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Cloning and Characterization of *ERG8*, an Essential Gene of *Saccharomyces cerevisiae* That Encodes Phosphomevalonate Kinase

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***Saccharomyces cerevisiae* strains that contain the *erg8-1* mutation are temperature sensitive for growth due to a defect in phosphomevalonate kinase, an enzyme of isoprene and ergosterol biosynthesis. A plasmid bearing the yeast *ERG8* gene was isolated from a YCp50 genomic library by functional complementation of the *erg8-1* mutant strain. Genetic analysis demonstrated that integrated copies of an *ERG8* plasmid mapped to the *erg8* locus, confirming the identity of this clone. Southern analysis showed that *ERG8* was a single-copy gene. Subcloning and DNA sequencing defined the functional *ERG8* regulon as an 850-bp upstream region and an adjacent 1,272-bp open reading frame. The deduced 424-amino-acid *ERG8* protein showed no homology to known proteins except within a putative ATP-binding domain present in many kinases. Disruption of the chromosomal *ERG8* coding region by integration of *URA3* or *HIS3* marker fragments was lethal in haploid cells, indicating that this gene is essential. Expression of the *ERG8* gene in *S. cerevisiae* from the galactose-inducible galactokinase (*GAL1*) promoter resulted in 1,000-fold-elevated levels of phosphomevalonate kinase enzyme activity. Overproduction of a soluble protein with the predicted 48-kDa size for phosphomevalonate kinase was also observed in the yeast cells.**

Phosphomevalonate kinase [ATP:(*R*)-5-phosphomevalonate phosphotransferase; EC 2.7.4.2] catalyzes the pyrophosphorylation of mevalonate in the second of three steps leading to the formation of isopentenyl diphosphate: (3*R*)-phosphomevalonate + ATP → (3*R*)-pyrophosphomevalonate + ADP. Although first identified as a cytoplasmic enzyme in *Saccharomyces cerevisiae*, phosphomevalonate kinase has been isolated from a variety of plant and animal sources (10, 24, 33, 50). This enzyme functions in the elaboration of isoprene subunits, which are used for the synthesis of a variety of essential compounds including sterols, dolichols, and ubiquinones. Isoprene-derived molecules are also used in some species for the covalent modification of tRNAs (17) and specific proteins, including ras and α-factor in *S. cerevisiae* (1, 27, 46). Phosphomevalonate kinase from pig liver has been purified to homogeneity and shown to be a monomeric protein with a molecular mass of 22 kDa (6, 32). It exhibits strong nucleotide specificity for ATP and utilizes Mg^{2+} as a preferred cofactor. Chemical modification studies have suggested that this enzyme requires both cysteine and lysine residues for full activity (7, 54), but little is known about the structure of the enzyme or its active site.

A large number of temperature-conditional *S. cerevisiae* mutants defective in ergosterol biosynthesis were isolated by Karst et. al (29, 48). These *erg* mutants required ergosterol supplementation for growth at 25°C but were inviable at 36°C even with supplementation. Mutant strains designated *erg8-1*, *-2*, and *-3* were found to be defective in phosphomevalonate kinase by labeling experiments (29). Whole cells failed to synthesize ergosterol from acetate, and extracts from *erg8* cells were unable to convert mevalonate to mevalonate-pyrophosphate in vitro. Genetic analysis showed that *erg8* mutations were recessive to the wild-type *ERG8* allele in heterozygous diploids. In addition, these experiments demonstrated that the thermosensitive and biochemical defects of *erg8* strains cosegregated, suggesting that both traits were the result of a single genetic defect. *erg8* strains were recently used to explore the regulation of ergosterol metabolism in *S. cerevisiae* (16, 47), but no detailed characterization of the yeast gene for phosphomevalonate kinase has been reported.

In mammalian cells, synthesis of sterols and isoprenoid compounds is regulated primarily through control of two key enzymes of mevalonate formation, HMG coenzyme A (CoA) synthase and HMG CoA reductase (15). The analogous yeast biosynthetic pathway may also be regulated at these steps (12, 47, 53). Some evidence suggests that in both yeast and mammalian cells, significant pathway regulation may be mediated through additional enzymes of sterol biosynthesis, including mevalonate kinase and squalene synthetase (18, 20, 52). To investigate these and other potential secondary control points in the pathway, we have initiated a molecular analysis of several of the *S. cerevisiae* enzymes of early sterol biosynthesis. In this report we describe the cloning of the yeast *ERG8* gene for phosphomevalonate kinase by using an *erg8-1* mutant complementation approach and the characterization of this gene by DNA sequencing and other analyses. We show that *ERG8* is a single-copy essential yeast gene that encodes a protein twice the size of the previously reported mammalian enzyme.

MATERIALS AND METHODS

Materials. D-Sorbitol, β-glucuronidase, D-galactose, Tween 80, and polyethylene glycol 4000 were obtained from Sigma Chemical Co. Glass milk (GeneClean) was from Bio101, LaJolla, Calif., and BioTrans membranes were from ICN Biomedicals, Inc., Irvine, Calif. Sequenase (modified T7 DNA polymerase) was from U.S. Biochemical Corp., Cleveland, Ohio. [α-^{32}P]dCTP, [α-^{35}S]dATP, and all ^{14}C-labeled sterol precursors were from Amersham Corp. 5-Fluoroorotic acid was from PCR, Inc., Gainsville, Fla., and silica gel LK6D thin-layer chromatography plates were from Whatman. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyrano-

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TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source
SP1	*MATα erg8-1*	F. Karst
AG8-6B	*MATa erg8-1 his3-11,15 leu2-3,112 trp1-1*	This study
AG8-2A	*MATa erg8-1 his3-11,15 ura3-1 leu2-3,112 ade2-1*	This study
ET7-6B	*MATα erg8-1 upc1 his3-11,15 leu2-3,112 ura1*	This study
ET15-6D	*MATa erg8-1 upc1 his3-11,15 leu2-3,112 trp1-1 ura3*	This study
ET20-2A	*MATa erg8-1 ura3-52 lys2-801*	This study
W303-1A	*MATa ERG8 ura3-1 his3-11,15 leu2-3,112 trp1-1 ade2-1 can1-100*	R. Rothstein
W303-1B	*MATα ERG8 ura3-1 his3-11,15 leu2-3,112 trp1-1 ade2-1 can1-100*	R. Rothstein
JT35-1A	*MATα ERG8 upc1 ura3-1 his3-11,15 trp1-1 ade2-1*	J. Tkacz
JT35-6B	*MATα ERG8 upc1 ura1 his3 leu2-3,112 trp1-1*	J. Tkacz
JT42-5B	*MATα ERG8 ura3-1 LYS2*	J. Tkacz
Y294	*MATa ERG8 his3d ura3-52 leu2-3,112 trp1-1 GAL*	M. Haffey
YM197	*MATa/MATα ERG8/ERG8 ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2*	J. Rine
ET16	*MATa/MATα ERG8/erg8-1 ura3-1/ura3-1 his3-11,15/his3-11,15 ade2-1/ade2-1 leu2-3,112/leu2-3,112 TRP1/trp1-1*	This study

side was from Bethesda Research Labs, Inc., Gaithersburg, Md. Reagents for the spectrophotometric assay of phosphomevalonate kinase were all obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind., except for *R,S*-5-phosphomevalonate, which was kindly provided by L. Parker, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, N.J.

