The Identification of Transposon-Tagged Mutations in Essential Genes That Affect Cell Morphology in *Saccharomyces cerevisiae*

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**ABSTRACT**

The yeast *Saccharomyces cerevisiae* reproduces by budding, and many genes are required for proper bud development. Mutations in some of these genes cause cells to die with an unusual terminal morphology—elongated or otherwise abnormally shaped buds. To gain insight into bud development, we set out to identify novel genes that encode proteins required for proper bud morphogenesis. Previous studies screened collections of conditional mutations to identify genes required for essential functions, including bud formation. However, genes that are not susceptible to the generation of mutations that cause a conditional phenotype will not be identified in such screens. To identify a more comprehensive collection of mutants, we used transposon mutagenesis to generate a large collection of lethal disruption mutations. This collection was used to identify 209 mutants with disruptions that cause an aberrant terminal bud morphology. The disruption mutations in 33 of these mutants identify three previously uncharacterized genes as essential, and the mutant phenotypes suggest roles for their products in bud morphogenesis.

A basic problem in biology is to understand the molecular mechanisms that generate specialized cell shapes. The yeast *Saccharomyces cerevisiae* provides a simple system in which to study this process. Early in the cell division cycle, the microtubule-organizing center (spindle pole body) is duplicated, and a ring of chitin forms in the wall of the mother cell (Pringle and Hartwell 1981). Cell wall growth is localized to the region within this ring, resulting in the formation of a small bud. A ring of 10-nm filaments appears in the cytoplasm next to the cell membrane between the mother cell and the bud and, as the cell cycle progresses, the bud continues to grow and eventually becomes the daughter cell. As the bud increases in size, DNA replication occurs, the spindle pole bodies separate, and the spindle is formed. The nucleus then migrates to the neck between the mother and daughter cells, the spindle elongates, and nuclear division takes place. Finally, with the formation of a cell membrane and cell wall barrier between the mother and daughter cells, cytokinesis physically separates the mother and daughter cells.

Mutations in a number of essential genes cause cells to form abnormally shaped buds, indicating that the products of these genes are required to make a normal bud. At the restrictive temperature, *cdc4*, *cdc34*, and *cdc53* mutants arrest late in G1-phase of the cell division cycle before the beginning of DNA replication (Byers and Goetsch 1974; Byers 1981; Pringle and Hartwell 1981; Adams 1985). In these mutants, the spindle pole body duplicates, but the poles fail to undergo the separation required for spindle formation. Although these mutants initiate bud emergence, they proceed to form abnormal (multiple and elongated) buds. Mutations that increase the activity of Cln1- or Cln2- (G1 cyclins) associated Cdc28 kinase or decrease the activity of Cib1- or Cib2- (B-type cyclins) associated Cdc28 kinase also cause cells to form elongated buds (Lew and Reed 1993). Moreover, a mutation in any of the genes that encode the three subunits of the replication factor-A homologue in yeast (*RFα1, RFα2, and RFα3*) causes cells to arrest with single or multiple elongated buds (Brill and Stillman 1991). Although the protein products of these genes are involved in a variety of functions, they are all required during the same period of the cell division cycle, late in the G1-phase, and may function in related pathways.

Mutations in several other genes whose products are required later in the cell division cycle also cause the formation of aberrant, elongated buds. The products of the genes *CDC3, CDC10, CDC11,* and *CDC12* are needed to assemble the ring of 10-nm filaments at the neck between the mother and daughter cell, and a mutation in any of these genes prevents cytokinesis (Haarer and Pringle 1987; Ford and Pringle 1991; Pringle and Hartwell 1981; Kim et al. 1991). These mutants continue to bud, replicate their DNA, and proceed through nuclear division in the absence of cytokinesis, thus forming cells with multiple, elongated buds and multiple nuclei. Deletion mutations of either *CDC3* or *CDC12* are lethal (J. Pringle, personal communication).
To gain further insight into the process of bud morphogenesis, we set out to identify additional genes required for this process. Previously, collections of conditional mutations have been screened to identify genes required for essential functions, but genes that are not susceptible to the generation of mutations that cause a conditional phenotype would not have been identified in such screens (Hartwell 1967; Moir et al. 1982; Kaback et al. 1984; Bartel and Varshavsky 1988; Riles and Olson 1988). However, a screen of mutants created by gene disruption could lead to the identification of a more comprehensive collection of essential genes, including those required for proper bud formation. This approach is not limited by the requirement that the mutated gene cause a conditional phenotype. In this report, we describe the use of transposon mutagenesis to generate a large collection of disruption mutations in essential genes and the identification of mutants that die with an aberrant bud morphology. The mutations in 33 of these mutants identify three previously uncharacterized genes as essential and suggest a role for their products in bud morphogenesis.

MATERIALS AND METHODS

Reagents: All chemical reagents, including those used for the preparation of media, were obtained from Sigma, Difco, or Boehringer Mannheim. All restriction endonucleases and other DNA modification enzymes were obtained from Boehringer Mannheim. Glusulase was obtained from NEN Research Products.

Media and growth conditions: Yeast strains were grown in yeast extract, peptone, dextrose (YPD) medium (Rose et al. 1990) at 30°C. To identify auxotrophic markers, yeast were grown on synthetic complete “drop-out” medium (Rose et al. 1990) in which all of the commonly encountered auxotrophies were supplemented except for those being used in the genetic selection. This medium was also supplemented with 2% glucose (SD medium). Presporulation medium consists of 1% yeast extract, 3% peptone, 5% glucose, and 2% agar. Sporulation media was prepared as described previously (Sherman 1991). Liquid cultures were grown with aeration at 30°C.

Escherichia coli strains were grown at 36°C in LB medium (Davis et al. 1980) supplemented with one or several of the following: ampicillin (50 μg/ml), kanamycin (80 μg/ml), streptomycin (100 μg/ml), and chloramphenicol (30 μg/ml).

