Isolation, Sequencing, and Disruption of the Yeast *CKA2* Gene: Casein Kinase II Is Essential for Viability in *Saccharomyces cerevisiae*

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Casein kinase II of *Saccharomyces cerevisiae* contains two distinct catalytic subunits, alpha and alpha', which are encoded by the *CKA1* and *CKA2* genes, respectively. Null mutations in the *CKA1* gene do not confer a detectable phenotype (J. L.-P. Chen-Wu, R. Padmanabha, and C. V. C. Glover, *Mol. Cell. Biol.* 8:4981–4990, 1988), presumably because of the presence of the *CKA2* gene. We report here the cloning, sequencing, and disruption of the *CKA2* gene. The alpha' subunit encoded by the *CKA2* gene is 60% identical to the *CKA1*-encoded alpha subunit and 55% identical to the *Drosophila* alpha subunit (A. Saxena, R. Padmanabha, and C. V. C. Glover, *Mol. Cell. Biol.* 7:3409–3417, 1987). Deletions of the *CKA2* gene were constructed by gene replacement techniques. Haploid cells in which the *CKA2* gene alone is disrupted show no detectable phenotype, but haploid cells carrying disruptions in both the *CKA1* and *CKA2* genes are inviable. Cells in which casein kinase II activity is depleted increase substantially in size prior to growth arrest, and a significant fraction of the arrested cells exhibit a pseudomycelial morphology. Disruption of the activity also results in flocculation. Yeast strains lacking both endogenous catalytic subunit genes can be rescued by expression of the alpha and beta subunits of *Drosophila* casein kinase II or by expression of the *Drosophila* alpha subunit alone, suggesting that casein kinase II function has been conserved through evolution.

Casein kinase II is a cyclic nucleotide-independent Ser/Thr protein kinase that is ubiquitous in eucaryotic organisms (15, 47). The structure and substrate specificity of casein kinase II have been extensively studied, but the physiological role of the enzyme has remained elusive. The enzyme from most sources is composed of alpha (37 to 44 kilodaltons [kDa]) and beta (26 to 30 kDa) subunits, which associate in vivo to form a native alpha₂beta₂ holoenzyme. The alpha subunit is catalytic and exists in at least two distinct forms (alpha and alpha') in several species, including *Saccharomyces cerevisiae* (30), cattle (45), and humans (26; F. Lozeman and E. G. Krebs, personal communication). The sequence of the catalytic subunit displays greatest similarity to the CDC28 family of protein kinases (8, 13). The beta subunit is required for maximal activity of the catalytic subunit (10) and becomes phosphorylated when the holoenzyme is allowed to undergo autophosphorylation. Although the beta subunit presumably plays a regulatory role, neither the function of this polypeptide nor the significance of autophosphorylation is well understood. The recognition site for casein kinase II consists of a serine or threonine residue located immediately N terminal to a cluster of acidic amino acids (22, 25). The list of known and potential substrates for the enzyme is extensive and diverse (47).

Although the physiological role of casein kinase II is unknown, a variety of data suggest that the enzyme plays a role in cell proliferation. First, the level of enzyme activity is high in actively dividing cells, including a variety of transformed cell lines (32, 34, 38) as well as normal cells of the developing mouse embryo (38). Second, a variety of growth

factors, including insulin, insulinlike growth factor, and epidermal growth factor, all stimulate casein kinase II activity, suggesting a role for the enzyme in the cellular response to mitogens (1, 21, 44). Third, serum stimulation of WI138 human lung fibroblasts yields time-dependent fluctuations in casein kinase II activity that appear to be associated with the cell cycle (7). Fourth, microinjection of purified casein kinase II into *Xenopus* oocytes inhibits progesterone-induced meiotic maturation but promotes meiotic maturation at the level of maturation-promoting factor (29). Fifth, a number of nuclear oncoproteins, including Myc, Myb, Fos, E1A, and simian virus 40 T antigen, contain a casein kinase II recognition motif. In the case of Myc, this site has been shown to be phosphorylated in vivo and to be a target for purified casein kinase II in vitro (24). In addition to its probable role in cell proliferation, casein kinase II also appears to be involved in cell differentiation (43).

In an effort to clarify the physiological role of casein kinase II, we are conducting a molecular and genetic analysis of the enzyme in the budding yeast *S. cerevisiae*. Purified yeast casein kinase II is composed of alpha (44 kDa), alpha' (35 kDa), beta (41 kDa), and probably beta' (32 kDa) subunits (30). Direct protein sequencing indicates that the alpha and alpha' proteins are not proteolytically related but rather encoded by separate genes. By using oligonucleotide probes derived from the protein sequence, the gene encoding the alpha subunit (*CKA1*) was isolated and sequenced (8). However, strains with null mutations constructed in this gene had no detectable phenotype, presumably because of the presence of the alpha' gene. We describe here the isolation and sequencing of the alpha' gene (*CKA2*) and its disruption in both wild-type and $\Delta cka1$ backgrounds. We demonstrate that strains lacking both the *CKA1* and the *CKA2* genes are inviable, indicating that casein kinase II activity is an essential enzymatic activity in *S. cerevisiae*. We also demonstrate that casein kinase II function appears

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TABLE 1. *S. cerevisiae* strains