Strains and genomic libraries. The *S. cerevisiae* strains used in this study are described in Table 1 and were all derivatives of strain S288C. The *Escherichia coli* strains used for cloning and plasmid propagation were RR1 [F$^-$ *pro leu thi lacY* Str^r *hsdR* (r_K^- m_K^-) *hsdM endo1*], DH5α [F$^-$ *supE44 thi-1 recA1 gyrA96 relA1 hsdR17* (r_K^- m_K^+) *endA1* j80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, and JM101 [*supE thi recA*$^+$ r_K^+ Δ (*lac-proA,B*)/F′ *traD36 proA,B*$^+$ *lacI*^{q}ZΔM15]. The two libraries of genomic DNA used in this work were the Nasmyth and Tatchell library (38), containing yeast DNA inserts in the high-copy-number vector YEp13 (obtained from the ATCC), and the Siede and Eckardt-Schupp library (49), containing yeast DNA inserts in the low-copy-number vector YCp50 (provided by W. Siede).

Genetic methods and media. Yeast cells were grown at 28°C unless otherwise indicated. Standard procedures were used for mating, sporulation, and tetrad analysis (37). Auxotrophic markers were introduced into the *erg8-1* mutant strain (SP1) in a cross with strain W303-1A. Yeast complete medium (YC) and yeast minimal medium (YM) were as previously described (40). YM was supplemented with nutrients as appropriate for selective growth of strains. YME medium was prepared by supplementation of YM with 20 μg of ergosterol per ml from a fresh ergosterol stock solution prepared in Tween 80–ethanol (1:1, vol/vol); YCE medium was YC comparably supplemented. Where indicated, 2% galactose was substituted for 2% glucose as the carbon source in YM. Growth of *S. cerevisiae* for induction of the *GAL1* promoter was as described previously (57). *E. coli* strains were grown at 37°C on LB medium (35) supplemented with 50 μg of ampicillin per ml to select for plasmid transformants.

Transformations. All yeast strains were transformed by the spheroplast method (25). *erg8* and *erg8 upc1* mutant strains were grown overnight in YCE at 25°C before spheroplasting, and the transformants were harvested from YME regeneration agar after 6 days of incubation at 25°C. *E. coli* strains were transformed by the $CaCl_2$ method (41).

Preparation of DNA. Crude plasmid DNA was prepared from yeast transformants by rapid glass bead disruption and phenol extraction (39). Yeast chromosomal DNA was prepared by the method of Struhl et al. (51). Large-scale preparations and minipreparations of plasmid DNA were prepared from *E. coli* by the boiling method (26). Large preparations were further purified by centrifugation on cesium chloride-ethidium bromide gradients.

DNA manipulations. Restriction enzymes and other DNA modification enzymes were purchased from Bethesda Research Labs, Inc., and New England BioLabs, Inc., and used according to the specifications of the manufacturers. DNA restriction fragments for cloning were purified from agarose gels by the glass milk-sodium iodide procedure (55). Radiolabeled *ERG8* probes for Southern analysis were prepared by random oligomer priming (19) of purified restriction fragments with a nick translation kit from Boehringer Mannheim. Unincorporated [^{32}P]dCTP was removed from the labeled probe with spin columns (Boehringer Mannheim).

Plasmid constructions. Plasmids used for localization of the *ERG8* gene were constructed from pET45 by insertion of restriction fragments (see Fig. 3) into compatible sites in the tetracycline resistance region of YCp50. Overhanging 5′ and 3′ ends of some restriction fragments were converted to blunt ends by using the respective polymerase or 3′-exonuclease functions of T4 DNA polymerase. Plasmid pET58 contained the same *Sal*I-*Bam*HI fragment as pET53 (see Fig. 3), except that it was cloned into the multicopy vector YEp352. Plasmid pET48 was made by cloning the 4.5-kb *Bam*HI fragment of pET45 into pBR322. A 2.1-kb *Xho*I-*Bam*HI fragment derived from pET48 was used as an *ERG8* probe for Southern analysis.

Plasmids pET61 and pET62, used for the production of respective *URA*$^+$ and *HIS*$^+$ disruptions of the chromosomal *ERG8* gene, were constructed from pET48 by replacement of a 1.0-kb *Xho*I-*Eco*RV internal fragment of the *ERG8* gene with the appropriate prototrophic marker fragments (see Fig. 6A). Plasmid pET61 was constructed in a three-piece ligation of a 2.0-kb *Sph*I-*Sma*I *URA3* fragment from Ylp5 (51), a 3.7-kb *Sph*I-*Pst*I fragment from pET48 that contained the *ERG8* 5′ end, and a 4.2-kb *Pst*I-*Eco*RV fragment from pET48 that contained the *ERG8* 3′ end. Plasmid pET62 was constructed in an analogous three-piece ligation of a 2.1-kb *Sph*I-*Eco*RV *HIS3* fragment from YEp6 (13) and the two pET48 fragments described above. Linear, disrupted *ERG8* fragments for transformation of diploid yeast cells were

obtained from plasmids pET61 and pET62 by digestion with *Pvu*II and *Xba*I.

Plasmid pET77, used for the overproduction of phosphomevalonate kinase, was constructed by insertion of a 2.4-kb *Xho*I-*Sal*I *ERG8* fragment from pET48 into the *Sal*I site of pMH101 (21). The *ERG8* coding region was properly oriented with respect to the *GAL1* promoter of this plasmid (see Fig. 7A).

DNA sequence analysis. The *Bgl*II-*Xba*I fragment from pET45 was sequenced by the dideoxy-chain termination method of Sanger et al. (45) (see Fig. 3B). DNA restriction fragments subcloned into M13mp18 or M13mp19 were primed for chain extension with the 17-bp universal primer, and [^{35}S]dATP was incorporated with Sequenase as recommended by the manufacturer. Sequences were compiled and analyzed with the Intelligenetics software programs. The EMBL/GenBank and PIR data bases were searched for homologies to the DNA and protein sequences of phosphomevalonate kinase and the 5′ open reading frame (ORF) peptide.

Southern analysis. Southern blot hybridization analysis was performed on yeast chromosomal DNA as described previously (40). Genomic DNA was digested to completion with *Eco*RI, and restriction fragments were separated on a 0.8% agarose gel. The gel was blotted to a BioTrans membrane, which was hybridized with a ^{32}P-labeled *ERG8* probe and autoradiographed.

Preparation of crude extracts. Enzyme extracts were prepared from late-logarithmic-phase yeast cells by centrifugation and suspension of cells in one pellet weight (gram) equivalent of 4°C breakage buffer (100 mM Tris hydrochloride [pH 7.5]–10 mM β-mercaptoethanol). Two pellet weight equivalents of acid-washed glass beads (diameter, 0.45 μm) were added, and the cells were disrupted by vigorous mixing on a Vortex Genie (three 45-s pulses). The disrupted cell suspension was centrifuged at 2,400 × *g* for 10 min, after which the supernatant was removed and centrifuged at 4,000 × *g* for 15 min. The 4,000 × *g* supernatant (S_{4K}) fraction was used directly in the ^{14}C-labeled substrate conversion assay or was centrifuged at 100,000 × *g* for 60 min to obtain a soluble S_{100K} fraction, which was used in the spectrophotometric assay for phosphomevalonate kinase. Protein concentrations in S_{4K} and S_{100K} fractions were determined by the method of Bradford (14). Enzyme extracts were frozen and stored at −70°C before assay with negligible losses in phosphomevalonate kinase activity.

