Constitutive pseudohyphal growth yeast mutants

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Constitutive pseudohyphal growth yeast mutants

Abstract
An isolated gene and mutations thereof capable of imparting constitutive pseudohyphal growth to S. cerevisiae is provided. The isolated wild type gene referred to as ELM1 is also capable of coding for a novel protein kinase that determines the yeast morphology and specific physiological properties.

Keywords
Biochemistry Biophysics and Molecular Biology

Disciplines
Biochemistry, Biophysics, and Structural Biology

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CONSTITUTIVE PSEUDOHYPHAL GROWTH YEAST MUTANTS

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Assignee: Iowa State University Research Foundation, Ames, Iowa; Institut Pasteur; Institut National de la Santé et de la Recherche Médicale, both of Paris, France

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U.S. Cl. 435/254.21; 435/172.3; 435/254.2

Field of Search 536/20.7; 435/172.3; 435/254.2, 254.21, 255.2

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(List continued on next page.)

Primary Examiner—Nancy T. Vogel
Attorney, Agent, or Firm—Muting, Raasch Gebhardt & Schwappach, P.A.

ABSTRACT

An isolated gene and mutations thereof capable of imparting constitutive pseudohyphal growth to *S. cerevisiae* is provided. The isolated wild type gene referred to as ELM1 is also capable of coding for a novel protein kinase that determines the yeast morphology and specific physiological properties.

13 Claims, 18 Drawing Sheets
OTHER PUBLICATIONS


Fig. 1a

A

Fig. 1b

B

Fig. 1c

C
Fig. 2a

A

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Fig. 2b

B

\(clm1::URA3\) -- URA3 -- 0.5 kb

\(ELM1\)

\(clm1::HIS3\) -- HIS3
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**Fig. 3d**
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EcoRI
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    ELPPFFGGENEFETYHKII

GTCATTAAAGGTTATTGTATAAAGACGTTACTTTACGCATAAGTATTCA
    VIKRLYKDBVTLRISIQ

ACAGTCAAATTTCATCGTCCAAGTGTAACCCCCGTAAAGAAAACGAAGGGCTCTG
    SQISSSSVNPVRNEGVP

CTCAGGAAAAAGGGAAAAGCAAGGTATTGGTATGCAGCAACTAGTAAGTAAC
    SGKGBKDKVLVSATSKVT

Fig. 3f
ACCTTCCATATCGAGGAAACCCTGATTTCTTCTGGCA

PSITHIDENSFPDKCFTS

GAGGAAGCCATTCAGGTGTTGCACTTTTATCTGTTGCTAA

EFQVTDFTCNSRN

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KTDRKVILSWP

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HindIII

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HindIII
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EcoRI
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NTYQDHDHTKTKEFPP

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Fig. 3h
Fig. 9

NΣDeim1/Δeim1  NΣ

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CONSTITUTIVE PSEUDOHYPHAL GROWTH YEAST MUTANTS

STATEMENT OF GOVERNMENT RIGHTS

The present invention was made with the support of the National Institute of Health under Contract No. SR29GM3925405. The Government has certain rights to this invention.

BACKGROUND OF THE INVENTION

Several fungal organisms are dimorphic, i.e., capable of existing in two forms. Such dimorphic fungi exhibit distinct morphologies in response to specific cellular signals. Typically, dimorphic fungi display either an egg-shaped, unicellular, yeast-like form, or a filamentous, mold-like form having attached and elongated cells. One example of such a dimorphism exists in the fungus Usitlagio maydis in which haploid sporiada exhibit a yeast-like morphology. Such haploid sporiada may fuse to form an elongated dikaryon filamentous form if they bear distinct alleles at both the a and b compatibility loci. In contrast to the unicellular form, the filamentous form of Usitlagio maydis, for example, causes corn smut.

A second well characterized example of dimorphism occurs in Candida albicans. This species of fungus exhibits a basic dimorphism between a budding yeast and a filamentous hyphal form. Several signals have been implicated in the switch between these two forms, including temperature, pH, nutrients, and exposure to serum factors. Mutants of C. albicans are known which are locked in either the yeast form or the hyphal form. Exploiting these observations for any useful purpose by classical genetic analysis is difficult, however. This is at least in part because C. albicans has only been observed as a diploid, and a sexual cycle has not been identified.

Saccharomyces cerevisiae (S. cerevisiae), also known as brewer’s yeast or baker’s yeast, is also a dimorphic species capable of displaying an egg-shaped yeast-like form and a filamentous mold-like form. Unfortunately, however, laboratory isolates of the fungus present a great variability in their ability to display this dimorphic characteristic. In this organism, nitrogen starvation in the presence of glucose is a natural inducer of the formation of the filamentous form, which is more appropriately termed pseudohyphal. See C. J. Gimeno et al., Cell, 68, 1078 (1992). Stimulation of a signal transduction pathway referred to as RAS2 facilitates this pseudohyphal response, i.e., the formation of a filamentous form.

S. cerevisiae pseudohyphal cells have an elongated morphology, and stay attached to each other presumably by their cell wall. Furthermore, a unipolar budding pattern occurs in which daughter cells bud, and bud, away from their mother cell in the great majority of the cell divisions. The result is a filamentous, mold-like structure growing away from the center of the colony. Of particular note is that the pseudohyphal form of S. cerevisiae forage deeply into agar media, possibly as a result of degrading polysaccharides into energy producing monosaccharides. Thus, the pseudohyphal form of S. cerevisiae could be used in the fermentation of complex polysaccharides for the production of ethanol, for example. Unfortunately, however, the wild type S. cerevisiae only undergoes the pseudohyphal response in near-starvation conditions. Exploiting these observations could lead to significant utility in commercial fermentation applications.

SUMMARY

The present invention provides a genetically modified S. cerevisiae yeast strain containing a constitutive pseudohyphal growth mutant gene, wherein the yeast strain exhibits constitutive pseudohyphal growth. Also provided is a constitutive pseudohyphal growth mutant gene capable of causing constitutive pseudohyphal growth on S. cerevisiae. Preferably, the constitutive pseudohyphal growth mutant gene is a deletion allele elm1::URA3, an insertion allele elm1::HIS3, or a nonsense allele elm1::R117. The present invention also provides an isolated DNA sequence capable of controlling pseudohyphal growth in S. cerevisiae. The isolated DNA sequence also codes for a Ser/Thr protein kinase, which is involved in the control of pseudohyphal growth.

