformation of strain EY057 with *Sph*I-*Sal*I-digested EB0149 and selecting for integrants on medium lacking histidine. Integration of *HIS3* at *PCL1* was confirmed by PCR. Deletion of *PCL2* was performed by transformation with *Kpn*I-*Xba*I-digested EB0226 and selecting for integrants on medium lacking uracil. Integration of *URA3* at *PCL2* was confirmed by PCR. One such strain was named EY0535. *PCL9* was deleted by transforming EY0535 with EB0727 that had been digested with *Not*I and *Xho*I and selecting for integrants on medium lacking tryptophan. Integration of *TRP1* at *PCL9* was confirmed by PCR. One such strain was named EY0552.

A deletion of the *EFR3* locus marked with the *LEU2* gene was generated by transforming EY0099 with MP0030 cut with *Sal*I and *Bam*HI and selecting for integrants on medium lacking leucine. Integration at the *EFR3* locus was confirmed by PCR.

Cloning of transposon insertions: The genomic position of transposon insertions that give rise to an Efr phenotype was determined by first using T4 DNA ligase to circularize fragments obtained by digesting genomic DNA from the Efr strains with *Csp*6 I. Primers that hybridize to the sense strand of the transposon just downstream of the 5′ end or to the antisense strand just upstream of the transposon's first *Csp*6 I site were used to perform PCR with the circularized DNA fragments, and the resulting PCR products were sequenced directly. We identified the genomic sequence adjacent to the transposon end by searching the yeast genome sequence database (Cherry *et al.* 2000).

RESULTS

Identification of mutants that require *PHO85* for viability: To screen for Efr mutants we constructed a strain in which expression of *PHO85* is galactose dependent and identified strains that can grow only in the presence of galactose. We mutagenized the *GAL1-10::PHO85*

TABLE 1

Yeast strains used in this study

Strain	Genotype	Source
EY0057	K699 *MAT***a** *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3*	Lab collection
EY0099	K699 *MAT***a**/α	Lab collection
EY0135	K699 *MAT*α *pho80*Δ*::HIS3*	Lab collection
EY0140	K699 *MAT***a** *pho85*Δ*::LEU2*	Lab collection
EY0141	K699 *MAT*α *pho85*Δ*::LEU2*	Lab collection
EY0173	bf264-15D *MAT***a** *cln1*Δ *cln2*Δ	F. Cross
EY0230	bf264-15D *MAT*α *bar1 trp1 leu2 ura3 ade1 his2*	F. Cross
EY0233	bf264-15D *MAT*α *cln1*Δ *cln2*Δ *leu2::GAL1-10::CLN1::LEU2*	F. Cross
EY0234	YPH274 *ade2-101 his3*Δ*200 leu2*Δ*1 lys2-801 trp1*Δ*63 ura3-52 cln1*Δ*::TRP1 cln2*Δ*::LEU2*	A. Sil
EY0340	bf264-15D *MAT***a** *cln1*Δ *cln2*Δ *lys2*	EY0233 × EY0234
EY0409	bf264-15D *pcl1*Δ*::HIS3 pcl2*Δ*::LYS2 cln1*Δ *cln2*Δ *leu2::GAL1-10::CLN1::LEU2*	F. Cross
EY0478	*MAT*α *far1-c* Lys$^-$	L. Huang
EY0535	K699 *MAT*α *pcl1*Δ*::HIS3 pcl2*Δ*::URA3*	EY0099
EY0551	K699 *MAT***a** *pcl1*Δ*::HIS3 pcl2*Δ*::URA3 pcl9*Δ*::TRP1*	EY0552 × EY0057
EY0552	K699 *MAT*α *pcl1*Δ*::HIS3 pcl2*Δ*::URA3 pcl9*Δ*::TRP1*	EY0535
MY0114	K699 *MAT***a** *efr3*Δ*::LEU2*	EY0057
MY0126	bf264-15D *MAT***a** *cln1*Δ *cln2*Δ *leu2::GAL1-10::CLN1::LEU2 pho85*Δ*::TRP1*	EY0233
MY0147	K699 *MAT*α *pho85*Δ*::TRP1*	EY099
MY0158	K699 *MAT*α *pho85-9*	EY0141
MY0165	bf264-15D *MAT*α *pho85*Δ*::LEU2*	EY0230
MY0173	K699 *MAT***a** *pho85-9*	MY0158 × EY0150
MY0176	K699 *MAT*α *efr3*Δ*::LEU2 pho85-9*	MY0114 × MY0158
MY0186	K699 *MAT***a**/α *cir^0*	EY0099
MY0187	K699 *MAT***a** *cir^0*	MY0186
MY0188	K699 *MAT*α *cir^0*	MY0186
MY0190	K699 *MAT***a** *cir^0 TRP1*	MY0187
MY0192	K699 *MAT*α *cir^0 HIS3*	MY0188
MY0195	bf264-15D *MAT*α *pho85-9*	MY0165
MY0205	bf264-15D *MAT***a** *pho85-9 cln1*Δ *cln2*Δ	MY0195 × EY0173
MY0245	K699 *MAT*α *pho85-9 HIS3*	MY0173 × MY0192
MY0246	K699 *MAT*α *cir^0 HIS3 GAL1-10::PHO85*	MY0192
MY0248	K699 *MAT***a** *cir^0 TRP1 GAL1-10::PHO85*	MY0246 × MY0190
MY0251	K699 *MAT*α *cir^0 TRP1*	MY0190 × MY0246
MY0252	K699 *MAT***a** *pho85-9 TRP1*	MY0245 × MY0190
MY0260	bf264-15D *HIS2 his3 pcl1*Δ*::HIS3 pcl2*Δ*::LYS2 cln1* Δ *cln2*Δ *cln3*Δ *leu2::GAL1-10::CLN1::LEU2*	F. Cross
MY0261	bf264-15D *cln1*Δ *cln2*Δ *cln3*Δ *leu2::GAL1-10::CLN1::LEU2*	F. Cross
MY0263	W303 *MAT***a** *mpk1*Δ*::TRP1*	A. Straight
MY0265	K699 *MAT*α *mpk1*Δ*::TRP1 pho85-9*	MY0263 × MY0245
MY0276	K699 *MAT***a** *efr1::LEU2 pho85-9 HIS3*	Screen isolate × MY0252
MY0277	K699 *MAT***a** *efr1::LEU2*	MY0276 × MY0251
MY0278	K699 *MAT*α *efr6::pho85-9*	Screen isolate × MY0245
MY0282	K699 *MAT***a** *efr2::LEU2 pho85-9 TRP1*	Screen isolate × MY0252
MY0284	K699 *MAT*α *efr2::LEU2*	MY0282 × MY0192
MY0286	K699 *MAT*α *efr7::LEU2 pho85-9*	Screen isolate × MY0252
MY0290	K699 *MAT***a** *efr4::LEU2 pho85-9 TRP1*	Screen isolate × MY0245
MY0292	K699 *MAT***a** *efr5::LEU2 pho85-9*	Screen isolate × MY0245
MY0293	K699 *MAT***a** *efr5::LEU2*	MY0292 × MY0192
MY0296	K699 *MAT***a** *efr8::LEU2*	Screen isolate × MY0190
MY0321	K699 *MAT*α *efr8::LEU2 pho85-9*	Screen isolate × MY0252
MY0361	W303 *MAT***a** *cdc42-1*	S. O'Rourke
MY0362	W303 *MAT***a** *cla4*Δ*::LEU2*	S. O'Rourke
MY0363	W303 *MAT***a** *ste20*Δ*::LEU2*	S. O'Rourke