Strains and plasmids: The S. cerevisiae strains used in this study are listed in Table 1. A yeast strain lacking 2 μm plasmid DNA (KC107) was isolated using the method of Dobson et al. (1980) as follows. Strain FY23 was transformed (Schiestl et al. 1989) with pBD219 (kindly provided by B. Futterer), and Leu+ transformants were selected on synthetic complete medium lacking leucine. A single transformant was picked and grown at 30°C for 6 days in 25 ml of synthetic complete medium lacking leucine, with a 1:1000 dilution into fresh medium each day. The final culture was then diluted 1:1000 into fresh YPD medium and grown at 30°C for five more days, again with a daily 1:1000 dilution into fresh medium. Cells from this culture were then spread onto solid YPD medium, grown at 30°C, and replica-plated onto synthetic complete medium with and without leucine to identify Leu+ clones. Three Leu+ clones were identified, and Southern blot analysis (Genius I DNA labeling and detection kit, Boehringer Mannheim Corp.) was performed using the SpeI fragment from YEP24 (Botstein et al. 1979) as a probe to determine which strains no longer contain 2 μm DNA. Two of the three yeast clones were shown to lack 2 μm DNA. From one of these strains (KC107), genomic DNA was isolated and used to generate the bacterial plasmid library described below.

The diploid strain KC100 was constructed as follows. The plasmid YEp-CYH7 (kindly provided by Jonathan R. Warner) consists of YEP24 (a pBR325-based derivative of YEP24) with the 5.4 kb HindIII/BamHI fragment that contains cyh2' (Kauf et al. 1985). The 2-μm plasmid DNA was removed by standard techniques (Sambrook et al. 1989) from YEP-CYH7 by deleting the ~1.9-kb SpeI fragment. The resulting plasmid was linearized at a BglII site within the cyh2' coding region and used to transform strain FY24. Transformants with the plasmid presumably integrated at CYH2 were selected on synthetic complete medium lacking uracil. A single transformant was inoculated into YPD, grown to saturation, and then plated onto YPD supplemented with cycloheximide (10 μg/ml) to select for clones that had lost the plasmid DNA and replaced the wild-type CYH2 with cyh2'. Several cycloheximide-resistant colonies were picked and examined by Southern blot analysis to detect the presence of the YEP24 DNA in the original transformant and then its absence in the cycloheximide-resistant clones (data not shown). A cycloheximide resistant strain that lacks the vector DNA was named KC106. The plasmid pKP102 contains CAN1 on a SalI/BamHI fragment from TLC-1 (Broach et al. 1979) inserted into the SalI/BamHI sites of pRS305 (Sikorski and Hieter 1989). A 1.3-kb HindIII fragment was deleted from CAN1, and the resulting plasmid was linearized at an NsiI site within the mutated allele and used to transform FY23, which is isogenic with FY24. Transformants presumably containing the plasmid integrated at CAN1 were selected on synthetic complete medium lacking leucine. A single transformant was grown in YPD to saturation and then plated onto synthetic complete medium lacking arginine but supplemented with canavanine (90 μg/ml) to select for transformants that had lost the plasmid and replaced the wild-type CAN1 with can1Δ. Several canavanine resistant clones were examined by Southern blot analysis to confirm that they contain a deletion in can1 (data not shown). The resulting canavanine resistant strain, KC105, was mated to KC106 to generate KC100.

Construction of a bacterial library of yeast genomic DNA: Genomic DNA was isolated (Sherman et al. 1986) from a yeast strain lacking 2 μm plasmid DNA (KC107), digested with BamHI, and ligated to BamHI digested, dephosphorylated

| TABLE 1 |
| S. cerevisiae strains used |
| Strain | Genotype | Source |
| FY23 | MATa ura3-52 trp1Δ63 leu2Δ1 | F. Winston |
| FY24 | MATa ura3-52 trp1Δ63 leu2Δ1 | F. Winston |
| KC100 | MATa/can1Δ' CAN1 cyh2' CYH2 ura3-52 trp1Δ63 leu2Δ1 | This study |
| KC105 | MATa can1Δ' ura3-52 trp1Δ63 leu2Δ1 | This study |
| KC106 | MATa cyh2' ura3-52 trp1Δ63 leu2Δ1 | This study |
| KC107 | MATa can1Δ' ura3-52 trp1Δ63 leu2Δ1 | This study |
The DNA from two separate ligation reactions was used to transform competent DH1 plB101 (HOEKSTRA et al. 1991), and transformants were selected on LB medium supplemented with chloramphenicol and kanamycin. The colonies of cells transformed with each ligation reaction were pooled separately. The first pool consists of 1800 transformants; 77% of these contain recombinant plasmid with yeast DNA as judged by Southern blot analysis (data not shown). The second pool consists of 8500 different transformants, of which an estimated 57% of these contain recombinant plasmid. In total, the plasmid library comprised of these two pools contains ~6200 independent recombinant plasmids.

**Generation of heterozygous disruption mutations in yeast:**

This and the following two sections are summarized in Figures 1 and 2. The bacterial strains and methods of HOEKSTRA et al. (1991) were used to mutagenize each pool of the plasmid library of yeast DNA with the mini-Tn3 transposon m-Tn3 (URA3). From the first pool of the plasmid library, 6.6 × 10^6 transconjugants resulted, and ~80% have the mini-Tn3 inserted into the yeast DNA (Southern blot analysis, data not shown). From the second pool, 3.3 × 10^6 transconjugants resulted, and ~72% have the mini-Tn3 inserted into the yeast DNA. Plasmid DNA was isolated from each pool of transconjugants, and the yeast DNA was excised by digestion with NotI, which cleaves at sites flanking the inserted DNA. Mutated DNA was substituted for one copy of its wild-type homologue in yeast by transforming (SCHESTL and GRETZ 1989) diploid strain KC100 (Table 1) and selecting for Ura transformants. Since the first pool of transconjugants represents 22% of the bacterial library of yeast DNA, 22% of the yeast transformants were derived using this DNA pool, and 78% were derived using the second pool.