Enzyme assays for phosphomevalonate kinase. Complementation of an *erg8* mutant strain was assessed biochemically by measuring the ability of S_{4K} fractions to convert [^{14}C]phosphomevalonate into sterol precursors, predominantly squalene. This assay functions because uncomplemented *erg8* mutants contain all of the enzymes necessary for this conversion, except phosphomevalonate kinase. Reaction mixtures contained the following in a total volume of 0.2 ml: 5 mM $MgCl_2$, 4 mM ATP, 1 mM NADPH, 10 mM KF, 100 mM Tris hydrochloride (pH 7.5), 0.1 μCi of [5-^{14}C] *R*-phosphomevalonate (58 mCi/mmol), and 0.15 ml of the S_{4K} fraction. In some experiments, equivalent amounts of [2-^{14}C]*R*-mevalonate (57 mCi/mmol), [^{14}C]pyrophosphomevalonate (58 mCi/mmol), or [1-^{14}C]isopentenyl disphosphate (56 mCi/mmol) were substituted for labeled phosphomevalonate in the mixture. Reactions were incubated at 37°C for 20 min and terminated by the addition of 0.8 ml of $CHCl_3$-methanol (2:1, vol/vol). The phases were vortexed briefly and separated by low-speed centrifugation. The organic phase was removed, washed twice with methanol, and counted to determine the extent of ^{14}C incorporation into organic solvent-soluble products. Thin-layer chromatography was also used to examine the various products of in vitro reactions. After incubation of S_{4K} fractions with the ^{14}C-labeled substrates described above, aqueous reaction mixtures were fractionated on silica gel LK6D thin-layer chromatography plates with *n*-propanol–NH_4OH–water (6:3:1, vol/vol/vol) as the solvent system. The dried plates were examined by autoradiography.

Overexpression of phosphomevalonate kinase was estimated by using two enzyme assays. The more sensitive was an in vitro complementation assay, which utilized [^{14}C] phosphomevalonate and measured the conversion of this substrate into squalene as described above. Small amounts (ranging from 0.001 to 0.01 ml) of S_{4K} fraction from putative overproducing cells were added to reaction mixtures that contained a large amount (0.15 ml) of S_{4K} fraction from *erg8* mutant cells. Reactions were terminated at various times, and conversion rates were determined; the S_{4K} fraction from an untransformed wild-type (*ERG8*) yeast strain was used to calibrate this assay for plasmid-transformed strains. A spectrophotometric assay for phosphomevalonate kinase was used to measure enzyme overproduction in cells bearing pET77 or pMH101 plasmids. Conditions for this assay were as previously described (42), except 9 mM *R,S*-phosphomevalonate was used as the substrate. The generation of ADP from ATP in the phosphomevalonate kinase reaction was coupled to the reactions catalyzed by pyruvate kinase and lactate dehydrogenase, and the reaction rate was monitored at 340 nm with a double-beam spectrophotometer.

Determination of crude ergosterol content. Nonsaponifiable lipids were prepared, and the crude ergosterol content of yeast cells was determined by the method of Karst et. al (47).

Gel electrophoresis of yeast proteins. Cultures of yeast strain Y294 that contained the *ERG8* overexpression plasmid pET77 or the control plasmid pMH101 (21) were grown in either YM-glucose or YM-galactose for 18 h. Cells were harvested, and S_{100K} fractions were prepared as described above. Aliquots that contained 20 μg of protein were mixed with Laemmli sample buffer (30), boiled for 3 min, electrophoresed on 12% sodium dodecyl sulfate–polyacrylamide gels (Integrated Separation Systems) as described by Laemmli (30), and stained with Coomassie blue stain. Band intensities on stained gels were quantified by densitometric scanning.

RESULTS

Biochemical and genetic analyses of *erg8-1* mutants. Two temperature-sensitive haploid strains, AG8-2A and AG8-6B (Table 1), derived from the Karst *erg8-1* mutant strain SP1 were characterized biochemically before attempts to clone the *ERG8* gene were initiated. Cell extracts prepared from these *erg8* strains possessed no detectable phosphomevalonate kinase activity and were unable to convert [^{14}C] phosphomevalonate, a substrate of phosphomevalonate kinase, into any other product in the sterol pathway (Fig. 1 and 2). In contrast, extracts from *ERG8* wild-type yeast strains were capable of efficient in vitro conversion of this substrate into other products, chiefly squalene (Fig. 1), showing that they contained all of the sterol enzymes necessary for this conversion. *ERG8* and *erg8* extracts were equally capable of converting [^{14}C]pyrophosphomevalonate, a product of phosphomevalonate kinase, into squalene. Similar experiments with [^{14}C]mevalonate (Fig. 1) and [^{14}C]isopentenyl diphosphate (data not shown) as substrates confirmed that the

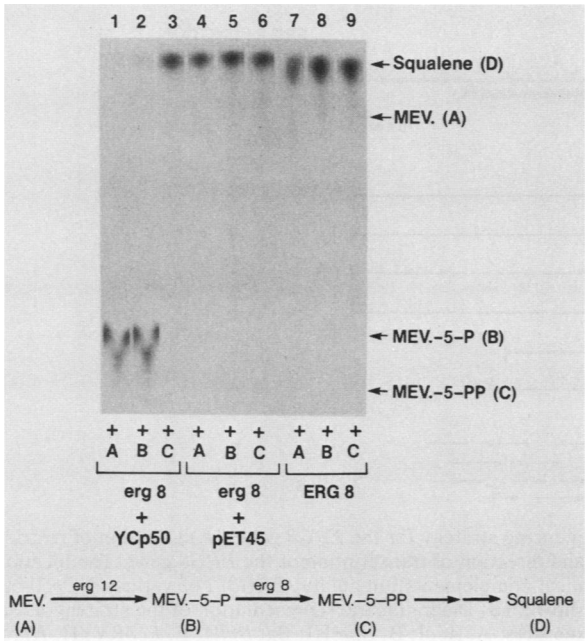

FIG. 1. Complementation of the biochemical defect in an *erg8-1* mutant strain. Cell extracts prepared from an *ERG8* (wild-type) yeast strain and from a YCp50- or pET45-transformed *erg8-1* mutant strain were incubated with the indicated ^{14}C-labeled sterol precursor (A, B, or C) for 20 min at 37°C. Equal amounts of total reaction mixture were applied to thin-layer chromatography plates, which were developed as described in Materials and Methods. The migration of standard compounds (A, B, C, and D) on the plates is indicated by arrows. Abbreviations: MEV., mevalonate; MEV.-5-P, phosphomevalonate; MEV.-5-PP, pyrophosphomevalonate.

enzyme defect of the *erg8* strains was restricted to phosphomevalonate kinase and affected none of the later steps of squalene synthesis.

Initial attempts to transform the *erg8* strains showed that even after growth in YCE they were poor recipients for plasmid DNA, yielding only 1 or 2 transformants per μg of DNA. Plating of the *erg8* strains on YME medium at temperatures above 25°C reduced their colony-forming ability, as expected, but additionally produced colony size heterogeneity that could complicate the screening of yeast libraries for plasmids capable of reversing the *erg8* temperature-sensitive phenotype. It appeared likely that some of these strain characteristics might be a function of both impaired synthesis of ergosterol and inefficient uptake of ergosterol, so the *upc1* mutation (34), which is known to enhance uptake of ergosterol in aerobically grown yeast cells, was crossed into the *erg8* strains. The resulting *erg8 upc1* strains, ET7-6B and ET15-6D (Table 1), not only grew better on YME at 25°C than the parent *erg8* strains but also formed colonies of more uniform size at all temperatures. Like the parent strains, the *erg8 upc1* strains formed fewer colonies on YME at elevated temperatures and none at 34°C. The *erg8 upc1* strains were much better recipients for plasmid DNA than the *erg8* strains, giving 20 to 50 transformants per μg of DNA.