TABLE 2

Plasmids used in this study

Plasmid	Contents	Source
EB0009	pRS316, *URA3 ARS/CEN*	Sikorski and Hieter (1989)
EB0049	*GPD1::PHO80 URA3* 2μ	Lab strain collection
EB0053	pAC800, pUC18 *PHO80*	Toh-e and Shimauchi (1986)
EB0098	pJJ215, pUC18-*HIS3*	Jones and Prakash (1990)
EB0103	pJJ248, pUC18-*TRP1*	Jones and Prakash (1990)
EB0128	*GPD1::PCL1-HA3 URA3* 2μ	This study
EB0149	*pcl1*Δ*::HIS3*	Espinoza *et al.* (1994)
EB0226	pBA634, *pcl2*Δ*::URA3*	Measday *et al.* (1994)
EB0327	pRS316 *PHO85.Py*^{2} *URA3 ARS/CEN*	This study
EB0373	pFHE27, pKS *PCL1*	Hernan Espinoza
EB0459	pJO21, YEp24 *CLN2 URA3* 2μ	Ogas *et al.* (1991)
EB0727	pKS *pcl9*Δ*::TRP1*	This study
MP0030	pUC18 *efr3*Δ*::LEU2*	This study
MP0054	pKS *pho85*Δ*::TRP1*	This study
MP0054	pKS *pho85*Δ*::TRP1*	This study
MP0056	pRS313 *PHO85*Δ*.Py*^{2} *HIS3 ARS/CEN*	This study (from EB0327)
MP0073	pKS *pho85-6.Py*^{2} *URA3*	This study (from EB0327)
MP0075	pKS *pho85-9.Py*^{2} *URA3*	This study (from EB0327)
MP0076	pKS *pho85-10.Py*^{2} *URA3*	This study (from EB0327)
MP0107	pBA945a, pRSETB-*PCL10*	Measday *et al.* (1997)
MP0108	pBA946a, pRSETC-*PCL8*	Measday *et al.* (1997)
MP0111	pBA950, pRSETB-*PCL9*	Measday *et al.* (1997)
MP0112	pBA619, *PCL2 LEU2* 2μ	Brenda Andrews
MP0113	PBA904, pRS426 *CLG1 URA3* 2μ	Brenda Andrews
MP0114	pBA906, *PCL5 TRP1* 2μ	Brenda Andrews
MP0115	pRS426 *PHO80 URA3* 2μ	This study (from EB0053)
MP0117	pRS425 *PCL6 LEU2* 2μ	Anita Sil
MP0120	pRS426 *PCL1 URA3* 2μ	This study (from EB0373)
MP0121	pRS426 *PCL2 URA3* 2μ	This study (from MP0112)
MP0126	pRS426 *PCL5 URA3* 2μ	This study (from MP0114)
MP0127	pRS426 *PCL6 URA3* 2μ	This study (from MP0117)
MP0133	YEp13 *PCL7 LEU2* 2μ	Nasmyth and Tatchell (1980)
MP0134	YEp13 *PCL9 LEU2* 2μ	Nasmyth and Tatchell (1980)
MP0136	pKS$^+$ *PHO85-5′::GAL1-10::PHO85 URA3*	This study
MP0143	CY676, YCp50 *PKC1 R398P URA3 ARS/CEN*	Nonaka *et al.* (1995)
MP0145	pRS426 *PCL7 URA3* 2μ	This study (from MP0133)
MP0146	pRS426 *PCL9 URA3* 2μ	This study (from MP0134)
MP0154	YEp13 *PCL8 LEU2* 2μ	Nasmyth and Tatchell (1980)
MP0155	YEp13 *PCL10 LEU2* 2μ	Nasmyth and Tatchell (1980)
MP0158	pRS426 *PCL8 URA3* 2μ	This study (from MP0154)
MP0159	pRS426 *PCL10 URA3* 2μ	This study (from MP0155)
pFV-17	*GAL1-10::FLP1*	Volkert and Broach (1986)

strain with transposon-containing fragments from a yeast genomic DNA library (Burns *et al.* 1994) on galactose-containing plates. These transformants were then replica plated to SD plates. Those colonies that could not grow on dextrose plates were retained for further characterization. Among 25,000 transposon transformants, 26 displayed a galactose-dependent phenotype.

In addition to identifying mutants requiring *PHO85*, our primary screen would also identify mutants unable to grow on dextrose even in the presence of *PHO85* (which we name *D*ead *O*n *D*extrose, or Dod mutants). To differentiate between these two classes of mutants, we crossed candidate Efr mutants to strains with a temperature-sensitive allele of *PHO85*. Eleven of the 26 mutants had a transposon-linked Efr phenotype: spores containing the transposon were dead or very sick in the presence of the *GAL1-10::PHO85* allele on dextrose but grew in the presence of the *pho85-9* allele at room temperature. These 11 mutants define seven complementation groups.

A temperature-sensitive *PHO85* mutation is suppressed by overexpression of Pho85-associated cyclins: To investigate which cyclins are required to perform the various functions of *PHO85*, we tested whether high-copy plasmids containing Pho85-associated cyclins

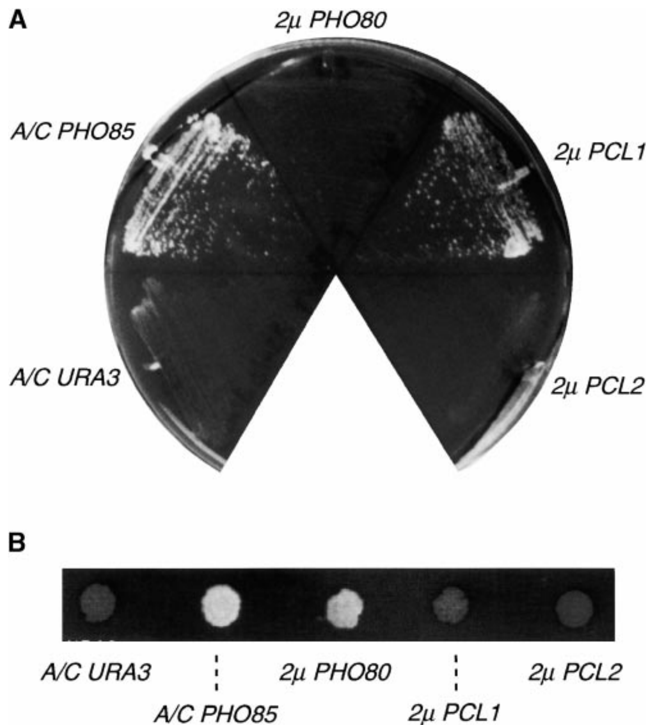

FIGURE 1.—Suppression of *pho85-9* temperature-sensitive phenotypes by high-copy cyclin plasmids. (A) Suppression of the synthetic temperature-sensitive lethality of the *pho85-9 cln1*Δ *cln2*Δ mutant. Strain MY0205 (*bf264-15D pho85-9 cln1*Δ *cln2*Δ) transformed with *ARS/CEN URA3* (EB0009), *ARS/CEN PHO85* (EB0327), 2μ *PHO80* (MP0115), 2μ *PCL1* (MP0120), or 2μ *PCL2* (MP0121) was streaked on SD-Ura plates and placed at 30° for 3 days. (B) Suppression of the temperature-sensitive constitutive acid-phosphatase expression phenotype of the *pho85-9* mutant. Strain MY0158 (*K699 pho85-9*; transformed with the same plasmids) was placed on SD-Ura and placed at 37° overnight and then stained for acid-phosphatase activity.