Strain ^a	Relevant genotype	Plasmid genotype	Reference
YPH250	a <i>CKA1 CKA2</i>		42
YPH252	α <i>CKA1 CKA2</i>		42
YPH274	a/α <i>CKA1/CKA1 CKA2/CKA2</i>		42
RPG22	a/α <i>CKA1/CKA1 CKA2/Δcka2::TRP1</i>		This paper
RPG22-1b	a <i>CKA1 Δcka2::TRP1</i>		This paper
RPG22-2a	α <i>CKA1 Δcka2::TRP1</i>		This paper
YDH30	a/α <i>CKA1/CKA1 Δcka2::TRP1/Δcka2::TRP1</i>		This paper
JC4	a/α <i>Δcka1::HIS3/Δcka1::HIS3 CKA2/CKA2</i>		8
RPG13	a/α <i>Δcka1::HIS3/Δcka1::HIS3 CKA2/Δcka2::TRP1</i>		This paper
JC31	a/α <i>Δcka1::HIS3/Δcka1::HIS3 CKA2/Δcka2::TRP1</i>	2μm <i>URA3 CKA1</i>	This paper
JC35	a/α <i>Δcka1::HIS3/Δcka1::HIS3 CKA2/Δcka2::TRP1</i>	<i>CEN/ARS URA3 GAL1-CKA1</i>	This paper
RPG41	a/α <i>Δcka1::HIS3/Δcka1::HIS3 CKA2/Δcka2::TRP1</i>	<i>CEN4/ARSH4 URA3 CKA2</i>	This paper
RPG52	a/α <i>Δcka1::HIS3/Δcka1::HIS3 CKA2/Δcka2::TRP1</i>	<i>CEN/ARS URA3 GAL10-Dma</i>	This paper
RPG62	a/α <i>Δcka1::HIS3/Δcka1::HIS3 CKA2/Δcka2::TRP1</i>	<i>CEN/ARS URA3 GAL1-Dma GAL10-Dmβ</i>	This paper
JC31-4c	a <i>Δcka1::HIS3 Δcka2::TRP1</i>	2μm <i>URA3 CKA1</i>	This paper
JC35-1b	a <i>Δcka1::HIS3 Δcka2::TRP1</i>	<i>CEN/ARS URA3 GAL1-CKA1</i>	This paper
RPG41-1a	a <i>Δcka1::HIS3 Δcka2::TRP1</i>	<i>CEN4/ARSH4 URA3 CKA2</i>	This paper
RPG52-1c	a <i>Δcka1::HIS3 Δcka2::TRP1</i>	<i>CEN/ARS URA3 GAL10-Dma</i>	This paper
RPG62-6b	a <i>Δcka1::HIS3 Δcka2::TRP1</i>	<i>CEN/ARS URA3 GAL1-Dma GAL10-Dmβ</i>	This paper

^a All strains used are isogenic to YPH250 and carry the additional mutations *ade2-101ochre his3-Δ200 leu2-Δ1 lys2-801amber trp1-Δ1 ura3-52* (42). YPH250, YPH252, and YPH274 were originally called YP6D, YP2A, and YPX, respectively. Strains RPG22-1b and RPG22-2a were obtained by sporulation of RPG22. Strain YDH30 was isolated by microdissection of a zygote from a mating between RPG22-1b and RPG22-2a.

to be highly conserved, since the *Drosophila* enzyme is capable of rescuing haploid yeast strains which lack both the *CKA1* and the *CKA2* genes.

MATERIALS AND METHODS

Yeast strains and media. The strains of *S. cerevisiae* used in this study are listed in Table 1. Yeast strains were routinely grown in either rich glucose medium (YPD medium [1% yeast extract, 2% peptone, 2% D-glucose]) or rich galactose medium (YPGal medium [1% yeast extract, 2% peptone, 2% D-galactose]). Other media were as described by Sherman et al. (41). Solid media contained 2% agar. All medium components were from Difco Laboratories.

Cloning and sequencing of *CKA2*. Oligonucleotide probes were synthesized at the University of Georgia Molecular Genetics Instrumentation Facility on an Applied Biosystems DNA synthesizer. The probes were 5' end labeled as described by Woods et al. (49) and used to probe blots of *S. cerevisiae* genomic DNA that had been digested with a battery of restriction enzymes. Conditions under which probe SCA2-P5 gave single bands were empirically determined. Nitrocellulose filters were hybridized in 10% formamide–5× SSC–5× Denhardt solution–50 mM sodium phosphate (pH 7.4)–0.2% sodium dodecyl sulfate at 42°C for 24 h (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate; 1× Denhardt solution is 0.02% Ficoll–0.02% polyvinylpyrrolidone–0.02% bovine serum albumin). The filters were then washed in 0.5× SSC–0.1% sodium dodecyl sulfate and autoradiographed. Probes SCA2-P6 and SCA2-P7 were hybridized and washed in the presence of tetramethylammonium chloride as described previously (8).

Probe SCA2-P5 was used to screen an *S. cerevisiae* genomic library in λgt11 (a gift from M. Snyder, Yale University) under the conditions described above. The four independent clones isolated were partially restriction mapped. A 2.4-kilobase (kb) *BglII-EcoRI* fragment of clone

SCA2-1 (Fig. 1) was subcloned into mp18 and mp19 (50), subjected to the deletion procedure of Dale et al. (11), and sequenced by the dideoxy-chain termination method (36) with [α-³⁵S]dATP and the Sequenase kit (United States Biochemical Corp.).

Chromosome mapping. *S. cerevisiae* chromosomes were separated either by orthogonal field alternation gel electrophoresis (6) or contour-clamped homogeneous electric field gel electrophoresis (9) as previously described. Yeast strain YP148 (courtesy of Phil Hieter, Johns Hopkins University) was used to resolve chromosomes VII and XV (chromosome VII is fragmented at *rad2* in this strain). Separated chromosomes were transferred to nitrocellulose and hybridized with either the 2.3-kb *EcoRI* fragment of λJC7 (*CKA1* [8]) or the 2.4-kb *BglII-EcoRI* fragment of clone SCA2-1 (*CKA2*). Filters were hybridized at 42°C in 50% formamide–5× SSC–5× Denhardt solution–50 mM sodium phosphate (pH 7.4)–0.2% sodium dodecyl sulfate–100 μg of sonicated and denatured salmon sperm DNA per ml. Filters were washed at 65°C in 0.5× SSC–0.1% sodium dodecyl sulfate and autoradiographed.

Disruption of *CKA2*. The strategy used to disrupt the *CKA2* gene is diagrammed in Fig. 2. An mp18 deletion clone that began close to the 3' end of the gene was chosen, and replicative form DNA was prepared (28) and digested with *HindIII* and *EcoRI*, both of which cut within the mp18 polylinker. The resulting fragment was isolated from an agarose gel with NA-45 DEAE paper (Schleicher & Schuell, Inc.; application update 364). The ends of the DNA were then repaired by incubation with the Klenow fragment of *Escherichia coli* DNA polymerase I, and the blunt-ended DNA was cloned into *SmaI*-cut pRS304 (42). The ligated DNA was then transformed into *E. coli* DH5α (Clontech Laboratories, Inc.), and white colonies were selected. Plasmid DNA was prepared and restriction mapped to determine the orientation of the insert. One transformant (pRS304-3) with the insert in the desired orientation was chosen for further