It was possible that digestion with NotI of the library plasmid DNA were incomplete, undigested plasmid would transform the yeast strain by integrating into the yeast genome. Such an event would generate a strain with a third, mutated copy of the DNA in question. To address this possibility, 10 transformants from each of seven separate yeast transformants were examined by Southern blot analysis, and it was determined that 93% of these transformants do not contain the pHSS6 vector DNA (data not shown). About 82% of the transformants contained the mini-Tn3 transposon (data not shown). Some of the other 18% might have become Ura by gene conversion of one of the ura3-52 alleles. Such mutants were eliminated in the subsequent screen for haploid-lethal disruptions. Because most of the transformants contain the transposable element and not the plasmid vector DNA, presumably most were indeed generated by the replacement of one copy of the wild-type DNA by the transposon-containing, mutated DNA.

**Screen for haploid-lethal mutations:** Putative transformants were transferred to a YPD grid and grown at 30°C for 2 days. They were then replica-plate onto sporulation medium, medium incubated at 30°C for 7 days. Sporulated cells were then replica-plate onto synthetic complete medium lacking uracil and arginine but supplemented with cycloheximide (10 μg/ml) and canavanine (90 μg/ml) and incubated at 30°C for 2–3 weeks until papillated patches appeared (Figure 1B). Approximately 34,500 putative transformants were screened; this number does not include the ~7% that contain plasmid DNA (data above). A total of 9900 failed to papillate, and the corresponding transformants were tested a second time. In the rest, 4672 transformants failed to papillate.

To eliminate mutants with disruptions that are not actually haploid-lethal, these 4672 transformants were tested for the ability to produce viable spores that lack the disruption. The diploid transformants were sporulated and then allowed to germinate and grow on the same medium as that described above, except uracil was included. All transformants should produce can1ΔΔ yeast spores that do not contain the URA3 tagged disruption mutation. Under these growth conditions, these spores should germinate, grow, and form papillations. However, 647 transformants did not papillate.
Identification of mutants with aberrant terminal morphologies: The 4025 transformants resulting from the previous screen were grown 1 day at 30°C on YPD medium, replica-plated onto sporulation medium and grown for an additional 2 days at 30°C. They were then replica-plated onto sporulation medium and grown for 1 day at 30°C and then 3 more days at 30°C. The sporulated cells were replica-plated onto synthetic complete medium lacking uracil but supplemented with cycloheximide (4 μg/ml) and incubated for 2 days at 30°C. Germinating cells were observed using a Zeiss microscope with a UD40 ×40 objective. Diploid transformants (495) whose segregants displayed aberrant terminal morphologies were transferred to YPD medium and characterized further.

Analysis of random spores from mutants with aberrant terminal morphologies: The 495 transformants were sporulated as described above. Spores were pretreated, separated as previously described (DAVISON and BYERS 1984), plated onto synthetic complete medium lacking uracil and arginine but supplemented with canavanine (90 μg/ml), and incubated at 30°C for 2–7 days before microscopic observation. Because cycloheximide affects the growth even of haploid cells with a cyp4 allele, it was preferable, when possible, to use canavanine alone to select against unsporulated diploid cells. In the previous screen, this was not feasible, because canavanine-resistant mutants arose spontaneously and grew to obscure the cells with disruption mutations. However, in the screen of random spores, sporulated cells were plated at a low density, so rare, spontaneously arising canavanine-resistant mutants were well separated from nearby cells. Diploid transformants whose segregants displayed aberrant terminal morphologies were transferred to YPD for further characterization.

To attempt to examine the nuclei of mutants with aberrant terminal morphologies, samples of these strains were sporulated (KASSIR and SIMCHEN 1991) and then allowed to germinate in synthetic complete medium lacking uracil and arginine but supplemented with canavanine (90 μg/ml) for 2 days at 30°C. Cells were then fixed with ethanol (HUTTER and EPEL 1979), stained with 1 μg/ml 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI), and observed using a Nikon Microphot-FXA fluorescence microscope with a ×20 or ×40 objective.

Photographing yeast cells: Images of mutant yeast cells were recorded using a Nikon Microphot-FXA microscope with a ×20 objective, a Pulinx TM-745 black and white video camera, an Apple Macintosh Quadra 700 personal computer, and the NIH Image 1.53b2 software. These images were assembled and labeled using Aldus Persuasion 2.1 and then transferred to black and white photographic paper.

Mapping insertion mutations to chromosomes: Intact chromosomal DNA was prepared (ROSE et al. 1990) from the 209 mutants containing a haploid-lethal mutation that causes an elongated bud morphology. The chromosomes of each mutant were separated on 1% agarose gels (ROSE et al. 1990) using a Bio-Rad CHEF-DR II pulse field electrophoresis system and a Pulsewave 760 Switcher. Gels were run essentially as described, except that electrophoresis was carried out for 17 hr with a 60-sec pulse frequency, followed by 11 hr with a 90-sec pulse frequency.

For Southern blot analysis, the DNA in each gel was transferred overnight to nylon membrane (Micron Separations Inc., Magna nylon transfer membrane, 0-45 micron) according to the directions of Schleicher and Schuell. The membrane was then hybridized with a digoxigenin labeled probe [labeled pGEM3 (Promega) for the bga gene in the mini-Tn3 transposon]. Each hybridizing chromosome band was aligned with one of the 13 chromosome bands visualized by staining with ethidium bromide. To correlate chromosome bands with chromosomes, chromosomal DNA from KCC100 was also analyzed as described above. A gene from each yeast chromosome was used as a probe to identify which yeast chromosomes correlate with which of the 13 chromosome bands.