could suppress phenotypes of a *PHO85* ts allele. This idea is based on the identification of *CLN1* and *CLN2* as high-copy suppressors of the *cdc28-4* temperature-sensitive G1 arrest (Reed *et al.* 1989). We first determined whether high-copy plasmids containing *PHO80*, *PCL1*, or *PCL2* suppress the ts growth phenotype of a *cln1*Δ *cln2*Δ *pho85-9* strain (Figure 1A) or the ts-Pho^c phenotype of a *pho85-9* strain (Figure 1B). High-copy plasmids containing *PHO80* but not plasmids containing *PCL1* or *PCL2* suppress the ts-Pho^c phenotype of a *pho85-9* strain. Since both *pho85*Δ *cln1*Δ *cln2*Δ and *pcl1*Δ *pcl2*Δ *cln1*Δ *cln2*Δ mutants are inviable, the function of *PHO85* that allows *cln1*Δ *cln2*Δ strains to grow requires Pcl1- and Pcl2-containing Pho85 kinases (Espinoza *et al.* 1994; Measday *et al.* 1994). High-copy plasmids containing *PCL1*, but not *PCL2* or *PHO80* suppress the ts growth phenotype of a *cln1*Δ *cln2*Δ *pho85-9* strain. Given the ability of plasmids containing *PCL1* to suppress the ts growth phenotype of a *cln1*Δ *cln2*Δ *pho85-9* strain, the inability of high-copy plasmids containing *PCL2* to suppress the same phenotype is surprising. This *PCL2* plasmid is functional as it suppresses the ts phenotype of an *mpk1*Δ strain (Madden *et al.* 1997, data not shown). High-copy plasmids containing each of the other seven Pho85-associated cyclins do not suppress either of these temperature-sensitive *pho85-9* phenotypes (data not shown).

Characterization of *efr pho85-9* strains: We next tested whether high-copy plasmids containing the Pho85-associated cyclins suppress the temperature-sensitive growth phenotypes of different *efr pho85-9* strains (Figure 2). High-copy plasmids containing *PCL1* suppress the ts growth phenotype of *efr1 pho85-9* and *efr8 pho85-9* strains, whereas plasmids containing *PHO80* suppress the *efr3 pho85-9* strain. None of the Pho85-cyclin-containing plasmids suppress the ts phenotype of *efr2 pho85-9*, *efr6 pho85-9*, or *efr7 pho85-9* strains. The ts phenotype of these strains is, however, *pho85-9* dependent as it is complemented by *PHO85* (Figure 2 and data not shown). We could not perform this analysis on the *efr5 pho85-9* strain as it is very slow growing.

The positions of the mutations responsible for the Efr phenotype are indicated in Table 3. The class of mutants that is not suppressed by cyclin overexpression contains mutations in a variety of genes involved in transcriptional regulation: *SPT7*, *HFI1* (*ADA1*), *SRB5*, and *GCR1*. With the exception of *GCR1*, which plays a specific role in regulating carbohydrate metabolism gene expression, the other genes encode general regulators of RNA polymerase II transcription. The *PCL1*-suppressible class of Efr mutants is due to mutations in *BEM2* and *BCK1*—genes involved in morphogenesis and cell-wall biosynthesis. Mutations in a previously uncharacterized open reading frame, *YMR212C*, that we have named *EFR3* accounts for the *PHO80*-suppressible mutant. Plasmids containing *EFR3* complement the *efr3* mutation. *efr3*Δ mutants also grow poorly on media containing acetate as a carbon source and *efr3*Δ diploids fail to sporulate (data not shown). High-copy plasmids containing *PHO80* suppress the *efr3*Δ *pho85-9* strain, but an *efr3*Δ *pho80*Δ strain exhibits no growth defects (data not shown). *efr3*Δ *pcl1*Δ *pcl2*Δ *pcl9*Δ strains also grow at a rate similar to that of wild type (data not shown).

Morphogenesis-related Efr mutants: Since Bck1 is the mitogen-activated protein kinase kinase kinase (MAPKKK) responsible for activation of Mpk1, and *bck1* mutants have an Efr phenotype, we examined synthetic interactions between *mpk1* and *pho85*. *mpk1*Δ *pho85*Δ strains are inviable (this result was subsequently reported in Huang *et al.* 1999) and *mpk1*Δ *pho85-9* strains exhibit a synthetic temperature-sensitive growth phenotype (data not shown).

Since high-copy *PCL1* plasmids suppress the ts lethality of *bem2 pho85-9* and *bck1 pho85-9* strains, we wondered if Pcl1-Pho85 complexes provide the *PHO85* function required for viability in these mutant backgrounds. *bem2 pcl1*Δ strains are inviable (data not shown). *mpk1*Δ *pcl1*Δ *pcl2*Δ and *bck1 pcl1*Δ *pcl2*Δ strains are inviable when