FIG. 1. Isolation of the *CKA2* gene. (A) Oligonucleotide probes used to isolate the *CKA2* gene. Amino acids in parentheses were not directly determined. Met-1 was assumed to have been posttranslationally removed, since proline was the first amino acid determined. Glu-32, Trp-34, and Glu-37 were assigned on the basis of homology with *CKA1* and the *Drosophila* alpha subunit (Fig. 4). (B) Mismatches between probe Sca2-P5 and the *CKA2* gene. Mismatches are shown in lowercase boldface letters. (C) Partial restriction map of the genome surrounding the *CKA1* gene (determined in part by genomic Southern analysis), partial map of λgt11 clone Sca2-1, and sequencing strategy. The thick black bar represents the coding region of the *CKA2* gene. Bs, *Bst*EII; Bg, *Bgl*II; C, *Cla*I; D, *Dra*I; E, *Eco*RI. Sites in parentheses are synthetic linkers added during cDNA cloning.

processing. An mp18 clone containing the entire 2.4-kb *Bgl*II-*Eco*RI fragment was digested with *Pst*I (which cuts within the mp18 polylinker) and *Cla*I. The resulting fragment was isolated from an agarose gel as before and cloned into *Pst*I-*Cla*I-cut pRS304-3. Transformants were isolated, and miniprep plasmid DNA was subjected to restriction analysis to identify the desired construction (pRP7). pRP7 was linearized by digestion with *Pst*I and used to transform yeast.

Yeast strains YPH274 and JC4 (see Table 1 for genotypes) were transformed by the lithium acetate method (41). Trp⁺ transformants were selected, and genomic DNA was prepared (17), digested with diagnostic restriction enzymes, and subjected to Southern blot analysis with the 2.4-kb *Bgl*II-*Eco*RI fragment as a probe. Filters were hybridized and washed as described above for chromosome mapping. Strains that showed the predicted bands were chosen for further analysis.

Tetrad analysis and other yeast genetic manipulations were performed by published procedures (41).

Construction of complementing plasmids. To construct plasmid pRS316-1, an mp18 subclone of the 2.4-kb *Bgl*II-

*Eco*RI fragment of Sca2-1 was cleaved with *Hind*III (which cleaves in the mp18 polylinker) and *Eco*RI and ligated into *Hind*III-*Eco*RI-digested pRS316 (42). pRS316-1 contains the protein-coding region of the *CKA2* gene plus at least 500 base pairs (bp) of both 5'- and 3'-flanking sequences. Plasmid pJCW11 was constructed by ligating the 2.3-kb *Eco*RI fragment of λJC7 (8) into *Eco*RI-cut YEp352 (16). pJCW11 contains the protein-coding region of the *CKA1* gene plus approximately 500 bp of both 5'- and 3'-flanking sequences. Plasmid pJCG32 was constructed by isolating a 1.78-kb *Afl*II-*Eco*RI fragment from λJC7, repairing the ends with the Klenow fragment, and ligating the fragment into *Bam*HI-cut and repaired pBM272. Vector pBM272 is identical to pBM150 (20) except for the addition of a *Hind*III site just distal to the *Bam*HI site (M. Johnston, personal communication). Transformants were isolated, and the orientation of the insert was determined by restriction analysis. Plasmid pJCG32 contains a 5'-truncated *CKA2* gene (*Afl*II cleaves at a site 61 bp upstream of the start site of translation) under control of the *GAL1* promoter.

Plasmids yielding *GAL*-regulated expression of *Droso-*

FIG. 2. Scheme for disruption of the *CKA2* gene. Symbols: ▨, 5'-flanking region; ■, coding region; ▩, 3'-flanking region of the *CKA2* gene. Restriction sites in parentheses are not found in the yeast genome but are present on the plasmids used to obtain the DNA fragments employed in the construction. The final linear DNA fragment that is used to transform yeast contains approximately 20 nucleotides at the 5' end and 8 nucleotides at the 3' end that are not present in the yeast genome. These do not significantly affect integration of the fragment at the *CKA2* locus.

phila casein kinase II subunits were constructed as follows. The *EcoRI* inserts from λ gt11 recombinants carrying either a *Drosophila* alpha (Dm92) or beta (Dm98) subunit cDNA (37) were isolated and cloned into the *EcoRI* site of pBM272. Recombinant plasmids bearing each cDNA in the desired orientation were identified by restriction analysis. Plasmids pDH1 (*Drosophila* alpha) and pDH3 (*Drosophila* beta) contained the respective cDNA in the correct orientation to allow galactose-dependent transcription from the *GAL10* promoter. The *EcoRI* fragment containing the *Drosophila* alpha cDNA was then ligated into *Bam*HI-cut pDH3 after the ends of both fragment and vector had been repaired. Recombinant plasmids containing the alpha cDNA in the desired orientation were determined by restriction analysis. Plasmid pDH5 contains the alpha cDNA in the correct orientation to allow transcription from the *GAL1* promoter.

Flocculation assay. Yeast strains were inoculated into 2 ml each of glucose (YPD) medium and galactose (YPGal) medium at an initial A_{600} of 0.05 and grown for 16 h at 30°C with

vigorous shaking. For each culture, flocculated cells were separated from free cells by removing the culture tube from the incubator and allowing it to stand at 23°C for exactly 5 min. Free cells were estimated by removing a small sample of the overlying suspension and determining the A_{600} of an appropriate dilution. The total number of cells was then estimated by adjusting the culture to 20 mM EDTA (pH 8.0), vortexing vigorously for 10 s to disperse all flocs, and determining the A_{600} of an appropriate dilution. The percent flocculation was then calculated as $100 \times [(A_{600} \text{ total} - A_{600} \text{ free})/A_{600} \text{ total}]$.

RESULTS

Isolation and sequencing of the *CKA2* gene. On the basis of information derived from N-terminal amino acid sequencing of the alpha' subunit of *S. cerevisiae* casein kinase II (30), two degenerate 17-base and one unique 44-base oligonucleotide probes were synthesized (Fig. 1). The codons used in the unique 44-nucleotide probe (ScA2-P5) were selected on the basis of codon usage in *S. cerevisiae* (40). Each probe was hybridized to Southern blots of yeast genomic DNA cut with a battery of restriction enzymes. A single prominent signal was detected in each digest, and the patterns obtained with all three probes were identical (data not shown). Probe ScA2-P5 was chosen to screen an *S. cerevisiae* genomic library in λ gt11 (a gift from M. Snyder, Yale University). Restriction mapping of four clones (ScA2-1 to ScA2-4) indicated that they were independent but contained overlapping yeast DNA fragments (not shown). A 2.4-kb *Bgl*II-*Eco*RI fragment from clone ScA2-1 was subcloned into mp18 and mp19 and sequenced as described in Materials and Methods. The sequencing strategy is outlined in Fig. 1C.