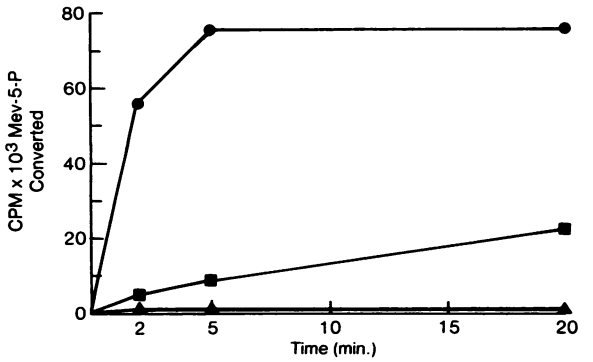

FIG. 2. Effect of *ERG8* plasmid copy number on phosphomevalonate kinase activity. Equivalent, small amounts of cell extract from either an untransformed *ERG8* strain (■) or from an *ERG8* strain transformed with the multicopy *ERG8* plasmid pET49 (●) were mixed with an excess of *erg8-1* mutant extract to measure enzymatic complementation. Mixed extracts were incubated with ^{14}C-labeled phosphomevalonate for various times at 37°C, and conversion into products was measured as described in Materials and Methods. Unsupplemented *erg8-1* mutant extracts (▲) converted <2,000 cpm into products at all times of incubation.

Cloning of the *ERG8* gene by functional complementation. Yeast recombinant DNA libraries were screened to isolate candidate *ERG8* plasmid clones. The *erg8* strain ET7-6B was transformed to leucine prototrophy with the high-copy-number Nasmyth and Tatchell library (38), and strain ET15-6D was transformed to uracil prototrophy with the low-copy-number Siede and Eckhardt-Schupp library (49). Approximately 35,000 *LEU*$^+$ transformants from the Nasmyth and Tatchell library and an equal number of *URA*$^+$ transformants from the Siede and Eckhardt-Schupp library were separately pooled for screening.

Transformant pools were screened for the *ERG8* gene by spreading cells onto YME and selecting for cells capable of colony formation after 2 days at 34°C. With the *LEU*$^+$ transformant pool, 2 to 5 cells formed colonies for every 10,000 cells plated. Extracts prepared from these colonies were assayed biochemically for their ability to convert [^{14}C]mevalonate into organic solvent-soluble products (Materials and Methods). None of the *LEU*$^+$ candidate cells was more efficient at this conversion than untransformed *erg8 upc1* cells, so these colonies were not further characterized. With the *URA*$^+$ transformant pool, 5 to 10 cells formed colonies at 34°C for every 10,000 cells plated. Cell extracts from these *URA*$^+$ candidates were tested biochemically, and some of them were found to be as efficient as wild-type cells in their ability to convert [^{14}C]mevalonate into squalene.

Plasmid DNA extracted from 22 *ERG8* candidate colonies was amplified in *E. coli* and examined by restriction enzyme analysis. Three unrelated plasmids were identified. Cells that carried either of two plasmids were partially complemented for the *erg8* biochemical defect and were not analyzed further. Cells that carried the third plasmid were completely complemented. This latter plasmid, designated pET45, contained a 4.5-kb insert with the restriction sites shown in Fig. 3A. To verify that it contained the *ERG8* gene, pET45 was transformed into an *erg8* strain that lacked the *upc1* mutation of strain ET15-6D. The pET45 transformants of strain AG8-2A were viable on YM at 37°C, and extracts from these cells showed the ^{14}C-labeled substrate conversion ability characteristic of wild-type cells (Fig. 1). To further test the premise that pET45 contained the *ERG8* structural gene, the entire 4.5-kb insert was subcloned onto YEp24 to generate the high-copy-number plasmid pET49. This plasmid was transformed into the wild-type strain W303-1A, and extracts were prepared from pET49 transformants and untrans-

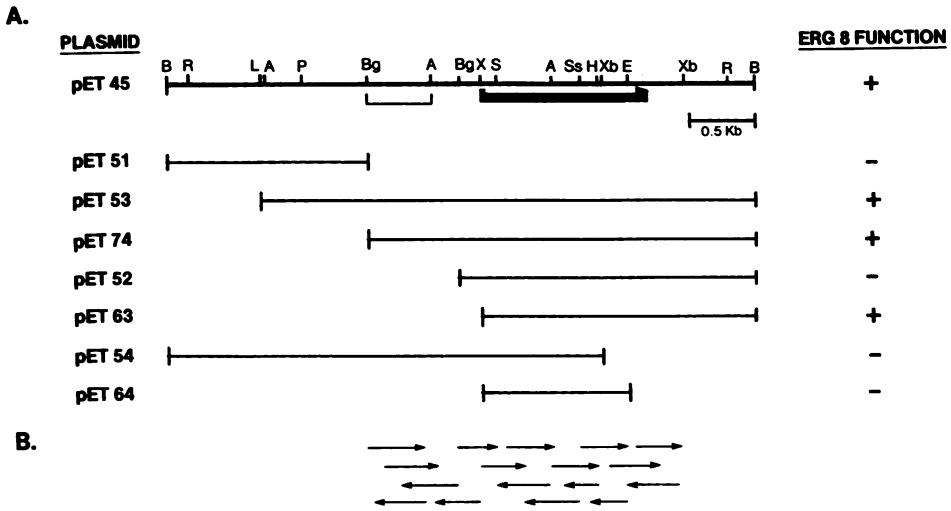

FIG. 3. Restriction map of pET45 and various deletion plasmids and sequencing strategy for the *ERG8* gene. (A) Location of restriction sites on the 4.5-kb insert of pET45. The thick arrow indicates the location and direction of transcription of the *ERG8* gene. The location of an incomplete ORF is indicated by a bracket. The phenotypic and biochemical complementation ability (*ERG8*$^+$) of various YCp50-based deletion plasmids and the extent of *ERG8* region DNA present in each are shown. (B) Diagrammatic representation of the strategy used for sequencing the *ERG8* gene and flanking regions on pET45. Restriction enzyme sites: A, *Acc*I; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RV; H, *Hin*dIII; L, *Sal*I; P, *Pvu*I; S, *Sph*I; Ss, *Ssp*I; R, *Eco*RI; X, *Xho*I; Xb, *Xba*I.

formed cells. As shown earlier, *erg8* extracts contain all of the enzymes of squalene synthesis except phosphomevalonate kinase, but it should be possible to complement this deficiency in vitro by the addition of small quantities of extract from *ERG8* cells. Using this approach, the relative amount of phosphomevalonate kinase in extracts was estimated by measuring the increased rate of [^{14}C]phosphomevalonate conversion (Fig. 2). From such mixing assays, it was determined that pET49 resulted in an approximately eightfold overproduction of phosphomevalonate kinase. Analogous experiments have been described for other sterol biosynthetic genes of *S. cerevisiae* and similar gene-dosage effects have been reported (2, 43).

Genetic verification of the *ERG8* clone. To establish the identity of the putative *ERG8* clone, the ability of pET45 DNA to target integration of a plasmid via homologous recombination to the yeast *erg8* locus was examined. For this purpose, a *Sal*I-*Bam*HI fragment of pET45 was subcloned into YIp5 (Materials and Methods), a vector that contains the yeast *URA3* gene but lacks a yeast replication origin (51). The resultant plasmid, pET46, was cut within *ERG8* sequences at a unique *Sph*I site and transformed into the *erg8* strain ET20-2A. Stable *URA*$^+$ transformants selected at 25°C grew well at 37°C and were biochemically *ERG8*. One such *URA*$^+$ integrant was crossed to the wild-type strain JT42-5B to give diploids, which were sporulated and subjected to tetrad analysis. In all of the tetrads examined, 4:0 segregation for *ERG8*/*erg8* was seen at the biochemical level, indicating close linkage between the *ERG8* gene on the integrated plasmid and the *erg8* chromosomal allele (Table 2). These data provided genetic evidence that pET45 contained the *ERG8* gene.