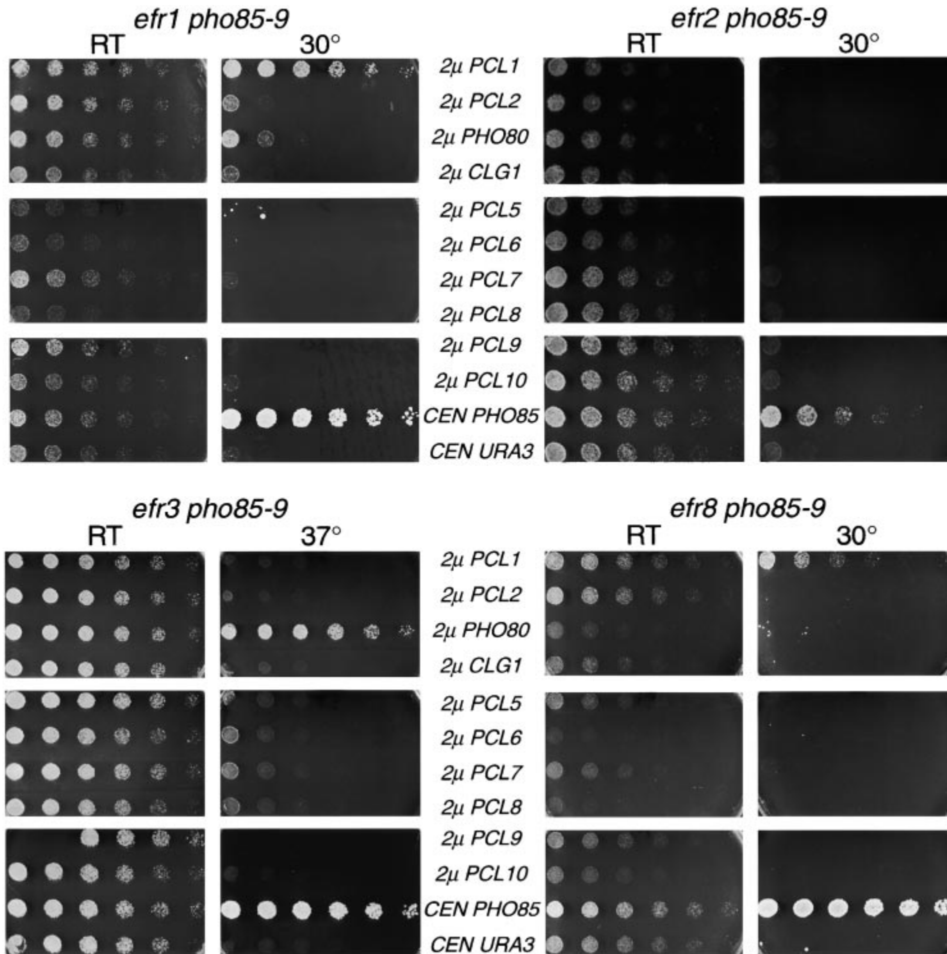

Figure 2.—Suppression of the synthetic temperature-sensitive lethality of various *efr pho85-9* double mutants by high-copy cyclin plasmids. Strains MY0276 (*efr1 pho85-9*), MY0282 (*efr2 pho85-9*), MY-0176 (*efr3*Δ *pho85-9*), and MY0321 (*efr8 pho85-9*) transformed with high-copy plasmids containing each of the Pho85-associated cyclins or low-copy plasmids containing *PHO85* (EB0327) or *URA3* (EB0-009) were diluted from an OD_{600} = 0.3 by threefold serial dilutions and plated on SD-Ura plates. Plates were placed at the indicated temperature for 3 days.

TABLE 3

Cloning of Efr mutants

Complementation group	No. of alleles	Gene disrupted	Position of transposon relative to start codon[a]
EFR1	4	*BEM2*	3033/6503
EFR2	2	*SPT7*	1750/3998
EFR3	1	*YMR212c*	See note[b]
EFR5	1	*GCR1*	3013/3108
EFR6	1	*SRB5*	−15/923
EFR7	1	*HFI1*	358/1466
EFR8	1	*BCK1*	1789/4436

[a] When more than one allele was identified, the transposon position is reported for the allele used in subsequent experiments.

[b] We had identified UV-induced alleles of *EFR3* in an earlier screen and generated an *efr3*Δ strain during the course of those experiments. The transposon-insertion allele of *EFR3* was identified by noncomplementation of this strain and was not characterized further. The deletion allele is used in subsequent experiments.

dissected onto standard medium, but are viable on medium containing 1 m sorbitol (Figure 3). This phenotype is different from the *bem2 pcl1*Δ phenotype because *mpk1*Δ *pcl1*Δ or *bck1 pcl1*Δ strains have growth rates similar to those of *mpk1*Δ or *bck1* strains. Furthermore, sorbitol does not rescue the inviability of *bem2 pcl1*Δ strains

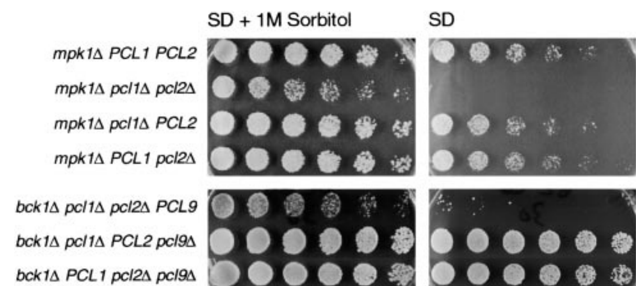

Figure 3.—Sorbitol-suppressible synthetic-lethal phenotype of the *pcl1*Δ *pcl2*Δ *mpk1*Δ mutant. Progeny of a cross between MY0263 (*mpk1*Δ) and EY0535 (*pcl1*Δ *pcl2*Δ) or of a cross between MY0296 (*efr8*) and EY0552 (*pcl1*Δ *pcl2*Δ *pcl9*Δ) were diluted from an OD_{600} = 0.3 by threefold serial dilutions and plated on SD-complete plates or SD-complete plates that had been supplemented with 1 m sorbitol. Plates were placed at 30° for 2 days.

(data not shown). Removing *PCL9*, a cyclin highly homologous to *PCL2*, does not exacerbate the sorbitol-requiring phenotype of *mpk1Δ pcl1Δ pcl2Δ* or *bck1 pcl1Δ pcl2Δ* strains nor do *bck1 pcl1Δ pcl9Δ* or *bck1 pcl2Δ pcl9Δ* strains exhibit any growth defects (data not shown). We also tested for genetic interactions between *spt7*, *gcr1*, *srb5*, or *hfi1* and *pcl1Δ pcl2Δ pcl9Δ*. All of these *efr pcl1Δ pcl2Δ pcl9Δ* strains grow similarly to the corresponding *efr* strain (data not shown), suggesting that the cause of the synthetic lethality in these Efr mutants is distinct from that of the *PCL1*-suppressible Efr mutants.

Suppression of *cln1Δ cln2Δ pcl1Δ pcl2Δ* lethality by sorbitol-containing medium: Our screen identified two types of *efr pho85-9* mutants that are suppressed by high-copy *PCL1* plasmids. *bem2 pcl1Δ* mutants are inviable while *bck1 pcl1Δ pcl2Δ* mutants are sorbitol requiring. Since high-copy *PCL1* also suppresses the *cln1Δ cln2Δ pho85-9* strain (Figure 1), we wondered if the lethality of a *cln1Δ cln2Δ pcl1Δ pcl2Δ* strain (Espinoza *et al.* 1994; Measday *et al.* 1994) would also be suppressed by sorbitol. We compared growth of a *GAL1-10::CLN1 cln2Δ* strain with *GAL1-10::CLN1 cln2Δ cln3Δ*, *GAL1-10::CLN1 cln2Δ pcl1Δ pcl2Δ*, and *GAL1-10::CLN1 cln2Δ cln3Δ pcl1Δ pcl2Δ* strains on YEPG, YEPD, and YEPD + 1 M sorbitol. While *GAL1-10::CLN1 cln2Δ pcl1Δ pcl2Δ* strains are unable to grow on YEPD plates, they grow on YEPD plates containing sorbitol (Figure 4A). In contrast, the *GAL1-10::CLN1 cln2Δ cln3Δ* strain is unable to grow on YEPD with or without sorbitol. Like the *GAL1-10::CLN1 cln2Δ* strain, our *WT* strain grows more slowly on YEPD + 1 M sorbitol than on YEPD (data not shown). We had found that the *PKC1 R398P* gain-of-function allele suppresses the ts phenotypes of *mpk1Δ* and *swi4Δ* strains (data not shown). This plasmid also suppresses the ts phenotype of a *cln1Δ cln2Δ pho85-9* strain (Figure 4B). These data indicate that the synthetic lethality of the *cln1Δ cln2Δ pcl1Δ pcl2Δ* strain has many similarities with the synthetic lethality of *mpk1Δ pcl1Δ pcl2Δ* and *bck1 pcl1Δ pcl2Δ* strains.