The present invention also provides a method of identifying constitutive pseudohyphal growth mutant genes in a yeast strain comprising: mutating the yeast strain; visually identifying mutant yeast strains having elongated cells; breeding the mutant yeast strains into defined genetic backgrounds; forming a hybrid diploid strain using the mutant yeast strains having a defined genetic background; and examining the hybrid diploid strain for pseudohyphal growth characteristics. This method could be used in any of a variety of yeast strains, such as S. cerevisiae, Ustilago maydis, and C. albicans, for example. Preferably, the yeast strain is S. cerevisiae. Any known method can be used to mutate the yeast, i.e., treat the cells with a mutagenic agent. Preferably, the method used is a chemical or irradiative method. More preferably, it is a chemical method.

The present invention is also directed to a method of regulating cellular dimorphism through the use of constitutive pseudohyphal growth genes. In this way, the present invention can be used in controlling pathogenic transformation in fungi. The present invention is also directed to a method of cloning constitutive pseudohyphal growth genes using the foraging characteristic as a genetic marker.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Plasmid pA2 suppresses the phenotype caused by the constitutive pseudohyphal growth mutant gene elm1. Strains were cultured in SDC liquid medium supplemented according to the auxotrophic requirements. Cells were photographed while in exponential growth using a phase contrast microscope. (A): Strain ct104W1, bearing elm1-1. (B): Strain A2 which was obtained by transformation of ct104W1 with the suppressor plasmid pA2; uracil was omitted from the medium. (C): The A2 strain was cultured in liquid medium containing uracil, allowing plasmid loss. The culture was spread on a plate while still in the presence of uracil. About 5% of the isolated colonies displayed a mutant morphology. Furthermore, the wild type looking colonies were uracil independent, while the mutant colonies required uracil indicating they had lost the pA2 plasmid. A representative uracil dependent segregant is shown.

FIG. 2. Restriction Map of the ELM1 Region. (A): Delineation of a genomic region suppressing the elm1-1 phenotype. The restriction maps of inserts from several plasmids are aligned, and the suppressing ability of the corresponding plasmid is indicated. Restriction sites are shown for EcoRI, Psil, SalI, SbaI, BglII, and HindIII. Sites in parenthesis are located in the multiple cloning region of the vector. Plasmids pA1 to pA4 were selected from a genomic library, based on their ability to suppress the elm1-1 defect of strain ct104W1. Inserts in pE104/ST1 and pE104/ST3 are the 1.8-kb Psil-SacI fragment and the 1.4-kb HindIII-EcoRI fragment from pA2, respectively (SacI and HindIII are located in the multiple cloning region of the vector). (B) Map of ELM1 and disrupted alleles. The region
common to the inserts of pA1 to pA4 is shown in the middle diagram. The ELM1 coding sequence is marked by the solid arrow. The upper and lower diagrams show the structure of the deletion allele elm1::URA3 and the insertion allele elm1::HIS3.

FIG. 3. 3α-3β Nucleotide sequence (SEQ ID NO:1) of the ELM1 locus and predicted amino acid sequence (SEQ ID NO:1) of ELM1p (protein kinase). Only the coding region is shown. The coding region is translated below the nucleotide sequence. The location of several restriction enzyme recognition sites are indicated for comparison to FIG. 2.

FIG. 4. The suppressor gene resides at the ELM1 locus. The suppressor locus, presumably ELM1, had been tagged by the URA3 marker in wild type strain aWΩ (see Experimental Procedures). This strain was mated with the elm1-1 strain a04W1 and meiosis was induced in the resulting diploid. Four spores from a single tetrad were separated and allowed to germinate. The resulting haploid strains were respread on YPD plates, cultured for 16 hours, then photographed in situ using an inverted microscope. Ura+ requirement was also scored. This tetrad is representative of the thirty tetrads analyzed from this cross. (A) and (D): Wild type morphology, uracil independent. (B) and (C): Mutant morphology, uracil dependent. The mutant colonies also are representative of the original collection of mutants obtained by visual screen of the ura3-501 strain.

FIG. 5a–5b ELM1p is homologous to Ser/Thr protein kinases. The deduced amino acid sequence of ELM1p (SEQ ID NO:1) is aligned with that of the protein kinase Cds28p (SEQ ID NO:1) (disclosed in A. T. Locnir et al., Nature, 307, 183–185 (1984)) and the relevant domain of the bovine cAMP dependent protein kinase catalytic subunit, α form (cAPK) (SEQ ID NO:1) (disclosed in S. Shoji et al., Biochemistry, 22, 3702–3709 (1983)). Identical residues are boxed and gaps are represented by dashes. Residues nearly invariant among protein kinases (disclosed in S. K. Hanks et al., Science, 241, 42–51 (1988)) are indicated by stars.

FIG. 6. Phenotype caused by elm1 deficiency in inbred and hybrid diploid yeast strains. The null alleles elm1::URA3 or elm1::HO were introduced in various strains by homologous recombination, and diploids homozygous for elm1 deficiency were obtained by mating the appropriate strains. Cells were cultured for 16 hours on a YPD dish and photographed with a regular microscope equipped with Nomarski optics. (A): WWΔelm1 (W303 background). (B): NNΔelm1 (NY13 background); the insert shows the elm1 phenotype in the haploid strain aNAΔelm1 (NY13 background). (C): ΔΣΔelm1 (21278b background). (D): Hybrid NNΔelm1. (E): Hybrid WWΔelm1. (F): Hybrid WWΔelm1. Colony morphology also was recorded in situ using an inverted microscope. (G): Hybrid ΔΣΔelm1, which is also representative of ΔΣΔelm1, NNΔelm1, NNΔelm1 and NWΔelm1 observed under the same conditions. (H): Inbred WWΔelm1 displaying some enlarged, round cells.

FIG. 7. The mutant genes elm2 and elm3 cause constitutive pseudohyphal growth. Diploid strains homozygous for either elm2-1 (aαELM2) or elm3-1 (aαELM3) were cultured on a YPD plate for 16 hours, then photographed in situ using an inverted microscope or at higher magnification with a regular microscope equipped with Nomarski optics. (A) and (B): aαELM2. (C) and (D): aαELM3.