DNA sequence analysis of disruption mutations: Disrupted genes were cloned and sequenced essentially as previously described (BURNS et al. 1994). Briefly, a yeast integrating plasmid pRS304 (SIKORSKI and HIETER 1989) was integrated into the bla gene of the transposable element linked to the disruption mutation in the diploid yeast mutant of interest. The plasmid DNA was linearized with the restriction endonuclease ScaI, which cleaves the plasmid once, within the bla gene. Transformants were selected on synthetic complete medium lacking tryptophan. Chromosomal DNA isolated from one of these transformants (ROSE et al. 1990) was digested with KpnI, which cleaves once in pRS304, does not cleave the transposable element, and cleaves somewhere in the adjacent yeast chromosomal DNA. This digestion produces a DNA fragment which contains the bla gene, an E. coli origin of replication (from pRS304), the URA3-containing end of the transposable element, and the disrupted yeast DNA next to the transposable element. After ligation under dilute conditions to promote self-ligation, this DNA was transformed into E. coli, and plasmids composed of this DNA were selected by incubation on LB medium containing ampicillin.

Plasmid DNA extracted from E. coli was used to determine the DNA sequence of the disrupted yeast DNA next to the transposable element. The DNA sequencing primer (mTrn3R, 5'-AGCTTGGGCTGAGGGTGAGGAT 3') anneals upstream of the 38-bp repeat at this end of the transposable element. Sequencing reactions were prepared using the Sequenase 1.0 kit (United States Biochemical). Using both the National Center for Biotechnology Information (NCBI) BLAST service (ALTSCHUL et al. 1990) to search GenBank and the Saccharomyces Genome Database 2.3 software (Department of Genetics, Stanford University School of Medicine), both the genomic location of this yeast DNA sequence and the position of the insertion mutation were determined.

PCR screen: Mutants with disruptions in chromosome II, V, VIII, or IX were screened using the polymerase chain reaction (PCR) for those with a disruption in one of the open reading frames YBR211, YER112, or YIL106, respectively. Briefly, PCR primers that anneal to the transposable element and a primer that anneals upstream of one of these open reading frames were used to determine whether the transposable element in the chromosomal DNA of a mutant lies in or near that open reading frame. Because each end of the transposable element contains a 38-bp repeat, the transposable element primers were designed to anneal upstream of each of these repeats and to direct DNA synthesis through each repeat and away from the transposable element. Chromosomal DNA was prepared from each mutant as described by ROSE et al. (1990).

DNAs from the seven mutants with disruptions in chromosome II were amplified using Taq DNA polymerase (Boehringer Mannheim), the transposable element primer mTrn3R (described above) and mTrn3L (5'-TGATATTCTCTACAGCCAAATCCC 3'), and a primer that anneals 565 bp upstream of the open reading frame YBR211 (5'-CGTGTAGCGAAGCTGTAGCAGATC 3'). DNAs from the five mutants with disruptions in chromosome V or VIII were amplified using the transposable element primers and a primer that anneals 1290 bp upstream of the open reading frame YER112 (5'-GAGGACAGAAGGAAATGACG 3'). DNAs from the 27 mutants with disruptions in chromosome IX were amplified using the transposable element primers and a primer that anneals 953 bp upstream of the open reading frame YIL106 (5'-GAGGTGTTTTATCCTTGGC 3'). The PCR reactions were carried out essentially as previously described (MATHIAS et al. 1994; except that 3.0
mM MgCl₂ was used) for 30 cycles (1 min 94°, 2 min 65°, 3 min 72°), and the products were separated in a 1% agarose gel using standard electrophoresis conditions (SAMBROOK et al. 1989).

RESULTS

Construction of a genomic library containing Tn3 insertions: To generate a library of disruption mutations in yeast (Figure 1; see MATERIALS AND METHODS for details), we first constructed a plasmid yeast genomic DNA. It should be noted that BamHI does not cleave rDNA, and it cleaves only a few Ty elements (mobile DNA elements present 30–35 times in the genomes of most laboratory strains) and only once in each known case (PHILIPPSEN et al. 1991). Whereas most yeast strains contain 50–100 copies of 2 µm plasmid DNA (PHILIPPSEN et al. 1991), KC107 lacks such DNA. Cleaving yeast chromosomal DNA from KC107 with BamHI thus generates relatively few repeated and identical DNA fragments and helps minimize the overrepresentation of repetitive DNA in this library. The resulting bacterial library contains 6200 recombinant plasmids, with the average size of the yeast DNA insert being ~7 kb. Therefore, with 95% confidence, this library is likely to include the entire yeast genome (MOORE 1987).

To generate insertion mutations in the library, it was mutagenized in E. coli with a mini-Tn3 transposon (HOEKSTRA et al. 1991). This transposable element contains both an E. coli selectable marker (bla) and a yeast selectable marker (URA3), which make it possible to select for DNA that has been mutagenized by the insertion of the transposon. The resulting mutagenized library contains ~10⁷ different plasmids, and in 77% of the transposition events, the mini-Tn3 element is inserted into the yeast DNA rather than into the plasmid vector. With 95% confidence, this library of insertion mutations should include a random collection of mutations dispersed every 5–6 nucleotides through the yeast genome (MOORE 1987).

Next, the mutagenized yeast DNA inserts were transformed into a diploid yeast strain, selecting for the URA3 marker in the mini-Tn3 element, to generate a collection of transformants, each with a heterozygous disruption mutation.