Other morphogenesis-related phenotypes of *pho85* mutants: Because *pcl1Δ* and *pcl1Δ pcl2Δ* are synthetically lethal with mutations in genes involved in morphogenesis, and some of these phenotypes can be reversed by osmotic stabilization, we wondered if mutations in *PCL1* and *PCL2* would show interactions with mutations in other morphogenesis genes. We were particularly interested in looking for interactions between *PHO85* and the p21-activated (PAK) kinases Ste20, Cla4, or Skm1 because mammalian CDK5, which is involved in regulat-

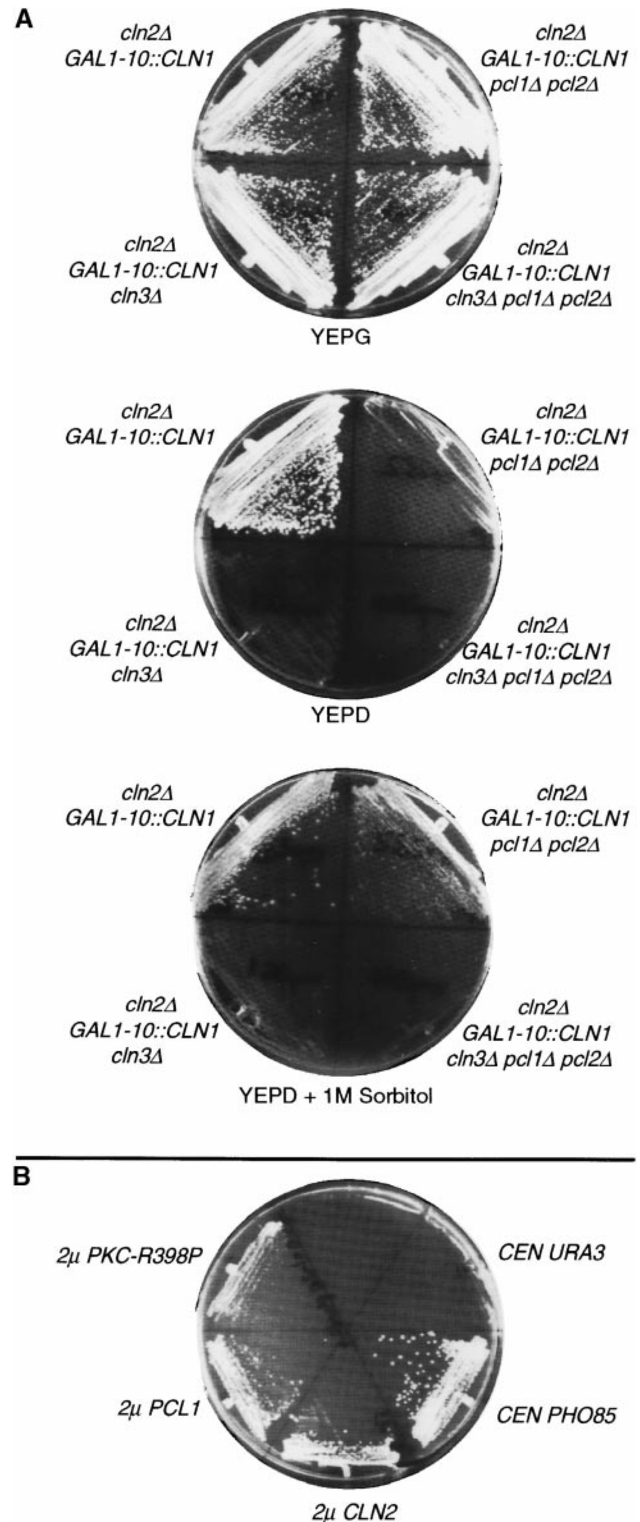

Figure 4.—Comparison of the phenotypes of the *pcl1Δ pcl2Δ cln1Δ cln2Δ* and the *pcl1Δ pcl2Δ mpk1Δ* mutants. (A) The lethality of *pcl1Δ pcl2Δ cln1Δ cln2Δ* is suppressed by 1 M sorbitol. The following strains were streaked on the indicated plates and placed at 37° for 2 days: *cln2Δ GAL1-10::CLN1* (EY0233); *cln2Δ GAL1-10::CLN1 pcl1Δ pcl2Δ* (EY0409); *cln2Δ GAL1-10::CLN1 cln3Δ* (MY0261); *cln2Δ GAL1-10::CLN1 cln3Δ pcl1Δ pcl2Δ* (MY0260). (B) The temperature-sensitive lethality of the *pho85-9 cln1Δ cln2Δ* mutant is suppressed by a PKC1 gain-of-function allele. Strain MY0205 (*cln1Δ cln2Δ pho85-9*) transformed with plasmids containing *PKC-R398P* (MP0143), 2μ *PCL1* (MP0120), 2μ *CLN2* (EB0459), *ARS/CEN PHO85* (EB0327), or *ARS/CEN URA3* (EB0009) was streaked on SD-Ura plates and placed at 30° for 3 days.

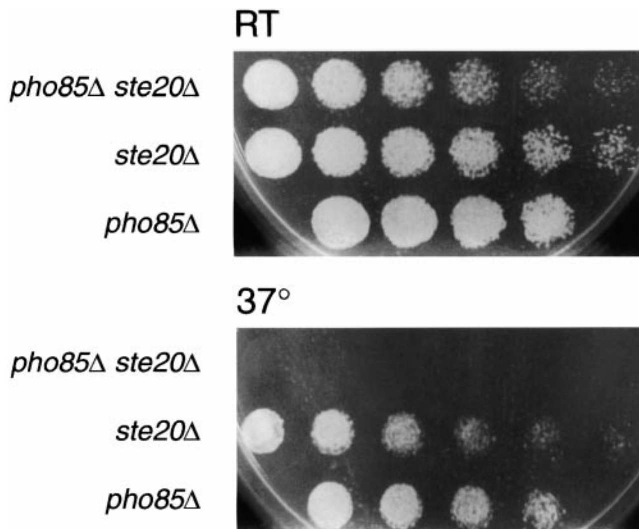

FIGURE 5.—The *ste20Δ pho85Δ* mutant is synthetically temperature sensitive. Progeny of a cross between MY0363 (*ste20Δ*) and MY0147 (*pho85Δ*) were diluted from an OD_{600} = 0.3 by threefold serial dilutions and plated on YEPD plates that were then placed at the indicated temperature for 2 days.