FIG. 8. Constitutive Pseudohyphal Forage Extensively in Agar Media. (A): Diploid strain homozygous for either elm1, elm2, or elm3, as well as two wild type control strains, were cultured for four days on a YPD plate, then photographed. Strains are 1) ΔW (wild type), 2) ΔWΔelm1, 3) NWΔelm, 4) aαELM2, 5) aαELM3, 6) NW (wild type). The elm1 strains are congruent with the wild type controls. (B): The plate was extensively washed under running tap water and photographed again. Cells invading the agar could not be washed off.

FIG. 9. Loss of ELM1 function causes constitutive pseudohyphal growth. Single cells of the indicated strains were isolated on a YPD plate using a micromanipulator, and incubated at 30°C. The developing colonies were photographed at the indicated times thereafter using an inverted microscope. ΔΣΔelm1/elm1 is homozygous for the deletion allele elm1::URA3, whereas ΔΣ is homozygous for the wild type allele ELM1; otherwise the two strains are genetically identical. Both strains are F1 hybrid diploids formed by mating haploids of the NY13 and 21278b backgrounds.

DETAILED DESCRIPTION OF THE INVENTION

Saccharomyces cerevisiae (S. cerevisiae) grows either as a unicellular, egg-shaped, yeast form or as a filamentous mold-like form, which is referred to as pseudohyphae. Although the yeast form usually prevails, pseudohyphal growth may occur during nitrogen starvation in the wild type S. cerevisiae strain. A general approach has been developed that allows for the isolation of genes involved in this dimorphic transition. An isolated wild type gene, referred to herein as ELM1 (Extended Morphology), is capable of coding for a novel protein kinase homolog, which is required for the yeast morphology.

The present invention is based on the discovery that deletion of the wild type gene ELM1 causes constitutive pseudohyphal morphology. Herein, “deletion” refers to the removal of the majority of the coding region of ELM1, or other forms of inactivation of ELM1 including: insertion of a foreign DNA sequence within its coding region; or changing a specific nucleotide sequence, such as converting the lysine codon at position 117 to an arginine codon. Furthermore, additional mutations of the wild type gene ELM1 and other specific genes, such as those referred to herein as elm1, elm2, and elm3, cause constitutive pseudohyphal growth. This is evidenced by mutant strains forming chains of connected and elongated cells that grow invasively into semisolid media, e.g., agar. It is believed that this occurs as a result of degradation of polysaccharides into energy-rich monosaccharides.

Thus, the present invention can be used in controlling pathogenic transformations in fungi. This is important in control of the prevalent human pathogen C. albicans, which can cause systemic infection when growing in the hyphal form. Such infections are frequent and life-threatening in immunosuppressed patients such as those with AIDS or undergoing chemotherapy treatment for cancer. Control of plant pathogens such as U. maydis also is possible, because prevention of the hyphal form precludes pathogenicity.

Furthermore, the present invention can be used to produce yeast that can degrade polysaccharides, feasibly even cellulose, in fermentation processes. Thus, for example, bulk ethanol could be prepared from corn silage or other agricultural plant byproducts using constitutive pseudohyphal S. cerevisiae strains. Such strains could also be used for production of alcoholic beverages using various cellulose sources as the substrate for fermentation.

Constitutive pseudohyphal growth mutant genes can be obtained by chemical mutagenesis of a wild type S. cerevisiae strain, e.g., the strain containing the wild type ELM1 gene. Cells are treated with the mutagenic agent, then
individual cells are separated on agar medium and allowed to form colonies. These are screened visually for the presence of elongated cells protruding from the body of the colony. Subsequent analysis of the mutant cells and their genetic properties can identify specific mutant genes that cause constitutive pseudohyphal growth.

The major characteristics imparted to yeast strains as a result of the incorporation of these mutant genes are as follows. Cells are elongated, growth occurs predominantly at the pole of the cell 180° opposite to its connection with its mother cell, and cell separation is delayed. This results in formation of expanded, branched chains of cells that grow outward from the center of a colony. These mutations are named generally elm (Elongated Morphology). Herein, a constitutive pseudohyphal growth mutant gene is referred to when this term is used in lower case letters. In contrast, the wild type gene is referred to when this term is used in upper case letters. Examples of three particularly effective mutant genes are referred to herein as elm1, elm2 and elm3.

A “constitutive pseudohyphal growth mutant gene” is used herein to refer to a gene that imparts filamentous pseudohyphal growth and polysaccharide degradation to a yeast strain in which the gene is incorporated. Preferably and advantageously the mutant genes impart constitutive pseudohyphal growth, including polysaccharide degradation, under substantially all yeast-growing conditions. Such a genetically modified yeast strain is referred to herein as a “constitutive pseudohyphal growth mutant yeast strain.”

In contrast to wild type S. cerevisiae, which only converts to the pseudohyphal form in near-starvation conditions, the genetically modified form described herein undergoes filamentous, mold-like growth to form elongated cells, and polysaccharide degradation under substantially all yeast-growing conditions. That is, the pseudohyphal mutant strains can degrade polysaccharides, as evidenced by their growing into agar as opposed to growing on the surface of agar, on nitrogen-rich media, on carbon-rich media, on liquid or solid media, etc., and under all temperatures capable of effecting yeast growth (typically about 15°–37° C). Although not intended to be limiting to the claims of the present invention, it is believed that the mutant S. cerevisiae strains described herein grow into agar media as a result of the excretion of a digestive enzyme capable of degrading polysaccharides, such as, for example, a glycohydrolase.

In addition to chemical mutagenesis of the wild type S. cerevisiae strain, a constitutive pseudohyphal growth mutant yeast strain can be obtained by incorporating a constitutive pseudohyphal growth mutant gene into an inbred diploid yeast strain or hybrid diploid yeast strain. Examples of inbred diploid yeast strains include, but are not limited to, ΣΣ and W303 (Table 1). Examples of hybrid diploid yeast strains include, but are not limited to, NW and ΣW (Table 1). Preferably, the mutant gene is incorporated into a hybrid diploid yeast strain. The use of hybrid diploid yeast strains imparts greater filamentous growth to the genetically altered yeast.