Identification of haploid-lethal mutations: To identify mutants with disruptions in essential DNA, ~34,500 transformants were screened for the presence of haploid-lethal mutations (Figures 1 and 2). Each transformant was sporulated and then allowed to germinate and grow on synthetic complete medium lacking uracil and arginine but containing cycloheximide and canavanine (HOLLINGSWORTH and BYERS 1989). The parent strain and thus the transformants are all heterozygous for the mutant alleles can1Δ' and cyh2', which confer resistance to canavanine and cycloheximide, respectively. Because sensitivity to these drugs is dominant, the canavanine and cycloheximide in the selective medium kill all diploid cells, and only the sporulated haploid cells that have both drug-resistance alleles can grow. In addition, because the medium also lacks uracil, only spores with a wild-type allele of URA3 (contained in the mini-Tn3 transposable element at the site of the insertion) can grow. Because only a fraction of the spores have the three selectable markers that allow them to grow, a collection of small colonies (papillations) is produced (Figure 1B, Plate B). Moreover, if a disruption is in essential DNA, the wild-type URA3 gene is tightly linked to a lethal mutation, so viable spores cannot be recovered, and no papillations are produced. Of the 34,500 putative transformants tested, this screen identified 4672 transformants that contain haploid-lethal mutations.

Of these transformants, 647 fail to produce viable spores without the transposon-linked mutation and therefore, appear to have mutations that are not haploid-lethal (see MATERIALS AND METHODS). There are a number of explanations for the presence of these transformants. Some might have a mutation that causes a dominant spo− phenotype (e.g., a mutation in the MAT' locus). Also, a transposon that disrupts essential DNA near the can1Δ' or cyh2' locus might make can1Δ' cyh2' spores without the transposon (viable spores) rare. In addition, a disruption of the cyh2' allele would preclude the existence of viable Cyh' spores. These mutants were removed, leaving 4025 transformants with haploid-lethal disruptions.

Identification of mutants with aberrant terminal morphologies: Mutations in many genes required for normal bud morphogenesis cause cells to die with an aberrant terminal morphology. We reasoned that other, novel genes that are also required for proper bud formation might, when disrupted, give rise to the same phenotype. To identify such mutants, the 4025 trans-

![Figure 2.—Summary of genetic screen to identify transformants with mutations in essential DNA.](image-url)
FIGURE 3.—Examples of mutants with aberrant cell morphologies. (A–C) Transformants were sporulated, replica-plated onto synthetic complete medium lacking uracil but supplemented with cycloheximide, and allowed to germinate and grow at 30° for 2 days. (A) mutant B59, (B) mutant B58, (C) mutant A2. (D–F) Transformants were sporulated, and the spores were separated and plated onto synthetic complete medium lacking uracil and arginine but supplemented with canavanine. The spores were then allowed to germinate and grow at 30° for 1–2 days. For each mutant, each cell or group of cells arose from a single spore. (D) mutant B54, (E) mutant B79, (F) mutant B5. (G–I) Transformants were sporulated, spores from intact tetrads were separated with a micromanipulator, and the spores were allowed to germinate and grow at 30° for 1 day. (G) mutant B77; disruption in YER112, (H) mutant B131; disruption in YIL106, (I) mutant B43, disruption in YBR211.

Transformants containing heterozygous lethal disruption mutations were screened microscopically. As before, each mutant was sporulated and then replica-plated onto synthetic complete medium lacking uracil but containing only cycloheximide, rather than both cycloheximide and canavanine, and the terminal morphologies of each mutant was then observed. [Under these conditions that require only URA3 linked to the disruption mutation and cyh' (and not can'), twice as many spores could grow and exhibit the mutant phenotype, thus increasing the sensitivity of the microscopic screen.] Because each patch of cells included a mixture of unsporulated diploids and both wild-type and mutant haploids, mutants arresting with a uniform but wild-type morphology, a characteristic of many cell-division-cycle mutants, were not conspicuous. However, mutants with abnormal terminal morphologies were obvious, and we observed several different aberrant cell shapes. Mutants (495) were observed to form elongated and abnormally shaped cells or buds (e.g., Figure 3, A and B). For mutants that appear to form elongated buds, the bud is narrower than the mother cell. However, for those that appear to form elongated cells, the bud is the same width as and therefore indistinguishable from the mother cell. We also observed six mutants that form enlarged cells whose diameter appears to be approximately three to four times that of wild-type cells (e.g., Figure 3C).

Analysis of random spores and tetrad analysis to characterize the phenotypes of individual cells: To more clearly observe the terminal morphologies of individual cells, spores from each transformant of interest were separated, plated at low concentration onto synthetic complete medium lacking uracil and arginine but supplemented with canavanine, allowed to germinate and grow at 30°, and then observed microscopically. The results of this analysis are summarized in Table 2. Of the 495 mutants initially classified to form an aberrant bud morphology, 209 were found to be inviable and form elongated, abnormally shaped buds (e.g., Figure 3D). Another 38 mutants were found to be inviable but have oblong or elliptically shaped cells. In addition, 48 mutants were found actually to grow very slowly (form visible colonies after 1 week at 30°). Of these, 32 form
Elongated cells or buds were observed to exhibit this same cell morphology, but all also proved to be viable and grow slowly. All of the six mutants classified as forming enlarged cells were separated by micromanipulation (Sherman and Hicks 1991), allowed to germinate and grow at 30°, and periodically observed microscopically for several days. Two different mutants with disruptions that were later determined to be in the same gene have different phenotypes. Mutant cells from one strain divide two or three times before dying, and about two-thirds of these cells are budded. About half of the mutant cells from the second strain grow slowly, while the other half divide three or four times before dying. Slow growing or not, about half of these spores give rise to at least one cell with an elongated-bud phenotype (Figure 3G). The mutant spores from the third strain fail to divide and instead give rise to cells with one or often two large buds (Figure 3H). The fourth mutant gives rise to cells that only divide once or twice before dying. Approximately one-quarter of these spores produce at least one elongated-bud cell (Figure 3I). The identification of the mutated gene in each of these strains is described below.