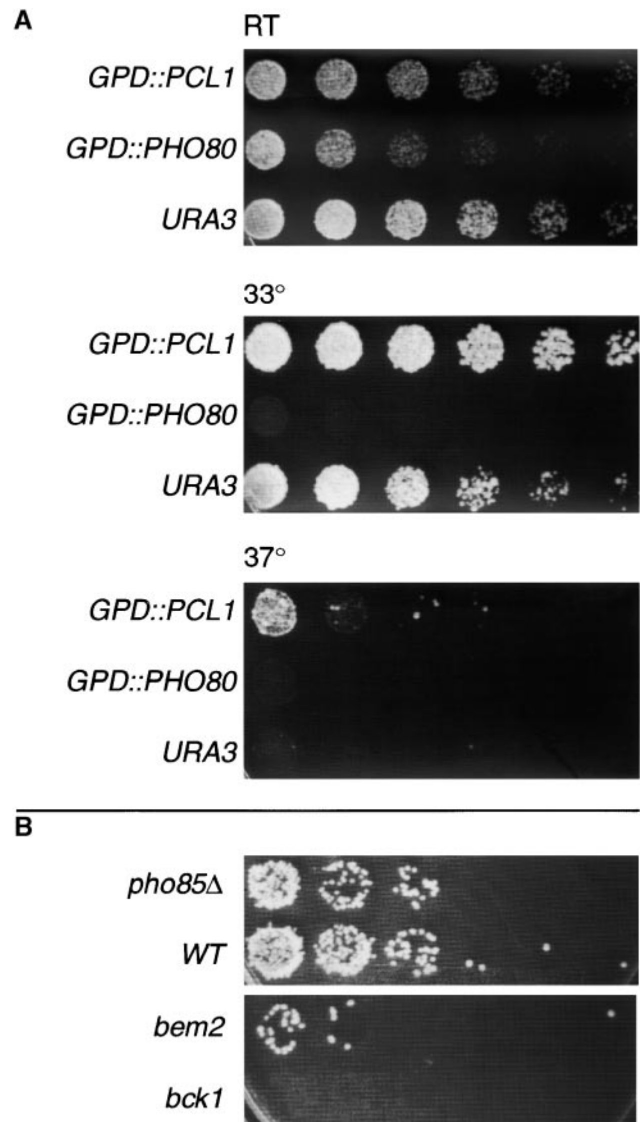

FIGURE 6.—Other phenotypic consequences of altering Pho85 activity. (A) The effect of Pho85-associated cyclin overexpression of the permissive temperature of *cdc42-1* strains. Strain MY361 (*MAT***a** *cdc42-1*) transformed with plasmids containing *GPD1::PCL1* (EB0128), *GPD1::PHO80* (EB0049), or *URA3* (EB0009) were diluted from an OD_{600} = 0.3 by threefold serial dilutions and plated on YEPD plates and placed at the indicated temperature for 3 days. (B) *pho85Δ* strains mate as efficiently as *WT* strains. Strains EY0140 (*MAT***a** *pho85Δ*), EY057 (*MAT***a** *WT*), MY0277 (*MAT***a** *bem2*), and MY0296 (*MAT***a** *bck1*) were diluted from an OD_{600} = 0.3 by threefold serial dilutions and plated on a lawn of 2.0 OD_{600} of EY0478 (*MATα far1-c* Lys^-) that had been spread on YEPD plates and placed at 30° for 6 hr. These plates were then replica plated to SD-Min plates (lacking amino acid supplements) and placed at 30° for 2 days.

ing PAK kinase activity in neurons (NIKOLIC *et al.* 1998), can complement a *pho85Δ* strain (HUANG *et al.* 1999; NISHIZAWA *et al.* 1999). *pho85Δ ste20Δ* strains are temperature sensitive (Figure 5). *cla4Δ pcl1Δ pcl2Δ* strains are inviable and are not cured by osmotic stabilization with sorbitol (data not shown). The *cla4Δ pcl1Δ pcl2Δ* inviability is therefore dissimilar from the sorbitol-requiring phenotype of strains carrying the *mpk1Δ*, *bck1*, or *cln1Δ cln2Δ* mutations in combination with *pcl1Δ pcl2Δ*.

As sorbitol suppresses the inviability of *cln1Δ cln2Δ pcl1Δ pcl2Δ*, *mpk1Δ pcl1Δ pcl2Δ*, and *bck1 pcl1Δ pcl2Δ* strains, *cln1Δ cln2Δ*, *mpk1Δ*, and *bck1* might have similar defects. Since *CLA4* is required for viability in the absence of *CLN1* and *CLN2* (CVRCKOVA *et al.* 1995), we wondered if *mpk1Δ* strains also require *CLA4*. Both *cla4Δ mpk1Δ* and *cla4Δ bem2* strains are inviable (data not shown). These data demonstrate another similarity between the phenotypes of *mpk1Δ* and *cln1Δ cln2Δ* mutants and suggest that despite the differences in synthetic lethality with *pcl1Δ pcl2Δ*, the defects of a *bem2* mutant are related to those of *mpk1Δ* and *cln1Δ cln2Δ* mutants.

Since *CDC42* is a GTPase that performs an essential function involved in actin polarization and is also required for the activation of Ste20 and Cla4 (ADAMS *et al.* 1990; PETER *et al.* 1996; BENTON *et al.* 1997), we tested whether mutations in *PCL1* or *PCL2* would alter the permissive temperature of strains carrying a ts allele of *CDC42*. *cdc42-1 pcl1Δ pcl2Δ* strains are inviable at all temperatures and are not rescued by sorbitol (data not shown).

The synthetic lethality between *cdc42-1* and *pcl1Δ pcl2Δ* could have many causes. Expression of *PCL1* from the *GPD1* promoter raises the permissive temperature of the *cdc42-1* strain (Figure 6A), suggesting that overexpression of *PCL1* either promotes a process that substitutes for *CDC42* function or promotes Cdc42 activity. In contrast, overexpression of *PHO80* lowers the permissive temperature of a *cdc42-1* strain, suggesting that *PHO80* is not able to perform the function *PCL1* pro-

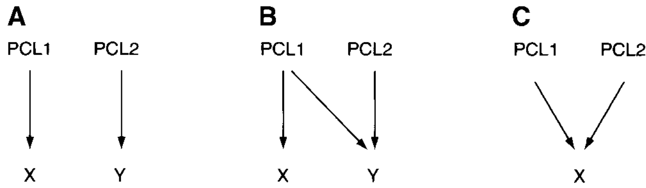

FIGURE 7.—Models explaining the functional relationships between Pcl1- and Pcl2-associated Pho85 kinase activities. (A) *PCL1* and *PCL2* perform nonoverlapping functions. (B) *PCL1* participates in multiple processes. Some of these are independent of *PCL2* (labeled X) and some are shared with *PCL2* (labeled Y). (C) The functions of *PCL1* and *PCL2* overlap.

vides. It also suggests that increasing Pho80 activity interferes with the Pcl1-mediated functions of Pho85, perhaps by decreasing the amount of free Pho85 available to associate with Pcl1.