Strain-dependent variability in the morphology caused by the elm mutations is to be expected, considering that great variability in competence for natural pseudohyphal growth has been reported among S. cerevisiae laboratory isolated. See, for example, C. J. Gimeno, et al., Cell, 68, 1077–1090 (1992). Presumably, pseudohyphal growth is less efficient in several inbred genetic backgrounds (which are expected to be homozygous at all genetic loci), owing to specific defects in genes required for this differentiation state. These defects could become fixed in particular reference strains, since there is no selection against such mutations in the laboratory environment. Expression of the pseudohyphal state in such defective backgrounds, owing to an elm mutation, would then result in an aberrant phenotype composed of defective pseudohyphae. In hybrid diploids formed by crossing two independently maintained laboratory isolates, defects impairing pseudohyphal growth most likely are heterogeneous, leading to a behavior closer to normal. Loss of ELM1 function in three different inbred diploid backgrounds can lead to three different phenotypes, with various degrees of pseudohyphal growth. In contrast, ELM1 loss in three different hybrid diploid backgrounds causes identical phenotypes, which closely resemble healthy pseudohyphal growth. Similarly, the most demonstrative pseudohyphal phenotypes caused by elm2 or elm3 are observed in hybrid backgrounds.

Pseudohyphal growth has not been reported for haploid strains of S. cerevisiae. The original elm mutants, however, are obtained by mutagenesis of an haploid strain. Although the axial budding pattern of haploids is inappropriate for pseudohyphal growth, the elm mutations always cause cell elongation even in haploid strains, allowing identification of the mutants. Several haploid elm mutants also display a unipolar budding pattern typical of diploid pseudohyphae.

The invention has been described with reference to various specific and preferred embodiments and will be further described by reference to the following detailed examples. It is understood, however, that there are many extensions, variations, and modifications on the basic theme of the present invention beyond that shown in the examples and detailed description, which are within the spirit and scope of the present invention.

**EXPERIMENTAL PROCEDURES**

Strains, Media and Genetic Methods

Yeast strains used in this study are described in Table 1 and were cultured at 30° C. unless specified otherwise. The following media were used: YPD (1% yeast extract, 2% peptone, 2% glucose); YPAD (YPD supplemented with 40 mg/l adenine); SD (2% glucose, 0.7% yeast nitrogen base without amino acids, supplemented as required with uracil, tryptophan, histidine, lysine, methionine, uracil and adenine at 20 mg/l each); SDC (SD supplemented with 0.5% casamino acids in addition to the auxotroph requirements); sporulation medium (1% potassium acetate, 0.05% glucose, 0.1% yeast extract); SLAD (nitrogen starvation media described by C. J. Gimeno et al., Cell, 68, 1077–1090 (1992), which is incorporated herein by reference). Solid media for yeast contained 2% agar.

Standard genetic methods were used for complementation analysis, mating, and tetrad dissection as disclosed in F. Sherman et al., Methods in Yeast Genetics, Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory, (1980), which is incorporated herein by reference. In those instances where auxotrophic markers were not available for selection of a diploid from a cross, isolated colonies of potential diploids were selected based on their increased growth rate relative to the haploids parents. In all instances diploidy was verified by the ability of the selected strains to sporulate.
<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<td>NY13 Background:</td>
<td></td>
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<td>Mata ura3</td>
<td>B. Goud et al., Cell, 53, 753–768 (1988)</td>
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<td>NY180</td>
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<td>MAα/MATα ura3 ura3 ura3 leu2/leu2 his3/his3 trp1/trp1 ade2/ade2</td>
<td>J. Wallis, Cell, 58, 409–419 (1989)</td>
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<td>W303-1A</td>
<td>MAα ura3 leu2 his3 trp1 ade2</td>
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<td>W303-1B</td>
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<tr>
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<tr>
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<td>MAα ura3 leu2 his3 trp1 ade2</td>
<td>Segregant from WWΔel1IU/+</td>
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<td>W303-1AΔ2URA3</td>
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<td>Defined Hybrid Backgrounds:</td>
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<td>NWAel1</td>
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<tr>
<td>NWAel1</td>
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<tr>
<td>NWAel1</td>
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<tr>
<td>NWAel1</td>
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<tr>
<td>D273-10B/A1</td>
<td>MATα met6</td>
<td>A. Tzagoloff, FEBS lett., 65, 391–396 (1976)</td>
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<td>E104</td>
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<tr>
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<td>MATα ade2 leu2 met6 elm2-1</td>
<td>Segregant from E124 × W303-1A</td>
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TABLE 1—continued

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<td>a124W1b × α23422</td>
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<td>El30</td>
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<td>Mutagenesis of D273-10B/A1</td>
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<td>MAα/MATAα ade2+ leu2+ met5+ try+ lys2+ elm3-1(elm3-1)</td>
<td>a130W1b × α13022</td>
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ELM1 Gene Isolation

Genes capable of restoring normal appearance, i.e., a normal morphology, to an elm1-1 mutant strain were selected from a yeast genomic library obtained from François Lacroute (Centre de Genetique Moleculaire du CNRS, Git-sur-Yvette, France). The library, which was stored in the plasmid vector pUC19, contains as insert the URA3 selectable marker as well as a centromeric sequence ensuring maintenance at high copy number in yeast. The genomic inserts were obtained by partial Sau3AI digestion of yeast DNA from the wild type S. cerevisiae, and were ligated to the BamHI site of pFL38.

The α1-1 mutant strain (α104W1) was cultured in 100 ml of YPAD medium and transformed with 50 μg of plasmid library DNA using a scaled up version of the lithium transformation procedure, as described in F. Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, NY (1989), which is incorporated herein by reference. Immediately after transformation, the cells were resuspended in 6 ml of 10 mM Tris-HCl pH 7.5, 1 mM EDTA (TE) buffer. Two ml of the cell suspension were added to each of three tubes containing 15 ml of the liquid medium SDC supplemented with histidine, leucine, tryptophan and adenine, but lacking uracil. The total number of uracil-independent transformants, 8×10^4, was estimated from a small aliquot of the TE suspension spread directly on selective dishes. The liquid cultures were incubated at 30°C with gentle shaking for three days. An aliquot of each saturated culture (5 μl) was inoculated into 5 ml of fresh SDC medium which was again grown to saturation. The dilution procedure was repeated several times in a row, every three or four days. At various times, samples from saturated liquid cultures were also spread on selective plates and morphology of individual colonies was scored. More than 50% of the colonies from the second or third cycle of liquid cultures displayed wild type morphology. Isolated wild type colonies were selected for further analysis. As a control, the α104W1 strain was also transformed with the pFL38 vector devoid of insert. These control cells never reverted to wild type, even after five cycles of liquid cultures.

