Temperature-sensitive Mutations of the CKA1 Gene Reveal a Role for Casein Kinase II in Maintenance of Cell Polarity in Saccharomyces cerevisiae*

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Asokan Rethinaswamy, Mark J. Birnbaum‡, and Claiborne V. C. Glover§

From the Department of Biochemistry and Molecular Biology, the University of Georgia, Athens, Georgia 30602-7229

Casein kinase II (CKII) of Saccharomyces cerevisiae contains two distinct catalytic subunits, α and α′, that are encoded by the CKA1 and -2 genes, respectively. We have constructed conditional alleles of the CKA1 gene. In contrast to cka1 cka2ts strains, which exhibit a defect in both G1 and G2/M cell cycle progression, cka1ts cka2 strains continue to divide for three cell cycles after a shift to restrictive temperature and then arrest as a mixture of budded and un budded cells with a spherical morphology. Arrested cells exhibit continued growth, a nonpolarized actin cytoskeleton, delocalized chitin deposition, and a significant fraction of multinucleate cell bodies, confirming the presence of a cell polarity defect in cka1ts strains. The presence of budded as well as un budded cells in the arrested population suggests that CKII is required for maintenance rather than establishment of cell polarity, although a role in both processes is also possible. The terminal phenotype of cka1ts strains bears a strong resemblance to that of orb5 strains of Schizosaccharomyces pombe, which carry a temperature-sensitive CKII catalytic subunit mutation, but the underlying mechanism appears to be different in the two cases. These results establish a requirement for CKII in cell polarity in S. cerevisiae and provide the first evidence for functional specialization of CKA1 and -2.

Casein kinase II (CKII)1 is an essential, highly conserved, acidic-directed protein kinase that is ubiquitous in eukaryotic organisms (1–4). The enzyme is composed of catalytic (α) and regulatory (β) subunits that associate to form an αβ2 holoenzyme. Distinct isoforms of one or both subunits occur in various species, and the three subunits of the mammalian enzyme (α, α′, and β) associate to form distinct αβ2, α′β2, and αα′β2 tetramers in vivo (5). Whether these distinct forms have unique functions is unknown. In vitro, CKII activity is inhibited by polyanions and activated by polycations, but the mechanism of regulation of the enzyme in vivo remains to be defined. CKII phosphorylates a broad spectrum of nuclear and cytoplasmic substrates (6) and has been implicated in the regulation of fundamental cellular processes, including transcription, translation, morphogenesis, and cell cycle regulation. Consistent with a role in the latter process, misregulated expression of CKII activity in lymphocytes of transgenic mice results in the stochastic production of lymphoma and, when combined with e-myc, leukemia (7).

Molecular/genetic techniques have been used to dissect the physiological role of CKII and its subunits in both budding and fission yeast. Purified CKII of Saccharomyces cerevisiae is composed of four subunits, α, α′, β, and β′, which are encoded by the CKA1, CKA2, CKB1, and CKB2 genes, respectively (8). The enzyme is exclusively tetrameric, but which of the nine possible isoforms occur has not been definitively established. Disruption of either catalytic subunit gene yields no obvious phenotype on normal media, but disruption of both is lethal (9, 10). Cells depleted of CKII activity arrest as a mixed population of budded and un budded cells, with a significant proportion of the budded fraction displaying an elongated morphology (10). The continued growth of the arrested cells suggests a cell cycle defect, and analysis of temperature-sensitive alleles of the cka2 gene confirms that CKII function is required for cell cycle progression at two points in the cell cycle, one in G1 and one in G2/M (11). Depletion of CKII activity also results in flocculation, a lectin-mediated, Ca2+-dependent cell-cell aggregation. The significance of this is unknown, but mutations in the global transcriptional repressor, Ssn6/Tup1, display a similar phenotype (12, 13). Rather surprisingly, disruption of CKB1 and/or CKB2 results in no overt phenotype on normal media; however, cells lacking either gene display specific sensitivity to Na+ and Li+ (14, 15).