Figure 3 shows the nucleotide sequence of the entire 2.4-kb fragment together with the deduced amino acid sequence. The restriction fragment contains an open reading frame of 1,017 nucleotides and encodes a protein of 339 amino acids with a calculated molecular mass of 39,408 daltons. The deduced amino acid sequence of the open reading frame matches perfectly the N-terminal amino acid sequence determined by direct sequencing of the alpha' protein (30), confirming that the gene encodes the alpha' subunit of *S. cerevisiae* casein kinase II. A stop codon is located 18 nucleotides upstream from the first methionine residue in the deduced amino acid sequence, consistent with translation initiating at that methionine. The restriction fragment does not contain other open reading frames of any significant length. The same restriction fragment also contains 520 nucleotides of 5'-flanking DNA and 863 nucleotides of 3'-flanking DNA. Figure 1B compares the actual nucleotide sequence with probe ScA2-P5 and shows that 37 of 44 nucleotides were correctly inferred. The usage of codons in the *CKA2* gene suggests that it may be expressed at low levels (40).

Alignment of alpha subunit sequences. Examination of the deduced amino acid sequence shows that the protein encoded by the *CKA2* gene shares extensive homology with other casein kinase II catalytic subunits. An alignment between the available sequences is shown in Fig. 4. Arrowheads in the figure indicate amino acid residues that are conserved in all protein kinases (13). Note that there are two casein kinase II-specific changes in these otherwise essentially invariant residues: valine at position 64 (V64; numbering relative to *Drosophila melanogaster*), in place of the alanine present in all other protein kinases except for KIN2 and HSVK, and W174 in place of F174, which is currently

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AGATCTACATACTGAATGAATTGTCCCTCAACTCTGAAGTTGATTACTTGTCTGAGGTCGATTGCTCCAAAGTTGGATATCCCTAATGACCAAACTTCCCTGCTTTCCGTAAGAGTA 120
AAATTTAGTGTGCATAATTTCCGTGACATCAAAAGTCAGGCGAATGGTGAATGTGTCCCTTTCAAGGCAGTCTATCATTTCTTAGTTACCTGCCAAGAGGCTTTATCAGGCTCAA 240
AGAAAAAATTTCCCTTGGTAGCCCGCTTTTCGCCGAACCTGACAAAGCAACAGACGATCGAAAAATTTCCAACGATCTTATCTAGGTGGAAGTTATAAAAAGAAGATAAAGCATCG 360
TCAACCTTTTTAACTGAAATGGGAAACAACGGGCACACATTTGTTGGAGTTTGTATGATGCTAAAAAATAAAAGTAATAAAAAACATCATAGAGTTGTAAGGTCTCAGGGAAA 480
TAGAAGGAACAATAAACCTAAAAGAATAGAAGAAACAGAATGCCATTACCTCCGTCACATTGAACCCAGAAATCTAATAGAGTCTACTCTGTAGCTAGGGTGTACAAGATGCCTGCCAG 600
M P L P P S T L N Q K S N R V Y S V A R V Y K N A C E 27
GAGAGACCACAAGAATACTGGGACTACGAACAAGGGGTGACCAATGATTGGGAAAGATTTCGAATTACGAAATATCAACAAAATTTGGAAGAGGAAATATTCGGAAGTGTTCAGCGGT 720
E R P Q E Y W D Y E Q G V T I D W G K I S N Y E I I N K I G R G K Y S E V F S G 67
AGATGTATTGTAACAACCAAGTGTGTTTATAAGTTTAAAAACAGTTAAAAATGAAAAAATTTATAGAGAGTTGAAAAATCTGACCAATCTAACAGGCGGCCCAATGTTGTTGGC 840
R C I V N N Q K C V I K V L K P V K M K K I Y R E L K I L T M L T G G P N V V G 107
CTTTATGATATAGTACAAGCGTGACTCCAAATACCTGCTTTGATCTTTGAGGAAATCAAAAATGTGATTTCAGAACTTTATATCTACATTCAAACCTCTGACATCCAGTATTAT 960
L Y D I V Q D A D S K I P A L I F E E I K M V D F R T L Y P T F K L P D I Q Y Y 147
TTCACGCAATTATTGATTGCGTTAGACTACTGTCACTCCATGGGCATAATGCACAGAGACGTAAGCCCTCAGAATGTGATGATGCTACGGAACGTAACCTAAGGCTGATCGATTGG 1080
F T Q L L I A L D Y C H S M G I M R D V K P Q N V M I D P T E R K L R L I D W 187
GGCCTGGCGGAGTTCTACCATCCAGGTGTAGATTACAACGTTCTGTCGCTTCGCGTTACCCACAAGGACCCAGAACTTTTAGTAACTTGAACCAATATGACTACTCCCTAGACTTATGG 1200
G L A E F Y H P G V D Y N V R V A S R Y E K G P E L L V N L M Q Y D Y S L D L W 227
TCAGTAGGATGCTAGCAGCTATGCTCTTCAAAAAGAACCTTTTTCAAAAGGGTCGCTAATCCAGATCAACTGGTAAAGATTGCCACAGTACTAGGAACCAAGGAAGCTGTTAGGC 1320
S V G C M L A A I V F K K E P F F K G S S M P D Q L V K I A T V L G T K E L L G 267
TATTGGGTAAAGTACGGGTTGCATTTACCATCTGAATACGACAACATTATGAGAGACTTTACAAAAAATCGTGGACACACTTTATAACCTCCGAGACCAATAGCTGTTCTGAAAGTG 1440
Y L G K Y G L H L P S E Y D N I M R D F T K K S W T H L L T S E T K L A V P E V 307
GTTGATTAACTGACAATTTATTAAGGTATGACCATCAAGAAAGATTAACAGCAAGGAGGCTATGGATCATAAGTTTTTCAAAACGAAGTTTGAATAATGGACAACAATACTCTTAGC 1560
V D L I D N L L R Y D H Q E R L T A K E A M D H K F F K T K F E * 339
AAGGCAATCTTTTTCCACCAGTATTTCCATTATATAAAAGCAGGGGTTTTCTTTTTTGTAGCTTGATATTAGAGAATATCCTTAATGTGAATATTAATGCAAACTCTATTTAACA 1680
ACGAATGATAGATTATATATGACCAGAAAATCACTTTATATAGATATAAGAGCCTTCGGTAATGCTATGCAAGTTTTGATGAATTATATATTGTCGTGAGGCGTGTCCGAGGAGTTTC 1800
GTCGCCATTGAGATAGAGATGCTCGTAATCGTACGTGTGGAAGTGCCTGCCGACGGTAGCGATGTCTACTCTCGTCCCAGACAATGGAATAGTTTTGAAATGTTTCTACATCATTAT 1920
TAGAACGCAGATTACGAAATCTCCACGTTTGCAATATCTCGCCTACGAACAGTGTCAATGATATGAAAGCTGAGAGAGCCATTACCTTTTGAGTAAATACGCTGCCTTT 2040
TTTCAGCAATGAAGAACCACCTCCCTCCTTTCTTACATCCAATAATGCTTCCCTTGTTCTTTATATCCCCATAGGAAAGTCCGTTGGAGTAGGTTGCTCTCTTTTCATGTATTCCAAC 2160
CAGATCAGGTCCGTAAGAACAGTAACGCTATTTCTTTATTCAGTTTATGCTTAGTGGATCAATTTGTATTATCTAAATGAAAGTGCCTTTATGGACCACTCTCCATATACAATG 2280
ATACAACCTAGCCATATATATGCTATAGTCAAACCTATTTCAGAGAGCAACTGGGAACCTCAATAATTTATGCTTGCCTATTTCAGCAACAGCGCCAGGTAGTGACACegaattc2401