DNA Manipulations and Allele Construction

DNA manipulations were performed by standard procedures as described in F. M. Ausubel, Current Protocols in Molecular Biology, New York: Greene Publishing Associates and Wiley-Interscience (1989), and J. Sambrook et al., Molecular Cloning, A Laboratory Animal, Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory (1989), which are incorporated herein by reference. Plasmid pUC118E is a modified version of pUC118 in which the multiple cloning site was replaced by a unique EcoRI site. See J. Vicira et al., Methods Enzymol., 153, 3–11 (1987), which is incorporated herein by reference. In each instance where a strain was constructed by gene replacement, Southern analysis (as disclosed in E. Southern, J. Mol. Biol., 98, 503–517 (1975), which is incorporated herein by reference) of the transformant was performed to confirm that integration by homologous recombination had occurred as expected.

The insertion allele elm1::HIS3 was constructed as follows. The 1.4 kb EcoRI fragment of the genomic insert in pA1 was subcloned in plasmid pUC118E, resulting in plasmid pELM1/ST13. The yeast HIS3 gene (which is disclosed in K. Struhl, Nucleic Acid Res., 13, 8587–8601 (1985), incorporated herein by reference) was available as a 1.7 kb genomic BamHI fragment cloned in pUC118, in the orientation such that the 1.2 kb Psil fragment containing the entire HIS3 promoter and coding region could be excised. This fragment was subcloned at the unique Psil site of pELM1/ST13, present at ELM1 codon 94, forming pELM1::HIS3. A 2.6 kb EcoRI fragment from pELM1::HIS3 was used for transformation of the his3his3 diploid strain W303 to histidine prototrophy.

The null allele elm1::URA3 was prepared as follows. The 2.8 kb genomic insert of pA1 was excised as a SacI-SalI fragment, and subcloned in pBLUESCRIPT SK+ (Stratagene Cloning System, La Jolla, Calif.). The resultant plasmid, pELM1/ST16, was digested at the unique Psil and BglII sites, removing ELM1 codons 94 to 487. A 1.2 kb HindIII fragment of yeast DNA bearing the URA3 gene (disclosed in M. D. Rose, et al., Gene, 29, 113–124 (1984), which is incorporated herein by reference) was inserted in pELM1/ST16 in place of the deleted sequence. The resulting plasmid, pELM1::URA3, was digested with XbaI, generating a 2.9 kb fragment used for DNA transformation of various ura3 strains to uracil prototrophy.

The chromosomal ELM1 locus was tagged with a genetic marker as follows. The insert of the suppressing plasmid pA2 was ligated into the integrative plasmid Yip352 (disclosed in J. E. Hill et al., Yeast, 2, 163–167 (1986), which is incorporated herein by reference) as a 2.4 kb BamHI-Sacl fragment. The resulting plasmid, pELM1::URA3, was linearized by digestion at its unique BglII site located within ELM1. The linearized plasmid was used to transform wild type strain W303-1A to uracil prototrophy. This type of integration results in duplication of ELM1, both copies being functional and separated from each other by the Yip352 linear plasmid which bears the URA3 marker.

The null allele cdc55::LEU2-2, similar to the cdc55::LEU2 allele described by A. M. Healy et al., Mol.
null
et al., Mol. Cell. Biol. 11, 5767–5780 (1971)). ELM1 was shown to be different from any of these CDC genes by its unique nucleotide sequence (see below).

**TABLE 2**

<table>
<thead>
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<th>MATa</th>
<th>MATa parent</th>
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<tr>
<td></td>
<td>104DS</td>
</tr>
<tr>
<td>E104</td>
<td>–</td>
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<tr>
<td>E105</td>
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<tr>
<td>E102</td>
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<td>E130</td>
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<td>E156</td>
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</table>

*The indicated strains were mated and diploids were selected based on complementing auxotrophs. “+” indicates the diploid had a mutant morphologic phenotype, and “−” indicates the diploid had wild type or near-wild-type appearance.*

*parents are progeny of the fifth backcross to E273-10B/A1. The original mutants were outcrossed to W303-1A prior to the backcrosses. These strains all contain a leu2 auxotrophic marker; 102DS also contains a his3 marker.*

Cloning of ELM1

The wild type ELM1 gene was selected from a genomic library based on its ability to complement the growth defect caused by elf1-1 in strain α104W1. This strain bears the ura3 marker allowing selection of URA3 plasmids. The elf1-1 mutation of α104W1 causes elongated morphology, chlumpiness, and a reduced growth rate (Fig. 1A). When DNA was stained with DAPI, a few very elongated cells seemed to bear several nuclei, suggesting that cytokinesis was impaired (data not shown). Cell viability was high despite the severely abnormal appearance of α104W1, and the phenotype was stable when the strain was maintained routinely on stock plates. A yeast genomic library based in the centromeric (low copy number) plasmid pFL38 was introduced into the α104W1 cells, and transformants were isolated en masse in liquid medium lacking uracil. Absence of uracil from the medium maintained a selection for transforming plasmids, and growth in liquid culture presumably would allow relatively rapidly growing revertants to overtake cells still suffering from the reduced doubling time associated with the mutant phenotype. Indeed, after about 30 generations, the majority of cells in the liquid cultures displayed wild type morphology. Liquid cultures were spread on agar medium lacking uracil, and four apparently reverted yeast colonies, named A1 to A4, were further characterized (Fig. 1B shows clone A2).