In contrast to S. cerevisiae, Schizosaccharomyces pombe appears to have a single gene encoding each subunit of CKII (16). Temperature-sensitive mutations of the cka1+ gene encoding the catalytic subunit have been isolated in a screen for morphological mutants (17). These mutations (orb5) are recessive lethal and confer a spherical morphology at the restrictive temperature. Although arrested cells display aberrant actin and tubulin cytoskeletons, analysis of double mutants between orb5 and various cell division cycle mutants indicates that CKII is required not for cell polarity per se but for the reestablishment of polarized growth following cytokinesis. No defects in cell cycle progression were defined for these mutants, and orb5 cells in fact undergo two to three cell divisions prior to arrest. In contrast to S. cerevisiae, null mutations of the ckb1+ gene encoding the regulatory subunit confer a slow growth phenotype, cell-cell aggregation, and cold sensitivity (16). Such mutants also display an abnormal rounded morphology, reminiscent of the orb5 phenotype.

The rather disparate results obtained in the two systems make it difficult to construct a unified model of the physiological role of CKII. In S. cerevisiae, CKII is implicated in cell-cell aggregation, ion homeostasis, cell cycle regulation, and perhaps morphogenesis. In S. pombe, CKII is implicated in cell-cell aggregation and polarized cell growth. Although cell-cell aggregation and morphological abnormalities are common to both

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‡ Present address: Dept. of Biology, Merrimack College, North Andover, MA 01845.
§ To whom correspondence should be addressed. Tel.: 706-542-1769; Fax: 706-542-1738; E-mail: glover@bscr.uga.edu.
1 The abbreviations used are: CKII, casein kinase II; DAPI, 4′,6-diamidino-2-phenylindole; 5-FOA, 5-fluoroorotic acid.

This paper is available on line at http://www.jbc.org
systems, CKII appears to be dispensable for cell growth in *S. cerevisiae* and for cell cycle progression in *S. pombe*. Furthermore, the morphological abnormalities observed in the two systems are opposite in sign, since the elongated phenotype observed in *S. cerevisiae* is indicative of hyperpolarization (18), whereas the spherical morphology of orb5 alleles reflects a loss of polarity (or the ability to grow in a polarized fashion). Although it is formally possible that the orb5 alleles are gain-of-function mutations, the recessive nature of these alleles makes this unlikely (17).

In the course of analyzing conserved elements in the N-terminal region of the CKII α subunit, we have constructed two temperature-sensitive alleles of the *S. cerevisiae* *cka1* gene. We report here that *cka1* mutants display an apolar phenotype, in marked contrast to the cell cycle phenotype displayed by *cka2* strains. Although this phenotype is similar in several respects to that observed in *S. pombe* orb5 mutants, we find that in *S. cerevisiae* CKII function is required not for polarized growth but for cell polarity itself. Our results also provide genetic evidence for functional specialization of the two catalytic subunit isoforms in budding yeast.

**EXPERIMENTAL PROCEDURES**

**Strains, Media, and Growth Conditions—**Principal *S. cerevisiae* strains and plasmids used in this study are listed in Table I. For routine work, yeast strains were grown at 29 °C in YPD medium (1% yeast extract, 2% peptone, 2% d-glucose) or in synthetic complete medium (SC) lacking specific components as necessary (19). Routine molecular cloning was carried out in *Escherichia coli* DH5α (CLONTECH). E. coli strains were propagated at 37 °C in Luria broth containing 50 μg/ml ampicillin as appropriate (20).

**Plasmid Construction—**Routine molecular biology procedures were according toAusubel et al. (20). Synthesis of oligonucleotide primers and sequencing were carried out on Applied Biosystems instruments at the University of Georgia Molecular Genetics Instrumentation Facility.

To construct the *cka1*Δ and *cka1ΔΔ* alleles, the wild-type *cka1* gene contained on the 2.3-kilobase pair *Eco*RI site in pRS315 necessitated a partial digestion with *AflI*, which cleaves in the 5′- untranslated region of the *cka1* gene, and used to transform YDH6 (Table I) to uracil prototrophy. Five colony-purified transformants were selected and grown nonselectively in YPD for 3 days and then plated on YPD at 29 °C. Cells that had lost the *cka2 LEU2* plasmid and only grown from YDH6 were identified by the slow growth rate conferred by the *cka1ΔΔ* allele. Replica-plating confirmed that such colonies were Leu-, Ura+, His+, and temperature-sensitive, consistent with construction of the desired tandem duplication. The duplication was resolved by plating on SC containing 5-FOA. All 5-FOA-resistant colonies were His+ as well as Ura and remained temperature-sensitive. Proper integration of the mutant allele was confirmed by genomic Southern hybridization.