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FIG. 3. Sequence of the *S. cerevisiae* *CKA2* gene. The nucleotide sequence of the 2.4-kb *Bgl*III-*Eco*RI fragment of λ gt11 clone ScA2-1 is shown, together with the deduced amino acid sequence of the encoded protein. Underlined amino acids indicate matches with the previously determined protein sequence (30). A potential TATAAA box and an in-frame translation stop codon located upstream of the initiating methionine are also underlined. The square brackets delineate the portion of *CKA2* deleted in the construction used to disrupt the gene (Fig. 2). Lowercase letters indicate a synthetic *Eco*RI linker. This sequence information has been submitted to the GenBank data base under accession number M33759.

believed to be unique to casein kinase II (8, 13). These changes may contribute to the unusual ability of casein kinase II to utilize GTP in addition to ATP as a phosphoryl donor.

The boxed areas in Fig. 4 show residues that are conserved among all of the available casein kinase II catalytic subunits. Of particular interest are two short blocks of sequence identity, ARVY and EYWDYE, which lie near the N terminus. Since these two sequence elements lie outside of the catalytic domain per se, their conservation presumably reflects some other critical function, perhaps interaction with the beta subunit. Also of interest is the highly basic motif, KKKIKR. This sequence is located just C terminal to the essential catalytic lysine (residue K66 of the *Drosophila* sequence) and has been postulated to form part of the binding site for protein substrate (8). The fact that the *CKA2* gene product contains two nonconservative replacements in this motif (KMKKIYR) and yet functions as the sole catalytic subunit in *S. cerevisiae* (8) indicates that at least two of these lysines are nonessential. Overall, 49% of the residues are identical in all of the sequences shown in Fig. 4 (ignoring the large insertion in the yeast alpha subunit and the C-terminal extensions present in several of the sequences). Pairwise comparisons yield the following percent identities: 60% (*CKA1* versus *CKA2*); 61% (*CKA1* versus *Drosophila* alpha); 54% (*CKA2* versus *Drosophila* alpha); 86% (*Drosophila*

phila alpha versus human alpha); 99% (rat alpha versus human alpha), 84% (human alpha versus human alpha'). If conservative changes are considered, the sequence similarity is even greater.

The available sequence data allow a comparison between alpha and alpha' sequences in two species, *S. cerevisiae* and humans (Fig. 4). Rather surprisingly, pairwise comparisons fail to reveal a clear-cut relationship between the two alpha sequences on the one hand and the two alpha' sequences on the other. In addition, the difference in molecular weight between the human alpha and alpha' subunits is due to the presence or absence of a C-terminal extension, while in the yeast case it is due to an insertion or deletion located between subdomains III and IV (8). These observations suggest that distinct gene duplication events may be responsible for the catalytic subunit heterogeneity in these two species.

Chromosomal location of the *CKA1* and *CKA2* genes. The *CKA1* gene was localized to chromosome IX and the *CKA2* gene was localized to chromosome XV by hybridization of genomic clones to electrophoretically separated yeast chromosomes (see Materials and Methods). The *CKA1* gene has been further localized as a result of recently published DNA sequencing studies of the *CAP2* gene (2). The DNA sequence upstream of *CKA1* (8) overlaps extensively with that downstream of *CAP2*. The sequence upstream of *CAP2* in

FIG. 4. Alignment of casein kinase II catalytic subunit sequences. Shown are the amino acid sequences of the following casein kinase II catalytic subunits: *S. cerevisiae* alpha (CKA1) (8), *S. cerevisiae* alpha' (CKA2) (this paper), *Caenorhabditis elegans* (C.elg) (18), *Drosophila melanogaster* (D.mel) (37), calf (Bovine) (45), rat alpha (26), human alpha (Human1) (26), and human alpha' (Human2) (Lozeman and Krebs, personal communication). Residues conserved in all casein kinase II catalytic subunits are shown boxed. Residues conserved in virtually all protein kinases are indicated by arrowheads (13). Symbols: -, deletion; . . ., partial sequence data; *, the C terminus. Numbers above the alignment correspond to the *Drosophila* alpha subunit.

turn overlaps with that downstream of *SRA1* (*BCY1*, *REG1* [2]). All three genes are thus transcribed in the same direction, with the intergenic region between *SRA1* and *CAP2* being 324 bp long and that between *CAP2* and *CKA1* being 317 bp long. The *SRA1* gene has been genetically mapped to chromosome IX between *lys11* and *his6*, 9 centimorgans from the latter (5). The *CKA1* gene must map within a few centimorgans of this location.