The reverted phenotype was caused by the plasmids because plasmid loss during mitosis resulted in reappearance of the mutant phenotype. Fig. 1C shows a derivative of clone A2 which presumably lost its plasmid. The four plasmids present in the reverted yeast clones were recovered, produced in E. coli, and named pA1 to pA4. Restriction enzyme mapping of the plasmids showed they all contain distinct genomic inserts that share an overlapping 2.1 kb sequence (Fig. 2). This common region was entirely sequenced and a single long open reading frame was observed, covering 1689 bp. Reintroducing plasmids bearing the entirety of this open reading frame into the elf1-1 strain α104W1 restored wild type morphology. Subclones containing only parts of the coding region, however, failed to restore wild type morphology (Fig. 2). Thus, this open reading frame corresponds to a yeast gene capable of suppressing the elf1-1 phenotype.

The “next door insertion” strategy, which is disclosed in R. Rothstein, Meth. Enzymol., 194, 281–301 (1991), and incorporated herein by reference, indicated the cloned suppressor gene is the wild type allele of ELM1. The insert of pA2 was subcloned in the integrative plasmid YIp352. The entire plasmid was then integrated in the genome of W303-1A by homologous recombination near the suppressing locus. Thus, in the resulting strain αW2 the suppressing locus is tagged by the URA3 marker. As expected, this strain displays wild type cell and colony morphology. The elf1-1 strain α104W1 was mated to αW2 and meiosis was induced in the resulting heterozygous diploid. Thirty tetrads were dissected, and in every cases, two spore-derived colonies had a wild type morphology and were uracil independent, whereas two spore-derived colonies displayed obvious morphological abnormalities and were uracil dependent (Fig. 3, 3a–3i). Thus, the cloned suppressor gene marked by URA3 and the mutation elf1-1 reside at the same genetic locus.

ELM1 Codes for a Putative Novel Protein Kinase

The nucleotide sequence of ELM1 (Fig. 3, 3a–3i) (SEQ ID NO:1) revealed an open reading frame coding for 563 amino acid residues (Fig. 5, 5a–5b) (SEQ ID NO:2, 3, 4). The predicted protein (ELM1p) sequence was used in a computer assisted search for related proteins. No close relative was detected, but significant homology was observed with several protein kinases. When ELM1p was compared with the available sequences of protein kinases, it appeared roughly equally diverged from all Ser/Thr kinases. Fig. 5, 5a–5b shows, as an example, ELM1p aligned with the CDC28 gene product Cdc28p (also known as p34 or histone kinase) and the catalytical region from the bovine cAMP dependent protein kinase CAPK. In this comparison, ELM1p is 23.7% identical to Cdc28p and 23.1% identical to CAPK, while these two reference sequences are 23.1% identical to each other. High conservation is observed in particular regions. For example, from residue 245 to 280, ELM1p is more than 45% identical to either Cdc28p or Cdc28p. In addition, the 15 invariant residues found in almost every protein kinases are also conserved in ELM1p. Thus, ELM1p bears a protein kinase catalytic domain, spanning approximately residues 90 to 400. The amino and carboxy terminal regions of ELM1p, where no significant homology has been detected, may provide regulatory functions. Two subdomains have been described in protein kinases, that display different consensus sequences in enzymes specific for either tyrosine or serine/threonine. See, S. K. Hanks et al., Science, 241, 42–51 (1988). At this first subdomain (residues 259–264), ELM1p bears DIKPSN (SEQ ID NO:5) which fits best the Ser/Thr kinase consensus DLKPEN (SEQ ID NO:6) as opposed to the tyrosine kinase signature sequence DLARN (SEQ ID NO:8) or DLRAN (SEQ ID NO:7). Likewise, at the second subdomain (residues 309–317), the ELM1p sequence GTPAFAPE (SEQ ID NO:9) match the consensus G-TS-X-X-F-Y-X-A-P-E (SEQ ID NO:10) for Ser/Thr specificity and is diverged from the tyrosine kinase consensus P-J-V-W-T-M-A-P-E (SEQ ID NO:110). Thus, ELM1p defines a novel branch in the Ser/Thr protein kinase family.

Inactivation of ELM1 Causes a Pseudohyphal Morphology

The W303 outcross progeny from the original E104 mutant showed an unusual variability in the severity of the elf1-1 phenotype (data not shown), even though a single mutation was known to cause the morphological defect. This suggested that the genetic background influences the elf1-1 phenoytye. To test this hypothesis, ELM1 was inactivated directly in several laboratory strains using the gene replacement technique disclosed in R. Rothstein, Meth. Enzymol., 194, 281–301 (1991), which is incorporated herein by
reference, and the phenotypes were compared. Two different disrupted alleles were constructed, namely elml1:URA3 and elml1:HS3, and were integrated by homologous recombination at the ELM1 locus of various strains. In the elml1:URA3 allele most of the ELM1 coding sequence is replaced by the URA3 gene, while ELM1 coding sequence is disrupted by HS3 in the elml1:HS3 allele (see Fig. 2). Replacement of ELM1 by either construct caused the same elongated morphology phenotype in haploid strains of the W303 background. Furthermore, diploids formed by mating elml1-1:URA3 or elml1-1:HS3 strains also displayed the mutant phenotype, confirming that the disruptions of ELM1 are allelic with elml1-1 (data not shown).

Inbred diploid strains deficient for ELM1 were obtained in the W303, NY13 and Σ12788 backgrounds (respectively WWΔelm1, NNΔelm1 and ΣΔelm1). All three strains presented elongated cells attached to each other, reminiscent of the elml1-1 phenotype (Fig. 6A, B, C). Strain-specific particularities were observed, however, confirming the elml1 phenotype is dependent at least in part upon the genetic background. In WWΔelm1, growth was slow, cell shape was irregular, cytokinesis was seemingly impaired and some round, enlarged cells were present (Fig. 6A, H). In contrast, NNΔelm1 strain displayed cells very regular in their elongated shape. Neither cytokinesis defects nor enlarged round cells were seen and the growth rate on plates was not significantly reduced when compared to a congeneric wild type strain. During exponential growth in liquid YPD medium, NNΔelm1 cells stayed attached presumably by their cell wall (Fig. 6B). This morphology, and in particular the budding pattern of NNΔelm1 resembles the recently described pseudoalgalae of S. cerevisiae; chains of elongated cells which stay attached to each other, where daughter cells bud opposite to their mother, while mother cells rebud near their daughter. The result is an expanded, highly branched, mold-like structure as disclosed in C. J. Gimeno, et al., Cell, 68, 1077–1090 (1992). In the haploid background of NY13 the phenotype caused by elml1 deletion was similar except for the budding pattern which was axial, as expected for haploid cells. See, for example, D. Freifelder, J. Bacteriol., 80, 567–568 (1960), and J. Chant et al., Cell, 65, 1203–1212 (1991). In this instance the elongated cells always formed buds near their mother. This resulted in small, star like clumps where each branch is composed of a single elongated cell (Fig. 6B insert). The diploid ΣΔelm1 strain had a phenotype close to NNΔelm1, except for less uniformity in the shape of individual cells (Fig. 6C).