Since many of the processes required for morphogenesis during the cell cycle are also required for the specialized morphogenetic processes that occur during mating, many mutants with defects in morphogenesis and cell-wall biosynthesis also have mating defects. To look for subtle mating defects, we compared the ability of *WT*, *pho85*Δ, *bem2*, and *bck1* mutants to mate to a *far1-c* strain (Valtz *et al.* 1995). *WT* and *pho85*Δ strains mate with similar efficiency whereas *bem2* and *bck1* mutants show a reduced mating ability (Figure 6B). This suggests that *pho85*Δ mutants are unlikely to have a defect in the physical processes of morphogenesis or cell-wall biosynthesis.

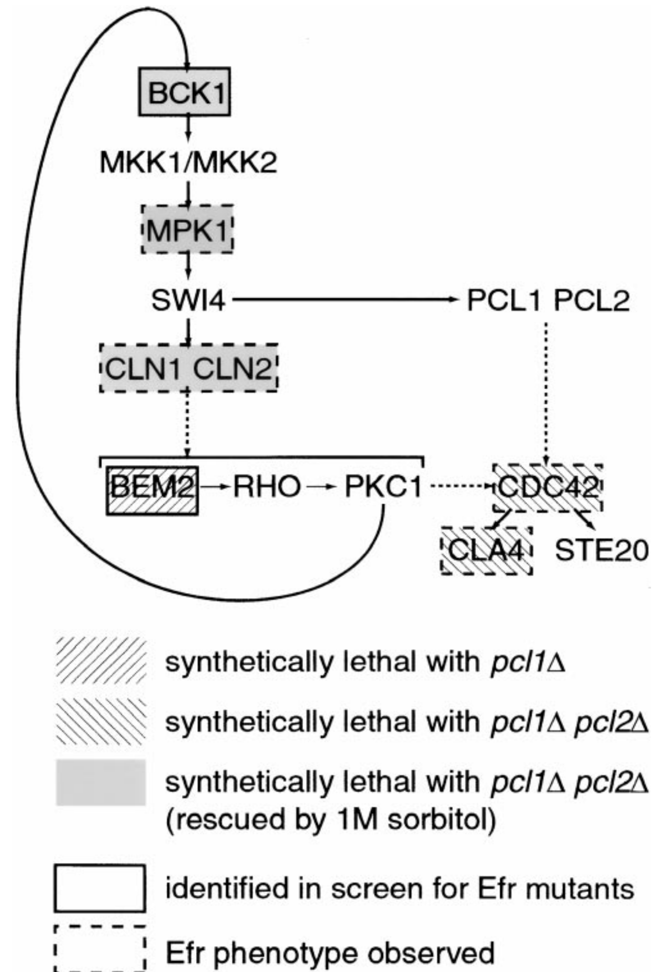

FIGURE 8.—A model explaining the functional relationships between Pcl1- and Pcl2-associated Pho85 kinase activities and some of the Efr mutants identified in our screen. This model hypothesizes that the underlying cause of many of the synthetic-lethal interactions is a defect in *CDC42* activity.

DISCUSSION

Our screen for mutants that require *PHO85* for viability was designed to survey the functions Pho85 performs in association with its 10 cyclin subunits. We identified mutations in seven genes. Because we found only one allele of many of these genes, and found additional synthetic-lethal interactions involving *PHO85* by directed tests, it is apparent that our screen was not saturating. A similar screen for Efr mutants using ultraviolet light as a mutagen uncovered 18 recessive mutations in 17 complementation groups, suggesting that the actual number of loci that can be mutated to give rise to an Efr phenotype is quite large. Two of these UV-induced mutants contained mutations in *EFR3*.

Our strategy for identifying the cyclins responsible for different functions of Pho85 involved determining if high-copy plasmids carrying the genes for the Pho85-associated cyclins could suppress various phenotypes of a temperature-sensitive allele of *PHO85*. This strategy was only partially successful as only *PHO80*- and *PCL1*-containing plasmids had activity in this assay, and some *efr pho85-9* mutants were not suppressed by either plasmid. Mutations in activators of transcription account for the largest class of non-cyclin-suppressible mutants. It is striking that our screen identified mutations in two different components of the SAGA histone acetylase complex (*SPT7* and *HFI1/ADA1*; Eberharter *et al.* 1998). We do not know if these synthetic-lethal interactions indicate a role for *PHO85* as a general regulator of transcription—like the yeast CDKs Srb10, Kin28, and Ctk1 (Liao *et al.* 1995; Sterner *et al.* 1995; Valay *et al.* 1995)—or if they cause a defect in expression of a particular gene with which *PHO85* is also synthetically lethal.

Several different classes of Efr mutants require *PCL1* and/or *PCL2*. One is *bem2*, which is synthetically lethal with *pcl1*Δ. *cla4* and *cdc42* represent a type of Efr mutant that is synthetically lethal with *pcl1*Δ *pcl2*Δ under all conditions tested. *bck1*, *mpk1*Δ, and *cln1*Δ *cln2*Δ form a third class of mutants that are sorbitol requiring when combined with *pcl1*Δ *pcl2*Δ. The inviability of *bem2 pcl1*Δ strains is the first example of a *pcl1*Δ phenotype that does not depend on also removing *PCL2*. Several models can account for the requirement for *PCL1 vs. PCL1* or

TABLE 4

Summary of synthetic-lethal interactions

Synthetic-lethal with *pho85Δ*	Recovered in Efr screen	Synthetic ts phenotype with *pho85-9*	ts phenotype suppressed by 2μ *PCL1*	ts phenotype suppressed by 2μ *PHO80*	ts phenotype suppressed by other 2μ *PCL* plasmids	Synthetic-lethal phenotype with *pcl1Δ pcl2Δ pcl9Δ*	Synthetic-lethal phenotype with *pcl1Δ pcl2Δ*	Synthetic-lethal phenotype with *pcl1Δ*	Synthetic-lethal phenotype with *pcl2Δ pcl9Δ*	Synthetic-lethal with *cla4Δ*	Other results
cln1Δ cln2Δ	No	Yes	Yes	No	No		Yes[a]	No		Yes[b]	*cln1Δ cln2Δ pho85-9* ts phenotype suppressed by *PKC-R398P*
bck1	Yes	Yes	Yes	No	No	Yes[a]	Yes[a]	No	No		Reduced mating to *far1-c*
mpk1Δ	No	Yes	No	No	No	Yes[a]	Yes[a]	No	No	Yes	ts phenotype of *mpk1Δ* mutant suppressed by *PKC-R398P*
bem2	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	Reduced mating to *far1-c*
cdc42-1	No						Yes	No			Mutant phenotype partially suppressed by *GPD1::PCL1* and exacerbated by *GPD1::PHO80*
cla4Δ	No	Yes					Yes	No			
efr3Δ	Yes	Yes	No	Yes	No	No	No	No	No		No growth defect of *efr3Δ pho80Δ* strain
spt7	Yes	Yes	No	No	No	No	No	No	No		
gcr1	Yes	Yes	No	No	No	No	No	No	No		
srb5	Yes	Yes	No	No	No	No	No	No	No		
hfi1	Yes	Yes	No	No	No	No	No	No	No		

[a] Synthetic-lethal phenotype with pcl1D pcl2D is rescued by growth in 1 M sorbitol.
[b] Cvrckova *et al.* (1995).