Disruption of the *CKA2* gene. One-step gene replacement was used to determine the phenotype of cells disrupted at the *CKA2* locus. The scheme outlined in Fig. 2 was used to obtain a linear disruption fragment. The construction results in the almost complete elimination of the protein-coding region (Fig. 3) and its simultaneous replacement by the entire sequence of pRS304, including the *TRP1* selectable marker. Disruption of *CKA2* was carried out both in a

TABLE 2. Plasmids that rescue the lethal phenotype

Strain	Plasmid	Plasmid genotype	Viability in medium containing:		% Flocculation ^a in medium containing:	
			Glucose ^b	Galactose ^b	Glucose	Galactose
RPG41-1a	pRS316-1	<i>CEN4/ARSH4 URA3 CKA2</i>	+	+	21 ± 1	5 ± 1
JC31-4c	pJCW11	2 μ m <i>URA3 CKA1</i>	+	+	2 ± 6	1 ± 3
JC35-1b	pJCG32	<i>CEN4/ARS1 URA3 GAL1-CKA1</i>	+ ^c	+	96 ± 6 ^d	5 ± 1
RPG52-1c	pDH1	<i>CEN4/ARS1 URA3 GAL10-Dma</i>	—	+	99 ± 1	0 ± 1
RPG62-6b	pDH5	<i>CEN4/ARS1 URA3 GAL1-Dma GAL10-Dmβ</i>	—	+	99 ± 1	4 ± 1

^a Percent flocculation following 16 h of growth at 30°C, calculated as described in Materials and Methods. The mean $\pm$ standard deviation for two identical experiments is shown. For comparison, the percent flocculation of a wild-type control strain (YPH250, [Table 1]) was 9 $\pm$ 1 on glucose and 0 $\pm$ 3 on galactose.

^b Glucose indicates YPD medium; galactose indicates YPGal medium.

^c Viable but exhibited slow growth. See text.

^d Gross flocculation of JC35-1b in glucose medium did not occur by 16 h. The original 16-h culture was diluted 1:100 in fresh glucose medium and grown for an additional 22 h at 30°C before being assayed.

diploid homozygous for the wild-type *CKA1* gene and in a diploid homozygous for a deletion of the *CKA1* gene (strains YPH274 and JC4, respectively [Table 1]). In each case, disruption of the *CKA2* gene on one homolog was confirmed by Southern hybridization (not shown).

Sporulation and tetrad analysis of strain RPG22 (*CKA1/CKA1 CKA2/ Δ cka2::TRP1*) resulted in four viable spores that showed 2:2 segregation for the *CKA2* disruption marker (*TRP1*). The *CKA1* Δ cka2::*TRP1* haploids showed no detectable phenotype with respect to growth rate in rich medium, resistance to heat shock, resistance to nitrogen starvation, or ability to mate. In addition, homozygous Δ cka2::*TRP1* diploids were readily constructed by mating disrupted haploids of opposite mating types, and these strains also displayed a wild-type phenotype, including normal sporulation behavior. In all these tests, the Δ cka2 strains behaved essentially identically to Δ cka1 strains (8), which also give no detectable phenotype when disrupted in a background wild type for *CKA2*. More stringent studies may reveal subtle phenotypic effects associated with disruption of one or the other of these genes.

In contrast, sporulation and tetrad analysis of RPG13 (*Δ cka1/ Δ cka1 CKA2/ Δ cka2::TRP1*) resulted in two viable and two inviable spores in 22 of 22 complete tetrads analyzed. In no case were any *Trp*⁺ spores found, indicating that the *CKA2* disruption is lethal in this background and hence that casein kinase II is essential for viability. Microscopic examination of the dissection plates revealed that the nonviable spores had gone through approximately two or three rounds of mitotic division before arresting. Such microcolony formation is frequently associated with disruption of essential genes in *S. cerevisiae* (12, 33, 48).

Rescue of haploids carrying the lethal double disruption. A number of different plasmid constructions were tested for their ability to rescue the lethal phenotype described above (Table 2). The process is outlined in Fig. 5 with the *CKA2* gene in a *CEN* plasmid as an example. Identical experiments were performed with all the other plasmids described in Table 2. Each plasmid was transformed into RPG13, and the resulting strains were subjected to tetrad analysis.

RPG41 was constructed by transforming RPG13 with pRS316-1, a *Ura3*-marked plasmid carrying the 2.4-kb *Bgl*III-*Eco*RI fragment of λ clone Sca2-1, i.e., carrying the entire coding region of the *CKA2* gene plus 500 to 1,000 bp of both 5'- and 3'-flanking sequences (Fig. 2). *Ura*⁺ transformants were selected and subjected to tetrad analysis. The results obtained for any given tetrad are obviously a function of how the plasmid segregates at each meiotic division (e.g., Fig. 5). Of eight tetrads analyzed, four gave four viable spores, while

the others gave two viable and two inviable spores. All viable colonies were tested for both the *CKA2* disruption marker (*TRP1*) and the plasmid marker (*URA3*). As expected, all viable *Trp*⁺ colonies were also *Ura*⁺. The fact that this plasmid is capable of complementing the *CKA2* disruption confirms that the lethal phenotype (in the Δ cka1 background) is in fact due to disruption of the *CKA2* gene (as opposed to some perturbation of a nearby gene on the chromosome). The result also suggests that the 2.4-kb *Bgl*III-*Eco*RI fragment contains the *cis*-acting regulatory elements necessary for transcription of the gene.

Similar rescue experiments were carried out with the *CKA1* gene with two different constructions (Table 2). The *CKA1* gene was placed on either a multicopy plasmid under the control of its own promoter (pJCW11) or a *CEN* plasmid under the control of the *GAL1* promoter (pJCG32). By using the scheme outlined in Fig. 5, rescue was obtained with both constructions. Conclusions similar to those just outlined for the *CKA2* gene thus apply.

The ability of *Drosophila* casein kinase II to complement the lethal phenotype of the yeast double disruption was also tested. For this purpose, two different *GAL* promoter plasmids were constructed, one carrying the *Drosophila* alpha and beta subunit cDNAs (pDH5) and the other carrying the alpha subunit cDNA alone (pDH1 [Table 2]). Successful rescue was obtained with both constructions, and the rescued strains exhibited normal morphology and growth rate. These results demonstrate that *Drosophila* casein kinase II is functionally equivalent to its yeast counterpart, at least with regard to all essential functions in *S. cerevisiae*.

The viability of all of the strains listed in Table 2 was plasmid dependent, as shown by growing each strain nonselectively for several days in either YPD or YPGal medium, isolating single colonies on plates, and then testing them for the presence of the *URA3* marker. In contrast to isogenic strains harboring a chromosomal copy of either *CKA1* or *CKA2*, in which fewer than 50% of the colonies typically remain *Ura*⁺, all of the strains in Table 2 exhibited 100% retention of the plasmid.