**Cell Biology Protocols—**To assay temperature-sensitive growth, cells were cultured overnight in YPD at 25–29 °C, diluted as necessary, and then spotted in triplicate (2000 cells/10-μl spot) on a series of YPD plates. Plates were incubated at the desired temperatures for 4 days.

For generation of growth curves, cells were grown overnight in YPD at 25–29 °C and then inoculated into fresh YPD at a starting concentration of 1 × 10⁶ cells/ml. Cultures were grown at permissive temperature (either 25 or 29 °C) with vigorous shaking (450 rpm). For temperature shift studies, parallel cultures were shifted to restrictive temperature (either 37 or 38.5 °C) when the culture reached a density of 5 × 10⁶ cells/ml. To determine cell number, aliquots were removed at the indicated times, fixed with 3.7% formaldehyde, and counted in a hemacytometer. Viable cells were determined by plating an appropriate dilution of an unfixed aliquot on YPD at 25 °C. Percent viability was calculated as 100 × (number of colonies)/(cell number).

To study cell morphology and budding pattern, formaldehyde-fixed cells from the growth curves were examined by phase contrast and Nomarski optics. Nuclear morphology was visualized by staining with DAPI (4′,6-diamidino-2-phenylindole) as described by Hanna et al. (11), and chitin distribution was visualized by staining with 0.1 mg/ml calcofluor as described by Pringle (23). Photographs were taken in a Zeiss IM 35 epifluorescence microscope fitted with Nomarski optics. F-actin was visualized by staining with rhodamine-conjugated phaloidin as described (24). Stained cells were examined by epifluorescence microscopy using the DeltaVision System (Applied Precision, Inc.). Digitized images were processed on a Macintosh computer running Adobe Photoshop.

**RESULTS**

The Terminal Phenotype of *cka1*ΔΔ Mutants Is Distinct from That of *cka2*ΔΔ Mutants—Our studies were initiated in an effort to analyze the function of the N-terminal region of the CKII catalytic subunit in vivo. This region is well conserved among different species (Fig. 1), with the highest conservation concentrated in two six-residue motifs (corresponding to the sequences SEARVY and EYWDYE of Cka1). We generated two deletion mutants of the *S. cerevisiae* *cka1* gene, one of which (*cka1ΔΔ*) removed the first element, and the other of which (*cka1Δ*) removed both, together with the intervening sequence (Fig. 1). The ability of these mutants to complement a null background was then tested by transforming each construct into RPG41-1a (Table I) and plating the resultant strains on 5-FOA to eliminate the original rescuing plasmid present in this strain. As shown in Fig. 2, the smaller deletion mutant (*cka1Δ*) supported viability in this assay (strain YAR106), whereas the larger deletion (*cka1ΔΔ*) did not (strain YAR107). YAR106 generated viable colonies at a frequency comparable with that of strains rescued by either wild-type *cka1* (strain YAR105) or *cka2* (strains YDH31 and YDH6) but exhibited a small colony size, indicative of a reduced growth rate. This small colony phenotype was reproducible, and subsequent analysis of this strain (designated YAR109 after eviction of the *URA3* plasmid) revealed a doubling time of 120 min in liquid YPD at 29 °C, compared with 90 min for the isogenic
LYS2–801amber trp1-contrast to five previously analyzed cka2, panel A cka1-D did not prove to be the case, however. Growth curves for the similar to previously characterized ance to nitrogen starvation or heat shock in stationary phase, nonfermentable carbon sources and exhibited normal resist- D2 cka1-D recessive nature of the mutation (data not shown) suggest that characteristic of reduced CKII activity (10, 11, 14). This and the tive flocculation (data not shown). Both of these phenotypes are wild-type control (YAR108). YAR109 also exhibited constitu- ture-sensitive, with a breakpoint between 35 and 36 °C (data not shown). This fortuitous temperature sensitivity afforded us the opportunity to explore the physiological role of the opportunity to explore the physiological role of the