Localization of the *ERG8* gene on pET45. To localize the *erg8-1* complementing region of pET45, subclones were constructed in YCp50 by using the restriction fragments shown in Fig. 3A. These subclones were tested for their ability to complement the temperature-sensitive phenotype and biochemical defect of strain AG8-2A. Data obtained from plasmids pET51, pET52, pET53, and pET74 indicated that one end of the *ERG8* gene was situated between the two *Bgl*II sites of pET45. Although pET52 failed to complement, this was at odds with the finding that pET63 fully complemented the *erg8* mutant even though pET63 contained a smaller pET45 fragment. A possible explanation for this anomaly was provided from the nucleotide sequence of the *ERG8* gene and is discussed below. Plasmids pET54 and pET64, which terminated at *Xba*I and *Eco*RV sites, respectively, failed to complement the *erg8* mutation, indicating that the other end of the *ERG8* gene was situated downstream of the *Eco*RV site on pET45.

Nucleotide and predicted *ERG8* polypeptide sequences. The strategy used for the determination of the *ERG8* DNA sequence is shown in Fig. 3B, and the complete nucleotide sequence is shown in Fig. 4. The 2,399-bp region sequenced spanned the most distal *Bgl*II and *Xba*I sites of pET45 (Fig. 3A) and included 846 bp of 5′-flanking sequences and 278 bp of 3′-flanking sequences. The presumptive *ERG8* coding region starts 3 bp after a *Xho*I site and continues for 1,272 bp, terminating at a TAA codon. The *ERG8* ORF is capable of encoding a 424-amino-acid polypeptide with a predicted molecular weight of 47,328. No TACTAAC-like intron con-

TABLE 2. Genetic analysis of *ERG8* plasmid integrants[a]

Segregation of *ERG* and *URA* phenotypes		Segregation of *LYS* and *URA* phenotypes	
Spore phenotype	No. of spores with phenotype	Spore phenotype	No. of spores with phenotype
ERG$^+$ URA$^+$	44	LYS$^+$ URA$^+$	25
ERG$^+$ URA$^-$	44	LYS$^+$ URA$^-$	19
ERG$^-$ URA$^+$	0	LYS$^-$ URA$^+$	19
ERG$^-$ URA$^-$	0	LYS$^-$ URA$^-$	25

[a] Plasmid pET46 (*ERG8 URA3*) was integrated into the *erg8* strain ET20-2A to obtain a *URA*$^+$ transformant that was crossed with the *ERG8* strain JT42-5B. The resulting diploids were sporulated and dissected. Nutritional markers segregated 2$^+$:2$^-$ in all four-spored tetrads, and the *ERG8* (or *erg8*) status of the spores was determined by biochemical analysis.

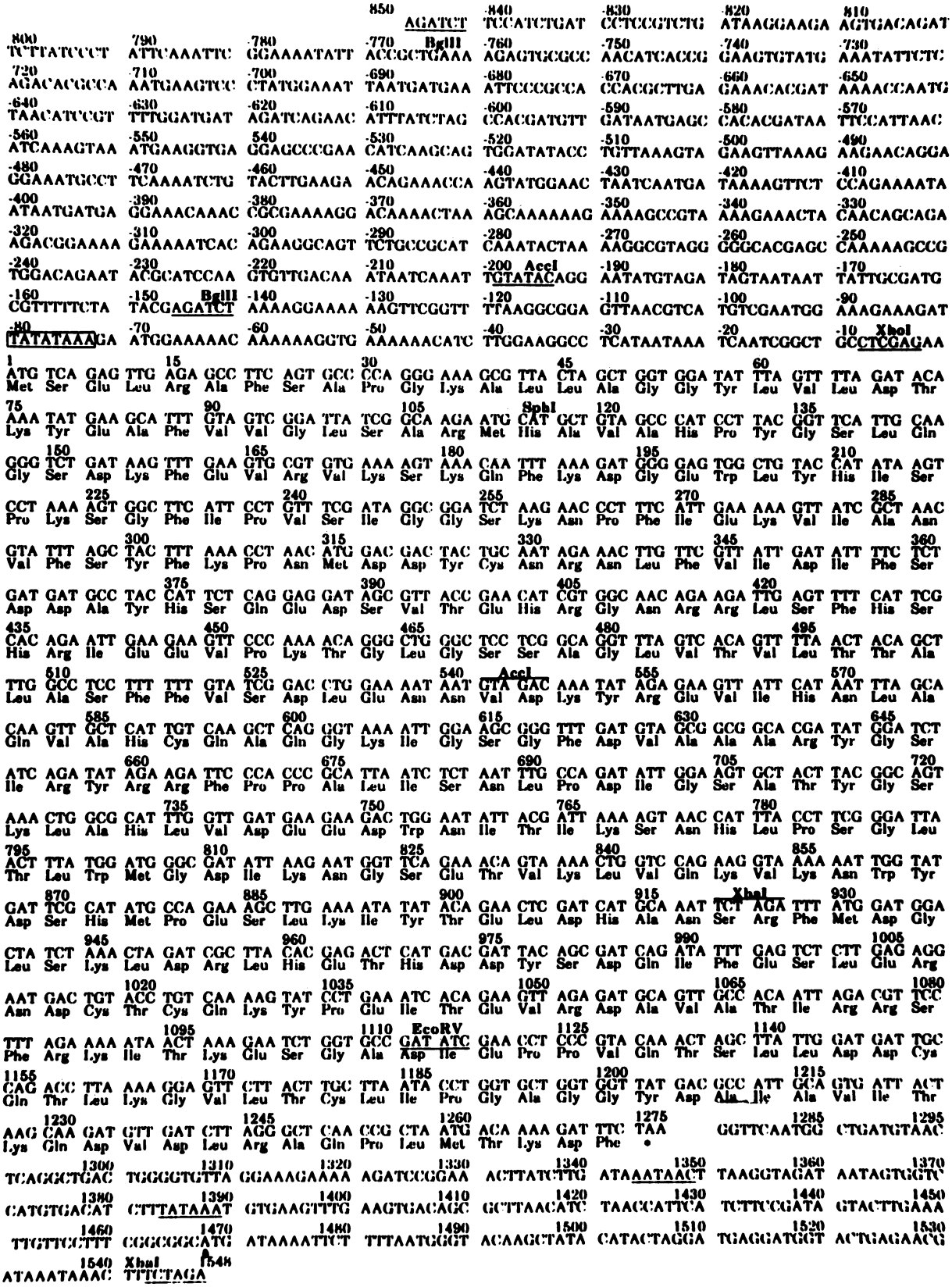

FIG. 4. Nucleotide and predicted amino acid sequence of the *ERG8* gene. Nucleotides in the DNA sequence are numbered starting at the *ERG8* translational start site and nucleotides in the 5′-untranslated region are designated by negative numbers. The predicted amino acid sequence for phosphomevalonate kinase is shown underneath. A consensus TATA box is enclosed in a box, and possible polyadenylation signals near the 3′ end are underlined. Restriction sites are labeled and either underlined or overlined.

Concensus		- - - - - G - G - - G - X(12-25).. K	
ERG8	150V	P - K T G L G S S A G ...X(25)...	K183
HSK1	90M	P I G S G L G S S A C ...X(16)...	K116
RAR1	140L	P I G A G L G S S A S ...X(24)...	K174
GAL1	162I	P T G S G L S S S A A ...X(12)...	K195

FIG. 5. Peptide homologies between phosphomevalonate kinase and other proteins. The consensus sequence for an ATP-binding site present in many protein kinases is aligned over the deduced amino acid sequence of yeast phosphomevalonate kinase (*ERG8*), *E. coli* homoserine kinase (*HSK1*), yeast *RAR1* protein, and yeast galactokinase (*GAL1*). Positions of the homologous peptides in the respective proteins are indicated by superscript numbers.

sensus sequence element (31) was found within this region, indicating that the *ERG8* mRNA is not spliced. An incomplete ORF of 637 bp (Fig. 3A; Fig. 4, positions −846 to −200) that begins at the 5′ *Bgl*II site and terminates near an *Acc*I site was also detected 207 bp upstream of the *ERG8* ORF. No function for the incomplete ORF was found in this study.