With the exception of pRS316-1, all of the constructions listed in Table 2 are expected to yield overexpression of the encoded polypeptide(s). For each of the three *GAL*-regulated constructions, overexpression was confirmed by Western blotting (immunoblotting) of whole-cell extracts (data not shown). The apparently normal phenotype of the rescued strains suggests that overexpression of either a free casein kinase II catalytic subunit (yeast alpha or *Drosophila* alpha) or the casein kinase II holoenzyme (*Drosophila* alpha plus beta) does not have any deleterious effects in this

FIG. 5. Rescue of lethal phenotype by plasmid-borne casein kinase II genes. Strain RPG13 was constructed by transforming strain JC4 with *Pst*I-cut pRP7 (Fig. 2). Doubly disrupted haploids derived from this strain are inviable (at left). Doubly disrupted haploids rescued by plasmid-borne casein kinase II genes were constructed by transforming RPG13 with various plasmid constructions (Table 2) followed by sporulation of the resulting transformants (at right). The procedure is illustrated for the case of pRS316-1, a *CEN*-based plasmid bearing the wild-type *CKA2* gene. A tetrad displaying favorable segregation of the *CEN* plasmid is shown, but this is only one of several possible outcomes. Strains transformed with plasmids carrying genes under the control of the *GAL* promoters were sporulated and grown on media containing galactose in place of glucose.

genetic background. Overexpression from the same three *GAL*-regulated constructions has also been carried out in an isogenic wild-type background, again without any obvious phenotypic consequences (unpublished results).

Phenotype associated with casein kinase II depletion. To obtain insight into the function of casein kinase II, the phenotype of cells arrested because of depletion of this activity was examined. Casein kinase II depletion was accomplished in three distinct ways: (i) by microdissection of strain RPG13 to yield spores lacking an intact catalytic subunit gene, (ii) by spontaneous loss of the rescuing plasmid from one of the strains shown in Table 2, and (iii) by shifting a strain rescued by a *GAL*-regulated construction to medium containing glucose. Except for the number of cell divisions completed before arrest, the consequences of casein kinase II depletion appear to be identical in each case.

Typical cell morphologies observed following arrest are illustrated in Fig. 6, which shows microcolonies formed following spontaneous plasmid loss from strain RPG41-1a (a strain rescued by a single-copy plasmid carrying the *CKA2* gene under control of its own promoter [Table 2]). The cells within a microcolony are greatly enlarged, having diameters at least two times, and hence volumes at least eight times, larger than those of the wild type. Many but not all of the cells are budded, and a variety of aberrant morphologies is observed. Perhaps most striking is a significant proportion

(10 to 30%) of pseudomycelial forms having a long bud. The buds in these cases occasionally contain multiple constrictions, but branched forms are rare. Unfortunately, we have been unable to analyze the nuclear morphology of casein kinase II-depleted cells because such cells exhibited a strong surface staining with DAPI (4',6'-diamidino-2-phenylindole). This artifact was not observed with control cells and may be related to changes in the cell wall responsible for flocculation (see below). The number of cells per microcolony formed by strain RPG41-1a varied from a few to a few hundred, the variability presumably reflecting the number of cell divisions between plasmid loss and isolation of the cell on the plate. The largest colonies observed indicate that in log-phase growth seven or eight cell divisions were required to deplete the mRNA and *CKA2* protein generated from a single copy of the *CKA2* gene. The four- to eight-cell microcolonies formed upon sporulation of RPG13 exhibited an identical array of morphologies (not shown).

By using the three strains that are rescued by *GAL*-regulated constructions (Table 2), it should be possible to deplete casein kinase II activity simultaneously in all cells of a culture via carbon source shift. To this end, all three strains were grown on rich galactose plates (YPGal agar) and then transferred to rich glucose plates (YPD agar). Somewhat surprisingly, one of the three strains (JC35-1b) failed to arrest when grown on YPD agar. This strain is discussed

FIG. 6. Morphology of cells arrested by casein kinase II depletion. Strain RPG41-1a, whose survival is dependent on a plasmid-borne copy of the *CKA2* gene (Table 2), was grown nonselectively in YPD medium for 24 h and then diluted 1:10 in sterile H₂O and spread on a layer of YPD agar on a microscope slide. The cells were allowed to grow for 2 days at 30°C and were then examined microscopically for the presence of microcolonies formed as the result of casein kinase II depletion following spontaneous loss of the rescuing plasmid. The frequency of microcolony formation was approximately 1 to 5%, consistent with the expected frequency of loss of a *CEN* plasmid. Microcolony formation was not observed in isogenic wild-type strains carrying the same plasmid. (A) An undisturbed microcolony flanked by two normal colonies. (B) A second microcolony and nearby normal colony, both of which have been micromanipulated to better display the cellular morphologies. Cells were photographed on Tri-X pan 400 film (Kodak) under bright-field illumination. Bar = 50 μ m.

further below. The other two, RPG52-1c and RPG62-6b, arrested after generating minicolonies consisting of a few thousand cells. The larger size of these colonies presumably reflects the larger initial pool of mRNA and enzyme, the kinetics with which *GAL*-dependent transcription is silenced, or both. Cells arrested in this fashion displayed the same array of morphologies already described.

Carbon source shifts were also carried out in liquid medium and revealed an additional phenotype associated with casein kinase II depletion. Approximately 12 to 18 h after a shift to YPD medium, both RPG52-1c and RPG62-6b underwent massive aggregation and ultimately precipitated

in the culture tube, yielding an essentially clear overlying medium (Table 2). Aggregation occurred before the morphological changes associated with growth arrest, which did not become evident until approximately 24 h after the shift. We identified this aggregation behavior as flocculation, as opposed to mating-type agglutination, on the basis of the following criteria: (i) the aggregation did not require mixing of opposite mating types, (ii) the aggregates readily reformed after their dispersal by mechanical agitation (vigorous vortexing or mild sonication), and (iii) the aggregates were sensitive to 20 mM EDTA but not to 0.1% sodium dodecyl sulfate or 30 mM dithiothreitol (23). Flocculation of RPG52-1c and RPG62-6b was not observed when either strain was grown in YPGal medium (Table 2) or when isogenic but *CKA2*⁺ strains harboring the same plasmids were grown in YPD medium. We presume that the same behavior also occurs in microcolonies produced by sporulation or plasmid loss, but this is not readily testable.

As noted above, strain JC35-1b, which carries a *GAL*-regulated copy of the *CKA1* gene, failed to arrest when grown on YPD agar. The morphology of the cells remained essentially normal, and the strain could be serially transferred on YPD plates for at least 3 weeks. However, JC35-1b did display two phenotypic deficits in YPD medium: (i) the growth rate was greatly reduced (as judged by colony size on plates), and (ii) when grown in liquid medium, the strain exhibited the same flocculation behavior as RPG52-1c and RPG62-6b (Table 2). Neither effect was seen when the same plasmid construction was present in a wild-type background. Because the survival of JC35-1b in YPD medium remained plasmid dependent, some residual transcription of the *CKA2* gene must have been occurring, sufficient to allow for survival but insufficient to achieve a normal growth rate or prevent flocculation. Both the growth deficit and the flocculation were reversed when the cells were returned to galactose medium.