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\text{FIG. 1. } S. \text{ cerevisiae Cka deletion mutants. The predicted N-terminal sequence of wild-type Cka1 and mutant derivatives Cka1-Δ2 and Cka1-Δ3 is shown. Regions deleted in the two mutants and the beginning of the protein kinase domain are indicated by brackets. Invariant residues of the CKII catalytic subunit (16) are boxed; invariant residues of the protein kinase domain (48) are marked with arrows.}
\]

With the exception of YPH499, all strains are isogenic to YPH250, which carries the additional mutations ade2–101ochre his3–Δ200 leu2–Δ1 lys2–801amber trp1-1::TRP1 ura3–52. YPH499 is identical to YPH250 except that trp1-1::TRP1 is replaced by trp1-63.

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\text{TABLE I}
\]

<table>
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<th>Strain</th>
<th>Relevant chromosomal genotype</th>
<th>Resident plasmid(s)</th>
<th>Ref.</th>
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<td>21</td>
</tr>
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<td>21</td>
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<td>pDH6 pRS316–1</td>
<td>This study</td>
</tr>
</tbody>
</table>

* With the exception of YPH499, all strains are isogenic to YPH250, which carries the additional mutations ade2–101ochre his3–Δ200 leu2–Δ1 lys2–801amber trp1-1::TRP1 ura3–52. YPH499 is identical to YPH250 except that trp1-1::TRP1 is replaced by trp1-63.
Asp-220 of cAMP-dependent protein kinase is invariant in all protein kinases and serves an essential structural role in stabilizing the catalytic loop (25). The resulting strain (YAR13) was indeed temperature-sensitive, although with a breakpoint approximately 2 °C higher than that of the corresponding cka2-13 strain, YDH13. Remarkably, the phenotype of YAR13 was virtually indistinguishable from that of YAR109.1 in all respects (data not shown), indicating that the novel behavior of the latter is not specific to the cka1-12 allele. An additional indication of the unique behavior of both cka1ts alleles was provided by an analysis of cell viability following a shift to restrictive temperature (Fig. 3, panel B). In contrast to cka2ts alleles, which lose viability within the first few hours (11) (Fig. 3, panel B), both cka1ts strains remain viable up to 10 h after the shift, a time approximately coincident with their growth arrest. Collectively, these results imply that the unique phenotype of the two cka1ts alleles is specific to the CK1 gene rather than to the nature of the alleles themselves.

A priori, the distinct phenotypes of cka1ts versus cka2ts strains could be due either to a quantitative difference in the level of the mutant activity (reflecting for example a difference in gene expression) or to a qualitative difference between the two catalytic subunits. In an effort to distinguish between these two possibilities, we examined each temperature-sensitive strain across a spectrum of permissive, semi-permissive, and restrictive temperatures. As noted above, the phenotype of YAR109.1 was identical at 37 and 38.5 °C, and in general the distinct phenotypes displayed by cka1ts versus cka2ts strains were independent of temperature (data not shown). The only variation noted was an increase in the frequency of hyperpolarization when cka1ts and cka2ts strains were grown at 29 °C (arrow). Cell number was monitored as described under “Experimental Procedures.” The data are plotted as a function of time after the shift to 37 °C.

Fig. 2. Functional analysis of cka1 deletion alleles in vivo. Plasmid shuffling was used to test the ability of cka1-Δ2 and cka1-Δ3 to complement a cka1 cka2 null background. Yeast strains were grown nonselectively in YPD for 3 days. Cells were adjusted to an identical concentration and then serially diluted in YPD and plated on YPD + 5-FOA, which selects against the URA3 gene. Plates were incubated at 29 °C for 4 days. All six strains shown contain chromosomal disruptions of both CK1 and CK2. Plasmid-borne CK1 or 2 alleles present in each strain are as follows, with those remaining after eviction of any URA3-marked plasmid shown underlined: YAR105 (CK1 CKA2); YAR106 (cka1-1 Δ2 CKA2); YAR107 (cka1-1 Δ3 CKA2); YDH31 (CKA2 CKA2); RFG41-1a (CKA2); and YDH6 (CKA2).