PCL2 (Figure 7). *PCL1* and *PCL2* might perform distinct functions: mutants such as *bem2* are sensitive to the loss of *PCL1*-mediated functions while mutants such as *mpk1*Δ are inviable only when both *PCL1*- and *PCL2*-mediated processes are perturbed. A similar model is that a subset of *PCL1* functions are distinct from those performed by *PCL1* and *PCL2*. Both models explain the inviability of *bem2 pcl1*Δ and mutants like *mpk1*Δ *pcl1*Δ *pcl2*Δ as being caused by different defects in the *pcl1*Δ as compared to the *pcl1*Δ *pcl2*Δ mutants. A third possibility is that *PCL1* and *PCL2* perform identical functions and that genes like *BEM2* and *MPK1* require different thresholds of this function for viability. We cautiously favor the possibility that *PCL1* and *PCL2* perform identical functions because it allows us to arrange the various Efr mutants along a phenotypic spectrum from more to less dependence on *PCL1*/*PCL2* and from this hypothesis make a prediction about the site of *PCL1*/*PCL2* action.

If *PCL1* and *PCL2* perform the same function, the requirement of *BEM2* mutants for *PHO85* is most severe because *bem2* mutants are inviable when just the *PCL1* fraction of Pho85 function is removed and the other mutants are inviable only when the *PCL1*- and *PCL2*-mediated functions are both removed. Similarly, the inviability of *mpk1*Δ *pcl1*Δ *pcl2*Δ strains can be rescued by sorbitol, while the inviability of the *cla4*Δ *pcl1*Δ *pcl2*Δ strain cannot. This could indicate that *cla4*Δ strains are more dependent on Pho85 function than are *mpk1*Δ strains. If these suppositions are correct we can arrange these Efr mutants along a phenotypic spectrum: *bem2* mutants have the strongest Efr phenotype, followed by *cla4*Δ and *cdc42-1*, and then followed by *mpk1*Δ, *bck1*, and *cln1*Δ *cln2*Δ.

A model to explain the defect that accounts for this spectrum is that *PCL1* and *PCL2* function together with the *PCL1*- and *PCL2*-requiring *EFR* genes to positively regulate Cdc42 activity (Figure 8). As the novel elements of this model are based on circumstantial evidence presented in this article, we have summarized our principal findings in Table 4. One aspect of our model proposes that *PKC1* positively regulates *CDC42* activity. A functional connection between *BEM2* and *CDC42* is supported by the observation that *GIC1* and *GIC2* are *CDC42* effectors and are also high-copy suppressors of the *bem2*Δ mutant (Chen *et al.* 1997). *PKC1* may function in between *BEM2* and *CDC42* as *in vitro* studies show Bem2 displays strong GAP activity toward Rho1 (Kim *et al.* 1994; Peterson *et al.* 1994), a GTPase that activates *PKC1* (Nonaka *et al.* 1995; Drgonova *et al.* 1996; Kamada *et al.* 1996). We have drawn a distinction between the functions of the Swi4-dependent genes *CLN1*, *CLN2*, *PCL1*, and *PCL2* because, of these, only *PCL1* and *PCL2* can high-copy suppress the temperature-sensitive phenotype of *mpk1*Δ strains (Madden *et al.* 1997). We propose that *PCL1* and *PCL2* high-copy suppress the *mpk1*Δ mutant as a result of increasing Cdc42 activity. We also hypothesize that *CLN1* and *CLN2* are positive regulators of *PKC1* function because gain-of-function alleles in *PKC1* suppress the temperature-sensitive phenotypes of *swi4*Δ and *mpk1*Δ strains (foreshadowed by Gray *et al.* 1997) and also suppress the synthetic temperature sensitivity of a *cln1*Δ *cln2*Δ *pho85-9* strain, suggesting that *PKC1* functions downstream of each of these genes. As *PKC1* is an activator of the *MPK1* MAP kinase pathway at the level of either *BCK1* or *MKK1 MKK2* (Huang and Symington 1995), overexpression of *CLN1* and *CLN2* fails to suppress the *mpk1*Δ phenotype because, in this mutant, increased Pkc1 function is unable to increase Swi4-dependent transcription.

The suppression of the *cln1*Δ *cln2*Δ *pcl1*Δ *pcl2*Δ G1 arrest by sorbitol suggests that these strains do not have an absolute defect in G1 progression but rather have a defect in a morphogenesis or cell-wall biogenesis related function that results in a G1 arrest. This phenotype is distinct from the non-sorbitol-suppressible G1 arrest of *cln1*Δ *cln2*Δ *cln3*Δ strains and the unbudded G2-phase terminal phenotype of *cln1*Δ *cln2*Δ *bud2*Δ strains (Cvrckova and Nasmyth 1993). Perhaps the morphogenesis defect in *pcl1*Δ *pcl2*Δ *cln1*Δ *cln2*Δ strains occurs upstream of a checkpoint that inhibits passage through START. Such a checkpoint might operate through stabilization of Sic1, an S-phase inhibitor, as *pho85*Δ *sic1*Δ cells are temperature sensitive for growth (Aerne *et al.* 1998) and Sic1 is more stable in *pho85*Δ strains (Nishizawa *et al.* 1998). This suggests that entry into S phase is delayed in the *pho85*Δ mutant and that removing this delay has deleterious consequences. Consistent with this hypothesis, while the synthetic lethality of *cln1*Δ *cln2*Δ *cln3*Δ is suppressed by deletion of *SIC1* (Schneider *et al.* 1996), the lethality of *pcl1*Δ *pcl2*Δ *cln1*Δ *cln2*Δ is not (M. Tyers and B. Andrews, personal communication).

In contrast to mutants with defects in morphogenesis and cell-wall biogenesis, *pho85*Δ strains mate efficiently. Furthermore, we have been unable to detect any defects in morphogenesis-related processes in either *pcl1*Δ *pcl2*Δ or *pho85*Δ mutants. Perhaps Pcl1- and Pcl2-associated Pho85 kinases regulate morphogenetic events specifically during G1—or perhaps a specialized G1.

Our data help to clarify the cause of inviability in *cln1 cln2 pcl1 pcl2* strains, as they suggest that *PCL1* and *PCL2* promote Cdc42 activity or promote a process that can substitute for Cdc42 rather than participating in the START step of G1 itself. We report a *pcl1*Δ phenotype that does not also depend on removing *PCL2*. It will be interesting to determine if this reflects the ability of Pcl1 and Pcl2 to phosphorylate different substrates or if instead it reflects differential requirements for phosphorylation of a common substrate. The challenge of understanding how cyclin binding confers functional specificity on Pho85 requires us to identify the *in vivo* substrates of these different Pho85-containing kinases.

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