The elml1 phenotype was also analyzed in the three hybrid diploid strains (NNΔelm1, WWΔelm1 and NWΔelm1) obtained by pairwise mating of haploid elml1 strains in the W303, NY13, and Σ12788 backgrounds. The phenotype of these three mutant strains was virtually identical, and resembles mostly that of NNΔelm1. Cell shape was very regular, and defective cytokinesis was not observed. Time-course examination of single cells on a plate for several generations showed the doubling time of ΝΔelm1 and WWΔelm1 to be approximately 1.5 hours, the same as congeneric ELM1/ELM1 strains (data not shown). Cell elongation in the hybrids, however, was not as extreme as in the inbred NNΔelm1 strain. The pseudoalgalae budding pattern and formation of branched structures were particularly obvious in all three elml1/elml1 hybrid diploids (Fig. 6D, E, F, G). The phenotype depicted by the hybrids, most likely represents the actual elml1 phenotype, while the inbreds probably bear some genetic defects responsible for their more or less aberrant phenotype.

celm2 and elml3 Also Cause Constitutive Pseudohyphal Growth

Many other elongated mutants obtained by mutagenesis of D273-10B/A1 behaved similarly to elml1 strains in the respect that the W303-1A outcross progeny displayed variable phenotypes, including some with pseudoalgalae morphology. Strains containing elm2 or elml3 mutations were characterized further in this regard. A diploid homozygous for elml2-1, DW2Δelm2-1/elm2-1, was formed in a largely hybrid background by mating a progeny clone from the outcross of the original mutant to W303-1A with one from the second backcross to Σ12788 (Table 1). Pseudoalgalae characteristics including cell elongation, cell attachment (after sonication), and formation of expanded, branched chains of cells all were obvious in DW2Δelm2-1/elm2-1 (FIG. 7), and the form of these cells was very similar to that of elml1 deletion mutants in F1 hybrid diploid backgrounds. Growth of single cell clones of the elml2 mutant DΔelm2-2/elm2-2 was observed over time on solid YPD medium. This strain exhibited the typical extended, branched chains characteristic of pseudoalgalae growth. Direct observation of clonal development showed the doubling time of DΔelm2-2/elm2-2 to be essentially the same as the congeneric wild type strain DD (data not shown). The same analysis was applied to the elml3 mutant DΔelm3-3/elml3-1 with similar results, except for more variability in cell length (data not shown).

Thus, mutations in ELM1, ELM2 or ELM3 all cause a dimorphic transition leading to a nearly identical constitutive pseudoalgalae growth phenotype.

celm1, elml2 and elml3 Mutants Grow Invasively in Agar Media

A distinctive property of the previously described pseudoalgalae form of S. cerevisiae is the ability to grow invasively under the surface of an agar medium, referred to herein as “foraging”. The foraging capacity of elml1, elml2, and elml3 strains was examined by culturing patches of cells for several days on YPD plates, then scrubbing the surface of the agar with a finger under running tap water to remove the cells from the plate’s surface. Haploid elml2-1, elml2-2, or elml3-1 strains in the D273-10B background could not be washed from the plate, whereas the congeneric wild type control strain was completely removed (FIG. 8). Observation with an inverted microscope showed most of the cells remaining after washing were located completely under the agar surface, with chains extending up to 5 cell lengths into the medium (data not shown). The ability to forage results from the elml2 or elml3 mutation, because this property consistently co-segregated with the cell elongation phenotype in at least 12 complete tetrads derived from elml2-2/ELM2 or elml3-1/ELM3 heterozygous diploids (data not shown). In the haploid D273-10B background elml1-1 and elml2-2 mutants also foraged, although to a lesser extent than the congeneric elml2 or elml3 strains (data not shown). WWΔelm1/Δelm1 and NWΔelm1/Δelm1 also exhibited obvious foraging behavior, whereas the congeneric wild type control strains were completely or nearly completely removed from the YPD plate by the washing procedure (FIG. 8).

ELM2 and ELM3 Function Affects Pseudoalgalae Differentiation in Response to Nitrogen Starvation.

The comprehensive phenotypic resemblance of the pseudoalgalae morphologies caused either by elml1, elml2, or elml3 mutations, and nitrogen starvation of wild type cells, suggested the mutations result in constitutive execution of the differentiation pathway that normally is triggered by nutrient availability. To test this hypothesis the effects of
ELM1, ELM2, and ELM3 gene dosage on the ability of a strain to form pseudohyphae in response to nitrogen starvation were examined. Congenic diploid strains were constructed in the D273-10B background that contained either one or two functional copies of each gene to be examined. All strains displayed typical yeast-like morphology in nitrogen-rich media such as YPD or SD. On the nitrogen starvation medium SLAHD the homozygous wild type strain DD failed to display pseudohyphal growth even after 14 days on SLAHD medium, which is typical of most inbred laboratory strains. In contrast, pseudohyphal differentiation was obvious in the congeneric strains DDelm2-2-1/4 and DDelm3-1/4 after three days on SLAHD medium. Morphologic differentiation of these two strains is dependent on the nutritional environment, because pseudohyphal cells transferred from an SLAHD plate to the nitrogen-rich medium YPD produced clones with typical yeast-like morphology; these clones again differentiated into pseudohyphae when they were replated on SLAHD. Thus, function of both ELM2 and ELM3 significantly affects the ability to flip a developmental switch in response to nitrogen starvation. This gene dosage effect was not observed for ELM1 in strain DDelm1-1/4.

A representative example of *Saccharomyces cerevisiae* E104: MA4 x elm1-1, was deposited on Sep. 9, 1996 with the American Type Culture Collection, Rockville, Md., 20852, and is available in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the purpose of Patent Procedure. This deposit has been assigned ATCC No. 74388.