Chromosome mapping of disruption mutations: Because each of these mutants has a lethal or nearly lethal disruption mutation, we cannot easily recover these mutations in haploid strains and define complementation groups or test for complementation with alleles of known genes. However, each disruption is marked by an E. coli transposable element that has a unique DNA sequence not normally found in the yeast genome. Therefore, we were able to use this DNA sequence as a probe to identify the chromosomal location of each of the 209 disruption mutations of interest (see MATERIALS AND METHODS; Table 3). They mapped to ~12 different chromosomes. However, because chromosomes V and VIII, VII and XV, and XIII and XVI do not resolve under standard conditions (Gerrinson et al. 1991), it was not possible to determine the precise chromosome position of a mutation that mapped to one of these chromosome pairs. Consequently, it was possible that the mutations mapped to as many as 15 different chromosomes, all but chromosome I. Therefore, this collection of mutants includes mutations in 12–15 different genes.

Mutations in a number of previously characterized genes cause an elongated-bud terminal morphology (see Introduction and Discussion). Our collection of mutants includes those with disruptions on at least six but perhaps as many as eight chromosomes (II, III, VII and/or XV, XIII and/or XVI, IX, and X) that are distinct from the seven chromosomes where these known genes reside. Therefore, our collection of mutants defines at least six novel genes.

In general, there is little correlation between the size of each chromosome and the number of mutations mapped there ($\rho^2 = 0.21$), indicating that the mutations are not randomly dispersed throughout the yeast genome. Because a nonrandom collection of mutations was analyzed (only those that cause an unusual morphology), this result is not surprising. Nevertheless, these mutations do appear to be scattered throughout the genome and are not clustered on a few chromosomes.

Identification of mutations in three novel open reading frames: To identify the mutated genes in a few of the 209 mutants, we cloned and sequenced portions of seven disruption mutations that lie on chromosomes II, V or VIII, and IX whose complete DNA sequences have been determined. Chromosomal DNA containing part of the transposable element and the adjacent yeast DNA was isolated and sequenced (see MATERIALS AND METHODS). Only ~20 bases of yeast DNA sequence was required to match the disrupted gene to the chromosome

### TABLE 2

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<th>Terminal morphology</th>
<th>No. of mutants</th>
<th>Phenotypes of mutants with aberrant terminal morphologies</th>
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<tr>
<td>Elongated cells or buds</td>
<td>209</td>
<td>Slow growth*</td>
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<tr>
<td>Elongated buds</td>
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<td>Slow growth*</td>
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<td>Elongated cells</td>
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<td>Slow growth*</td>
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*No visible colonies after 1 week.

### TABLE 3

<table>
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<th>Chromosome</th>
<th>No. of mutations</th>
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<td>IV</td>
<td>31</td>
<td>1600</td>
</tr>
<tr>
<td>V, VIII*</td>
<td>5</td>
<td>610, 555</td>
</tr>
<tr>
<td>VI</td>
<td>5</td>
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</tr>
<tr>
<td>VII, XV*</td>
<td>19</td>
<td>1100, 1100</td>
</tr>
<tr>
<td>IX</td>
<td>27</td>
<td>445</td>
</tr>
<tr>
<td>X</td>
<td>3</td>
<td>760</td>
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<tr>
<td>XI</td>
<td>5</td>
<td>690</td>
</tr>
<tr>
<td>XII</td>
<td>3</td>
<td>rDNA + 1100</td>
</tr>
<tr>
<td>XIII, XVI*</td>
<td>101</td>
<td>920, 960</td>
</tr>
<tr>
<td>XIV</td>
<td>2</td>
<td>800</td>
</tr>
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</table>

Chromosome sizes were from Gerrinson et al. (1991). *These pairs of chromosomes did not resolve.
sequence. For a mutation in chromosome V or VIII, the transposable element is inserted 130 bp downstream from the predicted translation start site of the open reading frame (ORF) of YER112 (which encodes the protein with Swiss-Prot accession No. P40070) on chromosome V. Four mutations in chromosome IX all lie in or near YIL106 (which encodes the protein with accession No. P40484). One mutation lies 101 bp upstream of the predicted translation start site, another lies 426 bp downstream of the start site, and the two others both lie 510 bp downstream from the start. The other two mutations analyzed lie in chromosome II, in or near YBR211 (which encodes the protein with accession No. P38313). One lies 17 bp upstream and the other 727 bp downstream of the predicted translation start site.

We next used a polymerase chain reaction (PCR) screen (see MATERIALS AND METHODS) to determine whether any of the other 32 mutants with disruptions in one of these four chromosomes also contains disruptions in or near one of these three genes. Chromosomal DNA from each mutant was tested by PCR using a primer that anneals upstream of the putative translation start site of the ORF and each of two primers that anneal to the ends of the transposable element. Products of different size (based on agarose gel electrophoresis) indicate disruption mutations at different positions within the gene. Of the remaining four mutants with disruptions in chromosome V or VIII, one has a disruption near the predicted translation start site of the ORF encoded by YER112. The insertions in 21 of the remaining 23 mutants with disruptions in chromosome IX also lie in or upstream of YIL106. The mutations appear to be in at least 11 different positions, six of which include 17 mutations that are within the ORF. Of the other five mutants with disruptions in chromosome II, four have disruptions in YBR211. The mutations appear to be in at least three different positions, all of which are within the ORF.

In addition to the elongated-bud mutants, we also mapped the mutations that cause an enlarged-cell phenotype. All six mutations are in FAB1 (GenBank accession No. U01017) on chromosome VI.

These putative genes were sequenced during the S. cerevisiae genome project (LEVY 1994) and little is known about their functions. Examination of their predicted amino acid sequences failed to reveal characteristic functional motifs. However, their predicted translations are homologous to those of a number of expressed genes. The predicted YER112 product is also similar to polypeptides predicted from ESTs from Arabidopsis thaliana, Homo sapiens liver, and Caenorhabditis elegans, suggesting that this gene is conserved among diverse species (Figure 4). The third ORF identified, YIL106, has a predicted product that is similar to that of an EST from A. thaliana, suggesting that this yeast ORF is probably an expressed gene and that it is conserved between yeast and plants (Figure 4).