Searches of the EMBL/GenBank and PIR data bases with the *ERG8* DNA or predicted protein sequence failed to identify closely related genes or proteins. However, a limited region of the *ERG8* protein beginning at amino acid 150 was found to share homologous sequences with several other proteins (Fig. 5). As indicated, all these proteins contain the motif Gly-X-Gly-XX-Ala-X(13–26)-Lys. A similar motif that contains a third glycine instead of the alanine residue has been described by others (23, 36). Considerable evidence suggests that this structure forms an ATP-binding pocket in many kinases (23, 36). Phosphomevalonate kinase and the proteins identified in Fig. 5 may use a modified version of this structure to serve a comparable function. Searches of the data bases with the incomplete 5′-ORF DNA or predicted protein sequence turned up no homologous sequences.

Many yeast genes contain TATA box-related sequences upstream of their coding regions that serve as basal promoter elements. They are typically found 60 to 120 bp upstream of the transcription initiation sites (22). A strong match to the consensus yeast TATA sequence is present at position −80 (TATATAAA) of the *ERG8* nucleotide sequence (Fig. 4). Weak matches to the consensus sequence were also found at positions −120 (TCTAAA) and −198 (TATACA). No significant inverted repeat sequences were found, but 8-bp perfect repeat sequences (at positions −447 through −455 and −483 through −491) and 20-bp imperfect repeat sequences (at positions −556 through −585 and −644 through −663) were detected upstream of the presumptive *ERG8* TATA boxes. The significance of all these sequences for transcription of the *ERG8* gene remains to be tested.

Sequences required for efficient termination of transcription and RNA polyadenylation have been described for a number of yeast genes. No tripartite termination elements of the type proposed by Zaret and Sherman (58) were found at the 3′ end of the *ERG8* gene. However, sequences similar to those of the putative consensus polyadenylation signal, AATAAA, were detected 72 and 113 bp downstream of the *ERG8* TAA stop codon (Fig. 4). The frequency of codon use in the *ERG8* coding region showed no striking bias toward codons most often utilized in highly expressed yeast genes (9). A codon bias index of 0.31 was calculated for the predicted *ERG8* protein, suggesting low to moderate expression of this gene in *S. cerevisiae*.

Disruption of the *ERG8* gene at its chromosomal locus. To determine whether the *ERG8* gene was essential for isoprene and sterol biosynthesis in *S. cerevisiae*, chromosomal disruptions of the gene were constructed by the method of Rothstein (44). A large internal region of the *ERG8* coding region was replaced with DNA fragments containing selectable markers (Fig. 6A). Constructs that contained the *ERG8* gene disrupted with either the *URA3* gene or the *HIS3* gene were transformed as linear DNA fragments into the diploid strain ET16, which was heterozygous for the *erg8* mutation (Table 1). Stable uracil or histidine prototrophs were selected; approximately half of each type were found to be temperature sensitive for growth at 37°C, suggesting that these contained a disruption of the *ERG8* allele in the chromosome. To confirm that the temperature-sensitive URA^+ and HIS^+ diploid cells contained the appropriate null alleles of the *ERG8* gene, a Southern analysis was performed. Genomic DNA from several disrupted and undisrupted strains was examined (Fig. 6B). DNA from three undisrupted strains yielded a single 3.6-kb band, demonstrating that the *erg8-1* and *ERG8* alleles present in the diploid strain were similar in size. Chromosomal DNA from the *ERG8*-disrupted diploid strains produced a second band of either 4.6 or 4.8 kb. The additional bands were the same size as the *ERG8*::*URA3* and *ERG8*::*HIS3* disruption fragments of plasmids pET61 and pET62 (Fig. 6B, lanes 7 and 8), proving that they represented two new alleles, *erg8-Δ1* and *erg8-Δ2*, of the *ERG8* gene.

To examine the phenotype of *ERG8* disrupted cells, temperature-sensitive URA^+ and HIS^+ prototrophs of strain ET16 were sporulated and dissected. For 10 complete tetrads from the *ERG8*::*URA3* diploid and 22 complete tetrads from the *ERG8*::*HIS3* diploid, spore viability segregated 2 live:2 dead on YCE (Table 3). The viable spores in all tetrads were either *ura* or *his*, respectively, for the two diploids, indicating that the inviable spores contained the deletion markers. These results were also obtained with tetrads derived from the *ERG8* homozygous diploid strain YM197 when one of the *ERG8* alleles was disrupted by the *HIS3* marker (Table 3). Together, these results confirmed the data from the Southern blot analysis and showed that *ERG8* was a single-copy essential gene of haploid yeast cells.

Transformation of strain ET16 with the *ERG8*::*HIS3* disruption gave rise to equal numbers of temperature-sensitive and non-temperature-sensitive diploids, suggesting that there was an equal likelihood of disrupting either the *ERG8* or *erg8-1* allele in this strain. To examine this premise, one temperature-sensitive and one non-temperature-sensitive *ERG8*::*HIS3* diploid were transformed with the multicopy plasmid pET58, which contained the *ERG8* and *URA3* genes, before tetrad dissection. Spore viability and the URA^+ marker segregated $4^+:0^-$ in all tetrads derived from both diploids (Table 3). Furthermore, none of the URA^+ spores was temperature sensitive. These results demonstrated that the *ERG8* gene had rescued the inviability of the *erg8-Δ2* disruption and complemented the *erg8-1* mutation. To verify these findings, isolated spores from the tetrads above were streaked onto YME plates that contained 5-fluoroorotic acid to counterselect cells that carried a URA^+ plasmid (11). Among all of the tetrads examined, only the two *his* spores from each tetrad were viable on 5-fluorootic acid plates. The viable spores were consistently either temperature sensitive or not, depending upon whether they