DISCUSSION

We report here the cloning, sequencing, and disruption of the *CKA2* gene, which encodes the α' subunit of casein kinase II in *S. cerevisiae*. We also demonstrate that casein kinase II is required for viability in *S. cerevisiae* and that the function of the enzyme appears to be conserved between evolutionarily divergent species.

Consistent with biochemical and molecular studies described previously (8, 30), the results presented in this paper confirm that *S. cerevisiae* casein kinase II contains two distinct catalytic subunits, α and α' , which are encoded by distinct genes (which we designate *CKA1* and *CKA2*, respectively). Both the biochemical and molecular-genetic data argue against the possibility that a third catalytic subunit gene remains to be discovered. As shown previously for the *CKA1* gene, disruption of the *CKA2* gene in a wild-type background yielded no significant effect on phenotype. In contrast, simultaneous disruption of both genes was lethal. These results imply that the yeast α and α' subunits are functionally equivalent. A number of essential proteins in *S. cerevisiae* appear to be encoded by a small family of functionally redundant genes. For example, the catalytic subunit of cyclic AMP-dependent protein kinase is encoded by three different genes (*TPK1*, *TPK2*, and *TPK3*), all of which must be disrupted before a significant effect on phenotype is observed (46). Of course, the possibility that more subtle tests will eventually reveal functional differences between the α and α' subunits cannot be

excluded. Indeed, phenotypic differences between the three cyclic AMP-dependent protein kinase catalytic subunits become readily apparent when disruptions are carried out in a *bcy1* background (4).

That casein kinase II is essential for viability in *S. cerevisiae* is perhaps the most significant result obtained in the present study. The essential nature of the activity of this kinase was first suggested by the inviability of doubly disrupted haploids derived by sporulation of RPG41. However, it could be argued that the inviability of such haploids is the result of some deficit acquired during meiosis or germination and hence that the enzyme is actually not essential for vegetative growth. In order to exclude this possibility, we constructed doubly disrupted strains carrying a plasmid-borne copy of either *CKA1* or *CKA2*. Such strains exhibited complete retention of the plasmid in the absence of selection, clearly indicating that casein kinase II is essential for mitotic growth. This is true of both haploid and diploid strains. We think it likely that doubly disrupted haploids derived by sporulation of RPG41 arrest mitotically in an identical fashion, i.e., such haploids probably retain sufficient maternal stores of catalytic subunit mRNA, protein, and phosphorylated substrates to complete meiosis, germinate, and carry out two or three mitotic divisions. Whether casein kinase II carries out an essential function in either meiosis or germination (or mating) has thus probably not been tested in the experiments described here and will require additional experiments.

The fact that the enzyme from *D. melanogaster* is capable of restoring viability to yeast cells that lack both endogenous catalytic subunits indicates that the function of casein kinase II, like its structure, has been well conserved over large evolutionary distances. A caveat to be kept in mind when interpreting these results is that the heterologous enzyme from *D. melanogaster* was overproduced, and it remains a possibility that overproduction is a prerequisite for complementation. The *Drosophila* alpha subunit by itself is also capable of complementation, although the same caveat applies. Complementation by the alpha subunit alone suggests either that the *Drosophila* alpha subunit can interact with one or both yeast beta subunits in vivo or that the free alpha subunit possesses sufficient activity to allow rescue. Biochemical studies do suggest that alpha subunits possess residual catalytic activity in the absence of beta subunits (10, 47; D. E. Hanna and C. V. C. Glover, unpublished observations). The functional similarity between the *Drosophila* and yeast enzymes is further underscored by the fact that both *Drosophila* constructions also prevent the flocculation behavior of doubly disrupted strains. Whether this conservation of catalytic function reflects an underlying conservation of a physiological role is unknown. The physiological role of casein kinase II might be more complex or possibly even different in higher eucaryotic systems, for example, as the result of the acquisition by the enzyme of either additional substrates or novel modes of regulation.

In order to probe the physiological role of casein kinase II in *S. cerevisiae*, the phenotype of cells depleted for this activity was examined. With each of three methods employed, cells arrested with similar morphologies following depletion of the activity. Two aspects of the morphology of the arrested cells are of interest. First, the arrested cells were greatly enlarged, suggesting that metabolism and growth continue after cell division is halted. Second, a significant proportion of the cells arrested with a pseudomycelial morphology that resembled the terminal phenotype of some cell division cycle mutants (14, 27, 31, 35). Both

observations suggest that casein kinase II may phosphorylate one or more substrates required for normal cell cycle progression. Such a result would be consistent with the broad substrate specificity of the enzyme as well as with diverse studies indicating an association between casein kinase II activity and cell proliferation (see the introduction to this paper). Construction of yeast strains temperature sensitive for casein kinase II activity will be required in order to explore the possible role of this enzyme in the cell cycle.

The flocculation behavior displayed by casein kinase II mutants provides an additional avenue of approach to the physiological role of the enzyme (for a review of flocculation in *S. cerevisiae*, see reference 3). Mutations which result in gross flocculation of *S. cerevisiae* have been identified in at least three other genes: *FLO1* (19), *SSN6* (also called *CYC8*) (39), and *TUP1* (also called *FLK1*, *CYC9*, and *UMR7*) (39). Flocculating alleles of *FLO1* are dominant, whereas flocculating alleles of *SSN6* and *TUP1* are recessive. *SSN6* and *TUP1* mutants are pleiotropic and exhibit very similar patterns of defects which, in addition to flocculation, include constitutive expression of glucose-repressible enzymes, elevated expression of iso-2-cytochrome *c*, and defects in mating and sporulation. It will be of interest to determine whether these additional phenotypes are also induced by disruption of casein kinase II activity.

The isolation of null mutations in the casein kinase II genes represents an important first step in the genetic analysis of this ubiquitous and conserved protein kinase. Mutants which are temperature sensitive for casein kinase II activity are currently under construction and should facilitate further analysis of the physiological role of the enzyme. The ability of *Drosophila* casein kinase II to substitute for its yeast counterpart suggests that the yeast system will prove a useful model for higher organisms.

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