DISCUSSION

In this report, we describe the use of transposon mutagenesis to identify essential genes that are required for normal bud morphogenesis in S. cerevisiae. We generated a large collection of disruption mutations in the genome and screened this collection microscopically for mutants that die with aberrant terminal morphologies. There are a number of advantages to this approach. Historically, studies of essential functions have been based primarily on the identification of conditional mutants. However, comparisons of different collections of conditional lethal mutants indicate that different collections identify different groups of genes (HARTWELL 1967; MOIR et al. 1982; KABACK et al. 1984; BARTEL and VARSHAVSKY 1988; RILES and OLSON 1988), presumably because genes differ in their susceptibilities to the creation of mutations that cause particular conditional phenotypes. For example, an analysis of yeast chromosome I suggests that it is difficult or impossible to isolate temperature-sensitive alleles of >75% of its single-copy, essential genes (KABACK et al. 1984; HARRIS et al. 1992). Therefore, any one conditional lethal screen will most likely identify only a subset of the essential genes. Because the use of disruption mutations involves no requirement that a gene be susceptible to the generation of conditional mutations, the resulting collection of mutants should not be subject to these biases. This should promote the identification of novel genes.

Our approach also takes advantage of the efficiency of transposon mutagenesis to generate a large number of random disruption mutations. The Tn3 transposon’s pattern of insertion into DNA is essentially random (KLECKNER 1981). The efficient mutagenesis makes it feasible to screen a large collection of mutants. Our original collection of 34,500 yeast mutants should represent disruptions randomly dispersed about every 1.2 kb through the yeast genome. Given the spacing of about one gene per 1.5–2 kb of the genome (OLSON 1991), it is likely that our collection has mutants with insertions in most genes of the genome. Finally, because each mutation is marked by the transposable element, the physical mapping and cloning of each mutation is relatively straightforward.
Novel Bud Morphology Genes

Yer112

<table>
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<tr>
<th>S.c.</th>
<th>1</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
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<td></td>
<td></td>
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</tr>
<tr>
<td>H.A.</td>
<td><strong><strong>S</strong><em>RT</em>Q<em>N</em>L</strong><em>Y</em><em><strong><strong><em><strong><em>TIN</em>VY<em>CT</em></strong>T</em>H<em>KQ</em></strong></strong><strong><em><strong>ICTSKGDRWMTC</strong></em>NT</strong>YLRT</em>EV**N*OEE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.e.</td>
<td>V<em><strong>X</strong>KT</em>QNHP<em>LX</em><strong><em><strong><em><strong><em>TYN</em>H<em>KAC</em>S</strong></em>IDXVD**********IFTSKGDG</strong>FF</em>MS*A</strong>V<strong>ST</strong>YLRIPETV*LQ</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**FIGURE 4.** Alignment of predicted amino acid sequences for Yer112, Yil106, and similar ESTs. A segment of the S. cerevisiae (S.c.) protein Yer112 is similar to the predicted amino acid sequences of ESTs from A. thaliana (A.t., GenBank accession No. Z27273), H. sapiens (H.s., GenBank accession No. T69207), and C. elegans (C.e., GenBank accession No. T01467). A segment of Yil106 is similar to the predicted amino acid sequence of an EST from A. thaliana (GenBank accession No. Z46539). Asterisks, amino acids that are identical to corresponding residues in the yeast sequence.

Burns et al. (1994) recently described the use of transposon mutagenesis to generate another large collection of yeast mutants, each with a random lacZ insertion mutation. As a result of these insertions, lacZ has been fused to the disrupted genes, and the β-galactosidase activity of each resulting fusion protein was used to determine when the mutant protein is expressed and its subcellular location. This collection of in-frame fusion/disruption mutations represents insertion mutations that are distributed approximately every 15 kb throughout the yeast genome (Moore 1987). Tetrad analysis was performed on 59 of these mutants and nine were found that failed to produce viable spores with disrupting mutations, indicating that they contain disruptions in essential DNA.

With our collection of mutants, we have focused upon the haploid-lethal class of mutations and have begun to assign functions to these disrupted genes. The mutated genes in 4025 mutants are essential or nearly so, and (given the arguments above) should identify most of the essential genes in yeast. This result indicates that 12% of the yeast genome is essential and is consistent with previous estimates (Burns et al. 1994; Goebel and Petes 1986). Whereas the insertions in the collection of fusion/disruption mutations are expected to be in the coding sequences of genes, some of the disruptions in our collection are in coding sequences, while others lie in essential, noncoding regions of DNA.

Mutations in a number of essential genes (see Introduction) cause cells to form buds with an aberrant morphology. To identify novel, related genes, we screened our collection of 4025 mutants with disruptions in essential DNA for those that die with an aberrant terminal morphology. A total of 209 mutants form elongated buds, an additional 38 die as elongated cells. In addition, 48 mutants with similar morphologies proved to be viable and grew slowly; apparently, the initial screen to identify mutants with haploid-lethal disruptions is not precise enough to distinguish between no growth and slow growth. We observed that our cycloheximide-resistant yeast strains grew slower in the presence of cycloheximide than in its absence. Perhaps, the presence of cycloheximide in the selective medium used in the initial screen, reduced the growth rate of slow growing mutants to the point where they were scored as inviable.