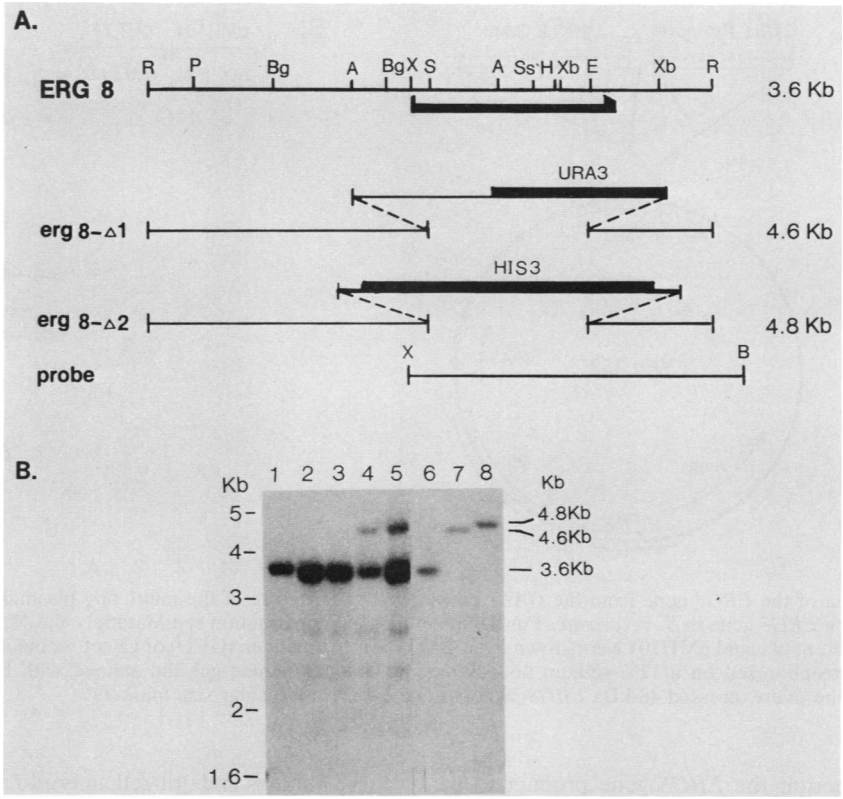

FIG. 6. Southern blot analysis of yeast genomic DNA. (A) Diagrammatic representation of the *ERG8* fragments from the pBR322-based plasmids pET61 (*ERG8*::*URA3*) and pET62 (*ERG8*::*HIS3*) that were used to create the *ERG8* chromosomal disruptions, *erg8*-Δ*1* and *erg8*-Δ*2*. The restriction map and symbols are as described in the legend to Fig. 3B. Fragment sizes listed to the right indicate *ERG8* band sizes expected for *Eco*RI digestion of plasmid or genomic DNA containing the disruptions. (B) Southern analysis of *Eco*RI-digested yeast genomic DNA (lanes 1 through 5) or plasmid DNA (lanes 6 through 8). Genomic DNA was from *ERG8* haploids (lanes 1 and 2), the *ERG8*/*erg8-1* diploid ET16 (lane 3), an *erg8*-Δ*1*/*erg8-1* diploid (lane 4), and an *erg8*-Δ*2*/*erg8-1* diploid (lane 5), and plasmid DNA was from pET45, pET61, and pET62 (lanes 6 through 8, respectively). All lanes were probed with the radiolabeled *Xho*I-*Bam*HI *ERG8* fragment shown in Fig. 6B.

came from a temperature-sensitive or non-temperature-sensitive diploid originally. Thus, both *erg8*-Δ*2* allele rescue and *erg8-1* allele complementation were dependent upon the continuous presence of an *ERG8* plasmid. The same results were obtained when the analysis was done with the low-copy-number *ERG8* plasmid pET63 (Table 3). Together these experiments indicated that alternate *ERG8* alleles had been disrupted in the heterozygous diploid strain.

Expression of the *ERG8* gene from the *GAL1* promoter. As described above, levels of phosphomevalonate kinase were increased severalfold in wild-type cells bearing the *ERG8* gene on a multicopy plasmid. However, this level of expres-

TABLE 3. Genetic analysis of *ERG8* gene disruptions

Diploid strain		Spore viability					Spore segregation[a]		
Disruption[b]	Plasmid[c]	4⁺:0°	3⁺:1°	2⁺:2°	1⁺:3°	0⁺:4°	*ERG8*:*erg8*	*URA3*:*ura3*	*HIS3*:*his3*
YM197									
ERG8::*URA3*	None	0	0	13	0	0	2:0	0:2	NA
ET16									
ERG8::*URA3*	None	0	0	10	0	0	0:2	0:2	NA
ERG8::*HIS3*	None	0	0	22	0	0	0:2	NA	0:2
ERG8::*HIS3*	pET58	7	0	0	0	0	4:0	4:0	2:2
ERG8::*HIS3*	pET63	5	0	0	0	0	4:0	4:0	2:2
erg8::*HIS3*	None	0	0	19	0	0	2:0	NA	0:2
erg8::*HIS3*	pET58	13	0	0	0	0	4:0	4:0	2:2

[a] *ERG8* (or *erg8*) status of the spores was determined by biochemical analysis. NA, Not applicable.

[b] The wild-type diploid strain YM197 (*ERG8*/*ERG8*) and the heterozygous diploid strain ET16 (*ERG8*/*erg8*) were disrupted in an *ERG8* (or *erg8*) allele by integration of a linear *ERG8*::*URA3* or *ERG8*::*HIS3* disruption fragment.

[c] Some of the *ERG8*::*HIS3* diploids were secondarily transformed with either pET63 (YCp50) or pET58 (YEp352) plasmids that contained the *URA3* and *ERG8* structural genes before sporulation and tetrad dissection.

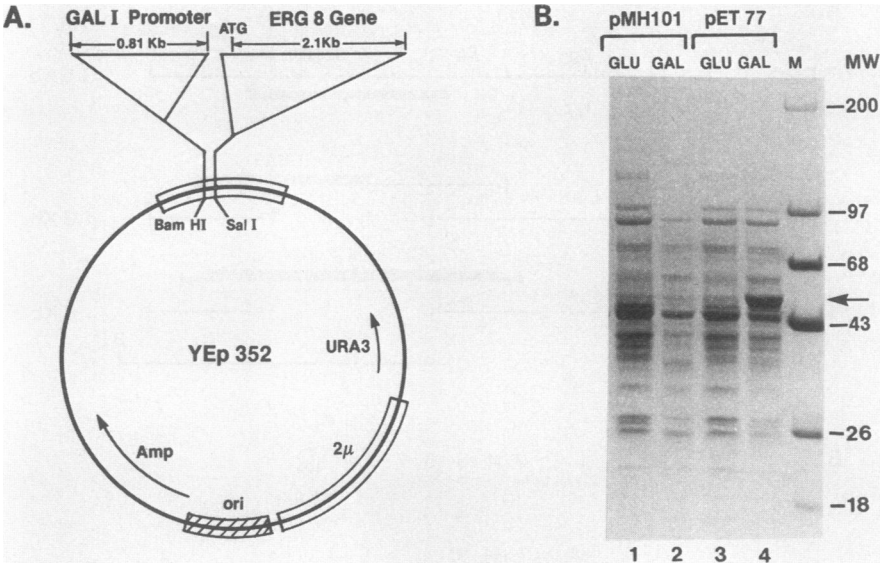

FIG. 7. Overexpression of the *ERG8* gene from the *GAL1* promoter. (A) Structure of the multicopy plasmid pET77 used for galactose inducible expression of the *ERG8* gene in *S. cerevisiae*. For details of plasmid construction see Materials and Methods. (B) Yeast cells that contained pET77 or the parent plasmid pMH101 were grown overnight in glucose medium (GLU) or galactose medium (GAL). Equal amounts of cell protein were electrophoresed on a 12% sodium dodecyl sulfate–polyacrylamide gel and stained with Coomassie blue. An arrow indicates the position of the overexpressed 48-kDa *ERG8* protein in lane 4. M, Molecular size markers.

sion was too low to permit the *ERG8* gene product to be identified on sodium dodecyl sulfate-polyacrylamide gels (data not shown). To obtain higher levels of expression, the *ERG8* coding region was placed under the regulatory control of the yeast galactokinase (*GAL1*) promoter on plasmid pET77 (Fig. 7A). The parent vector pMH101 (21) and pET77 were transformed into a GAL^+ strain, and the strain was grown in YM-glucose and then transferred to YM-galactose to induce expression from the *GAL1* promoter. Protein extracts from glucose- and galactose-grown cells were run on sodium dodecyl sulfate-polyacrylamide gels (Fig. 7B). Extracts from pET77-transformed cells that were grown on galactose contained a strong band of approximately 48 kDa. An analogous band was not seen in extracts from pET77-transformed cells that were grown on glucose or from cells bearing pMH101 grown on either carbon source. The size and galactose-regulated expression of this protein strongly implied that it was the predicted (47.3-kDa) *ERG8* gene product. To confirm this, extracts were analyzed by a standard spectrophotometric assay for phosphomevalonate kinase activity. Extracts from pET77-transformed cells grown on galactose had nearly 1,000 times more enzyme activity than did extracts from cells grown on glucose (Table 4). Simple chromatographic fractionation of extracts containing the overexpressed protein demonstrated that phosphomevalonate kinase activity copurified with the 48-kDa protein (data not shown). No excess ergosterol accumulated, and no deleterious effects of enzyme overexpression were observed, even after prolonged growth (72 h) of cells in YM-galactose.