The disclosures of all patents, patent applications, patent documents, and publications cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

**SEQUENCE LISTING**

(1) GENERAL INFORMATION:

(1.1) NUMBER OF SEQUENCES: 11

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 2055 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(x) SEQUENCE DESCRIPTION: SEQ ID NO:1

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(A) LENGTH: 563 amino acids
(B) TYPE: protein
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

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(2) INFORMATION FOR SEQ ID NO:4:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(i) MOLECULE TYPE: protein

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Asn Thr Ala His Leu Asp Glu Phe Arg Ile Lys Thr Leu Gly Thr 1 5 10 15
Gly Ser Phe Gly Arg Val Met Leu Val Lys His Met Glu Thr Gly Asn 20 25 30
His Tyr Ala Met Lys Ile Leu Asp Lys Gln Lys Val Val Lys Leu Lys 35 40 45
Gln Ile Gln His Thr Leu Asn Glu Lys Arg Ile Leu Gin Ala Val Asn 50 55 60
Phe Pro Phe Leu Val Lys Leu Glu Phe Ser Phe Lys Asp Asn Ser Asn 65 70 75 80
Leu Tyr Met Val Met Gln Tyr Val Pro Gly Gly Glu Met Phe Ser His 85 90 95
Leu Arg Arg Ile Gln Arg Phe Ser Glu Pro His Ala Arg Phe Tyr Ala 100 105 110
Val Gin Ile Val Leu Thr Phe Gin Tyr Leu His Ser Leu Asp Leu Ile 115 120 125
Tyr Arg Asp Leu Lys Pro Gin Asn Leu Leu Ile Asp Gin Gin Gly Tyr 130 135 140
Ile Gin Val Thr Asp Phe Gly Phe Ala Lys Arg Val Lys Gly Arg Thr 145 150 155 160
Trp Thr Leu Cys Gly Thr Pro Gin Tyr Leu Ala Pro Gin Ile Leu 165 170 175
Ser Lys Gin Tyr Asn Lys Ala Val Asp Trp Trp Ala Leu Gly Val Leu 180 185 190
Ile Tyr Gin Met Ala Ala Gly Tyr Pro Pro Phe Phe Ala Asp Gin Pro 195 200 205
Ile Gin Ile Tyr Gin Ile Val Ser Gly Lys Val Arg Phe Pro Ser 210 215 220
His Phe Ser Ser Asp Leu Lys Asp Leu Leu Arg Asn Leu Leu Gin Val 225 230 235 240
Asp Leu Thr Lys Arg Phe Gly Asn Leu Lys Asp Gly Val Asn Asp Ile 245 250 255
Lys Asn His Lys 260

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid

(i) MOLECULE TYPE: protein

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Asp Ile Lys Pro Ser Asn 1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid

(i) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Leu Lys Pro Glu Asn

1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Leu Ala Ala Arg Asn

1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Leu Arg Ala Ala Asn

1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Thr Pro Ala Phe Ile Ala Pro Glu

1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Xaa Xaa Xaa Xaa Xaa Xaa Ala Pro Glu

1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Xaa Trp Xaa Ala Pro Glu

1 5
What is claimed is:

1. A genetically modified *S. cerevisiae* yeast strain containing a ELM1 mutant gene in a genetic background selected from the group consisting of W303, NY13, \( \Sigma 1278b \), and combinations thereof.

2. The genetically modified *S. cerevisiae* yeast strain of claim 1 wherein the ELM1 mutant gene is a deletion allele.

3. The genetically modified *S. cerevisiae* yeast strain of claim 2 wherein the deletion allele is \( \text{elm} 1::HIS3 \).

4. The genetically modified *S. cerevisiae* yeast strain of claim 1 wherein the ELM1 mutant gene is an insertion allele.

5. The genetically modified *S. cerevisiae* yeast strain of claim 4 wherein the insertion allele is \( \text{elm} 1::HIS3 \).

6. The genetically modified *S. cerevisiae* yeast strain of claim 1 wherein the ELM1 mutant gene is a missense allele.

7. The genetically modified *S. cerevisiae* yeast strain of claim 6 wherein the missense allele is \( \text{elm} 1::R117 \).

8. The genetically modified *S. cerevisiae* yeast strain of claim 1 which is a diploid yeast strain.

9. The genetically modified *S. cerevisiae* yeast strain of claim 8 wherein the diploid yeast strain is a hybrid diploid yeast strain.

10. The genetically modified *S. cerevisiae* yeast strain of claim 9 wherein the hybrid diploid yeast strain is selected from the group consisting of NW\( \Delta \text{elm} 1 \), \( \Sigma W\Delta \text{elm} 1 \), and NW\( \Delta \text{elm} 1 \).

11. The genetically modified *S. cerevisiae* yeast strain of claim 1 wherein the diploid yeast strain is an inbred diploid yeast strain.

12. The genetically modified *S. cerevisiae* yeast strain of claim 11 wherein the inbred diploid yeast strain is selected from the group consisting of WW\( \Delta \text{elm} 1 \), \( \Sigma \Sigma \Delta \text{elm} 1 \), and NW\( \Delta \text{elm} 1 \).

13. A genetically modified *S. cerevisiae* yeast strain selected from the group consisting of NW\( \Delta \text{elm} 1 \), \( \Sigma W\Delta \text{elm} 1 \), and NW\( \Delta \text{elm} 1 \), WW\( \Delta \text{elm} 1 \), \( \Sigma \Sigma \Delta \text{elm} 1 \), NW\( \Delta \text{elm} 1 \), a/\( \alpha \text{elm} 2 \), and a/\( \alpha \text{elm} 3 \).

* * * * *
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page.
Item "[21] Applt. No.", delete "61,636", and insert -- 08/061,636 --;

Column 9.
Line 31, delete "("

Claim 3.
Line 2, delete "HIS3", and insert -- URA3 --; and

Claim 11.
Line 1, delete "1", and insert -- 8 --.

Signed and Sealed this
Fourteenth Day of August, 2001

Attest:

Nicholas P. Godici

Attesting Officer
Acting Director of the United States Patent and Trademark Office