The previously characterized mutants with lethal mutations that cause an elongated-bud terminal morphology can be grouped into at least two different classes. A mutation in CDC4, CDC34, CDC53, RFA1, RFA2, or RFA3 causes cells to arrest late in the G1-phase of the cell-division cycle, before DNA replication takes place (Byers 1981; Pringle and Hartwell 1981; Adams 1985; Bril and Stillman 1991). On the other hand, a mutation in CDC3, CDC10, CDC11, or CDC12 prevents cytokinesis (Pringle and Hartwell 1981; Haarer and Pringle 1987; Ford and Pringle 1991; Kim et al. 1991). These mutants continue to bud, replicate their DNA, and proceed through nuclear division; so they form multiply budded cells with multiple nuclei. A deletion mutation of either CDC3 or CDC12 is lethal (J. Pringle, personal communication). We have to date been unable to assign our 209 mutants to these classes, because we have been unable to visualize their nuclei (Materials and Methods). Instead of the localized staining that would identify nuclei, we observed diffuse staining throughout the cells (data not shown). Unlike analyses of temperature-sensitive mutants, where the mutant phenotype is observed several hours after mutant cells are exposed to the nonpermissive condition, the nuclei of our mutants were stained and examined two days after the sporulated, haploid cells were allowed to germinate and grow. Perhaps, during this extended period of time required for the aberrant terminal morphology to become apparent, the nuclei of these dying, mutant cells disintegrated. Conditional alleles may be required to classify and further characterize these mutants.

Because each of our mutant strains has a haploid-lethal disruption mutation, we cannot easily perform complementation analysis. However, we have determined the chromosome on which each mutation resides (Table 3). We found that our 209 mutations map...
to \( \geq 12 \) different chromosomes. The previously characterized lethal mutations causing similar phenotypes are in genes on seven different chromosomes: CDC34 and CDC53 are on chromosome IV (GOEBL et al. 1988; M. C. GOEBL, unpublished observations); CDC4, CDC3, and CDC12 are on chromosomes VI, XII, and VIII, respectively (PRINGLE and HARTWELL 1981; JOHNSON et al. 1987); and RFA1, RFA2, and RFA3 are on chromosomes I, XIV, and X, respectively (BRILL and STILLMAN 1991).

The collection of mutants described in this report includes disruptions in at least six (II, III, VII and/or XV, XIII and/or XVI, XI, and IX) and possibly as many as eight chromosomes that are distinct from those where the eight characterized genes map. Our mutants thus have disruptions in at least six to eight genes that appear to be different from those previously characterized.

Of our 209 mutants, we have determined that 33 have mini-Tn3 transposon-linked disruptions in or near three previously sequenced but otherwise uncharacterized genes (YBR211, YER112, and YIL106). There are at least four different mutations within the ORF of YBR211, plus one other insertion 17 bp upstream of the translation start site; one mutation within the ORF of YER112 plus another near the start site; and at least six different mutations within the ORF of YIL106, plus at least five upstream of the start site. The observation that numerous mutations were identified at different sites within each gene supports the hypothesis that this collection of mutants is comprehensive.

Disruptions in each of these genes cause aberrant cell morphologies. Mutations in YBR211 are lethal and cause some of its mutant cells to form elongated buds. Mutations in YIL106 are also lethal, and they cause the formation of one or often two large buds. Disrupting YER112 caused at least two different phenotypes. A disruption within the ORF is lethal and causes two-thirds of its mutant cells to die as budded cells. This is not an aberrant terminal morphology, but the preponderance of budded cells causes a microcolony to appear unusually spiked. A disruption near YER112's predicted translational start site causes death in some cells and slow growth in others. Perhaps this disruption alters gene expression in a way that makes the phenotype leaky. About half of these mutant spores give rise to some cells with an elongated-bud phenotype. All of these genes appear to be essential, and mutations in two of them affect the formation of normal buds. The YIL106 mutant forms apparently normally shaped buds, but it often buds more than once and fails to divide.

Two of these yeast genes are similar to ESTs from A. thaliana, and one is also similar to ESTs from human and C. elegans. The functions of the proteins corresponding to these ESTs are unknown, so these similarities do not suggest what the functions of the encoded yeast proteins may be. However, they do suggest that the products of these genes are involved in conserved, basic functions and are consistent with their playing a role in the fundamental process of cell morphogenesis.

We also identified six mutants with a disruption in FAB1, which encodes a protein that is similar to a human protein shown to have PI(4)P 5-kinase activity (YAMAMOTO et al. 1995). YAMAMOTO et al. (1995) report that a deletion or disruption of FAB1 causes the formation of apoid and binucleates (fab phenotype), enlarged vacuoles and defects in vacuole function, an aberrant cell shape, slow growth at 23° and death at 37°, and an osmoremedial phenotype. They do not report an enlarged-cell phenotype, but they observed fab1 phenotypes under different conditions, at 23° or 37° and after no more than 4 hrs at 37°.

In addition to identifying mutations that cause morphological phenotypes, the collection of 4025 mutants with haploid-lethal disruptions could also be screened under conditions that suppress otherwise lethal mutations (e.g., 1 M sorbitol to suppress osmoremedial phenotypes) to identify genes required for other cellular processes. In addition, it should be possible to transform a haploid yeast strain with the plasmid library of disruptions and screen the resulting mutants for those with defects in nonessential functions. Because each disruption mutation is marked by the transposable element, it is relatively simple to map the physical location and clone any mutated gene of interest. It may also be possible to use this library to clone the wild-type genes identified by recessive mutations that are difficult to clone by other methods. Using the mutagenized plasmid library, one could transform a diploid strain that is heterozygous for the mutation in the gene of interest and screen the transformants for those with defects in nonessential functions. Because each mutation is marked by the transposable element, one can quickly determine the DNA sequence adjacent to the disruption mutation and match this sequence to that of the originally mutated gene, and because this disruption is marked by the transposable element, it should then be straightforward to clone the gene by standard techniques.

Although much of the sequence of the yeast genome has been determined and nearly all of it is expected to be known by the end of 1996 (GOFFEAU 1994), little is known about the functions of many of the encoded gene products. Our collection of mutants with disruptions in essential DNA should facilitate the characterization of such functions. Because each mutation is marked by the transposable element, one can quickly determine the DNA sequence adjacent to the disruption mutation and match this sequence to that of the corresponding wild-type gene in the genomic DNA sequence database. As described above, the plasmid library of insertions in yeast genomic DNA could also be used to determine the functions of nonessential gene products. In these ways, we can begin to increase the pace of assigning functions to the proteins encoded by the yeast genome.
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