DISCUSSION

Studies by Karst and Lacroute suggested that yeast strains that contained *erg8* mutations were blocked in isoprene and ergosterol biosynthesis due to the presence of a thermolabile phosphomevalonate kinase (29). Our investigation confirmed these findings and utilized an *erg8-1* mutant to isolate a yeast library plasmid that complemented both the temperature-sensitive and biochemical defects of the mutant strain. This plasmid was shown by genetic analysis to contain the yeast *ERG8* gene. Southern analysis, gene disruption, and plasmid rescue experiments confirmed that the cloned gene contained sequences that corresponded to the *ERG8* chromosomal locus. Consistent with known gene dosage effects, when the *ERG8* gene was present in cells on a high-copy-number plasmid, a corresponding eightfold overproduction of phosphomevalonate kinase was observed.

Subcloning and nucleotide sequence analyses showed that the yeast *ERG8* gene encoded an unexpectedly large protein of approximately 47 kDa. Previous characterizations of the porcine phosphomevalonate kinase had shown it to be 22 kDa (6, 32). This difference in protein size was surprising, because to date comparisons between yeast and mammalian clones for other enzymes of sterol biosynthesis, including HMG CoA synthase (27a), HMG CoA reductase (5), mevalonate kinase (51a), farnesyl diphosphate synthetase (3), and lanosterol demethylase (28), have revealed strong conserva-

TABLE 4. Phosphomevalonate kinase activity and ergosterol content of yeast strains[a]

Strain	Plasmid	Medium	Ergosterol content (%)	Sp act (nmol/min/mg)
Y294	pMH101	Glucose	0.76	<15
Y294	pMH101	Galactose	0.80	<15
Y294	pET77	Glucose	0.75	<15
Y294	pET77	Galactose	0.82	13,200

[a] Yeast cells that contained pMH101 (without *ERG8*) or pET77 (with *ERG8*) were grown overnight in minimal medium with the indicated carbon source. Phosphomevalonate kinase activity and crude ergosterol content of cells (percentage of dry cell weight) were determined as described in Materials and Methods. A specific activity of 15 corresponds to the minimal amount of enzyme detectable in the spectrophotometric assay used.

tion in protein size if not in protein sequence. Molecular cloning of a cDNA encoding the mammalian phosphomevalonate kinase will be necessary to determine which regions of the yeast polypeptide have been conserved in the smaller mammalian protein. Potentially, the additional sequences present in the yeast phosphomevalonate kinase may confer on it novel properties not associated with the mammalian enzyme. This possibility will be explored during future characterization of the yeast enzyme.

Another feature of phosphomevalonate kinase deduced from the *ERG8* nucleotide sequence was the location of a small peptide that may represent a portion of the enzyme active site. Its importance was suggested from the finding that homologous peptides were present in similar positions in several other known or potential (*RAR1*) metabolic kinases. Further support for this idea came from the recognition that a related peptide motif has been identified in protein kinases (23). In tyrosine and serine or threonine protein kinases the lysine residue of the consensus motif, GXGX XG...K, is thought to serve as a contact point, and the glycine repeat is thought to serve as a pocket for ATP binding (23, 36). Phosphomevalonate kinase and the proteins shown in Fig. 5 that contain the GAGLGSSA...K motif are unlikely to possess protein kinase activity because they lack the additional peptide motifs that are conserved in all protein kinases. However, the motif in the *ERG8* protein may be involved in ATP binding, since alanine substitutions for the third glycine residue have been described for some nucleotide binding proteins (36). Modification of the motif in phosphomevalonate kinase by mutagenesis should permit the significance of this motif to be determined.

Although an *ERG8* promoter analysis was not conducted, it was found that truncation of the *ERG8* upstream sequences at position −140 rendered the gene unable to complement the *erg8* mutant. This result may indicate that sequences further upstream of the *ERG8* consensus TATA box are required for optimal expression of the gene. A puzzling finding was that the complete *ERG8* coding region carried on pET63, in the absence of upstream sequences, could complement the *erg8* defect. It seems likely that this reflected the joining of the *ERG8* coding region to a spurious promoter in vector sequences. A precedent for such vector artifacts has been established in other studies (8). We are currently examining the *cis*-acting elements of the *ERG8* promoter in greater detail.

Karst and Lacroute (29) postulated that exogenous ergosterol improved the growth of *erg8-1* mutants because it allowed them to divert their limited isoprene supply into the synthesis of essential products other than ergosterol. In the course of our cloning work, we compared the growth on ergosterol medium of several *erg8* mutants and found that *erg8 upc1* mutants were healthier than the parent *erg8* strains, as judged by a number of criteria. Unlike *erg8* strains, the *erg8 upc1* strains also formed uniformly sized colonies at permissive temperatures. We believe that these differences reflect more consistent uptake of ergosterol by the *erg8 upc1* cells. The *upc1* mutation was reported to have similar effects on growth of yeast mutants defective in oxidosqualene cyclase (34), another enzyme of ergosterol biosynthesis. Our observations on the growth of *erg8 upc1* mutant cells were thus consistent with the Karst and Lacroute hypothesis.

Even in the presence of ergosterol, growth of *erg8* or *erg8 upc1* cells is blocked at restrictive temperatures, indicating that phosphomevalonate kinase is essential for more than just ergosterol synthesis. Haploid *erg8* null strains likewise formed inviable colonies on medium that contained ergosterol, even when tetrad dissection was done under anaerobic conditions known to enhance ergosterol uptake in *S. cerevisiae* (4). Repeated attempts to rescue the inviability of *erg8* null mutants by the addition of dolichol and ubiquinone to the medium along with ergosterol were also unsuccessful. Such findings suggest a requirement for additional isoprene-derived products such as farnesol to support growth of *erg8* null mutants. In contrast to these *erg8* results, null mutants in lanosterol demethylase (28) or squalene monooxygenase (52a) were viable when grown on medium supplemented only with ergosterol. However, these enzymes are utilized exclusively for ergosterol biosynthesis. It should prove interesting to examine the effect of *erg8* mutations on other processes that rely on isoprene-derived products, such as protein farnesylation.

A curious finding of this study was that phosphomevalonate kinase could accumulate in *S. cerevisiae* to very high levels without toxicity. Densitometric scanning of protein gels indicated that phosphomevalonate kinase constituted approximately 20% of soluble protein after growth of cells in galactose medium. Despite this enormous elevation in enzyme levels, these cells appeared normal by visible and phase-contrast microscopy. Likewise, neither viability nor cell doubling time was appreciably altered in these cells. Moreover, ergosterol accumulation in these cells was no greater than that in control cultures, indicating that the overexpression of phosphomevalonate kinase had little effect on the synthesis of this isoprene-derived product. Similar findings were previously reported in studies in which the activity of acetoacetyl CoA thiolase (16) or HMG CoA reductase (56) was increased tenfold in yeast strains bearing the corresponding structural gene on a multicopy plasmid. From these studies and our own, it seems that yeast sterol production may be regulated primarily through other rate-limiting enzymes of sterol biosynthesis such as HMG CoA synthetase or squalene synthetase. Alternatively, in vivo mechanisms may exist for down-regulating the enzyme activity increases measured by in vitro assays. We have begun testing various sterol and isoprenoid compounds to see whether they can act as feedback inhibitors of phosphomevalonate kinase in a manner consistent with in vivo regulation of this enzyme.

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ADDENDUM

The *ERG8* protein sequence reported here contains four polypeptide domains out of six that are highly conserved between rat mevalonate kinase (52) and the yeast mevalonate kinase, recently cloned in our laboratory (42a).

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