Fig. 3. Temperature sensitivity of cka1ts strains. A, growth arrest of YAR109.1 (cka1-Δ2). Strains YAR108 (CKA1) and YAR109.1 (cka1-Δ2) were inoculated into YPD at a starting concentration of 1 × 10^5 cells/ml grown at 29 °C to a concentration of 5 × 10^6 cells/ml, and then shifted to 37 °C (arrow). Cell number was monitored as described under “Experimental Procedures.” B, viability of YAR108, YAR109.1, YAR13 (cka1-13), and YDH13 (cka2-13) at restrictive temperature. Strains were grown as described in A, and percent viability was determined as described under “Experimental Procedures.” The data are plotted as a function of time after the shift to 37 °C.

Stoichiometric amounts (8, 26). These results argue that the distinct phenotypes of cka1ts versus cka2ts strains are not due simply to quantitative differences in the amount of CKII activity in these mutants but rather to some qualitative difference in the two catalytic subunits (see “Discussion”).

cka1ts Mutants Exhibit a Defect in Cell Polarity—The spherical morphology conferred by temperature-sensitive cka1 mutants suggested that these mutants have a cell polarity defect. To test this proposition, we analyzed the distribution of nuclear chromatin, cell wall chitin, and actin in cka1ts and cka2ts strains grown at both permissive and restrictive temperature. At permissive temperature both mutants exhibited an essentially wild-type pattern (see below and data not shown).

DAPI staining of nuclear chromatin at restrictive temperature is illustrated in Fig. 5. Control strain YPH499 displayed a pattern of nuclear staining typical of dividing cells, with unbudded cells containing a single nucleus and budded cells containing either a single nucleus or a nucleus in both mother and daughter (panel A). In contrast, both cka1ts strains, YAR109.1...
Chitin deposition was normal in the cka1(ts) strains and that this behavior is specific to cka2(ts) strains and YDH13 strains. Despite the identical amino acid replacement in the cka1-13 and cka2-13 alleles, multinuclear cells were completely absent. Collectively, these results imply that the nuclear cycle is at least partially uncoupled from the budding cycle in cka1(ts) mutants and that this behavior is specific to cka1.

Cell wall chitin is ordinarily confined primarily to the mother-bud junction and to bud scars (23) but becomes delocalized and deposited at elevated levels in apolar mutants (27, 29). By using the chitin-specific stain, calcofluor, we visualized the distribution of chitin in cka1ts and cka2ts mutants (Fig. 6). Chitin deposition was normal in the cka1ts strain YAR13 at permissive temperature (panel A) but became delocalized and accumulated to high levels at restrictive temperature (panel B). Identical results were obtained with the cka1ts strain YAR109.1 (data not shown). For both cka1ts mutants, chitin deposition in budded cells was confined primarily to the mother cell, with staining of the bud becoming more pronounced as the size of the bud increased. Preferential staining of the mother cell has previously been observed in other apolar mutants (29). This behavior may represent overexpression of a normal pathway, as a low level of chitin deposition also occurs diffusely in the cell wall of wild-type cells, beginning in the bud at or immediately prior to septum formation (30). Surprisingly, chitin was also delocalized in YDH13 cells arrested at restrictive temperature (panel C). Cells with small buds were not present because of the large-budded arrest occasioned by G2/M arrest (11), and chitin was found equally distributed in both mother and daughter, including in the elongated buds of hyperpolarized cells. Delocalized chitin distribution in a polarized cell has been reported for mutations of the CDC3, -10, -11, and -12 genes, encoding septin proteins (31), and the BED1 gene (32). Extended incubation of a wild-type control strain at restrictive temperature resulted in only a modest perturbation of calcofluor staining (panel D).

The distribution of filamentous actin was visualized by staining with rhodamine-phalloidin (Fig. 7). At permissive temperature, YAR109.1 (panel A), YAR13 (panel B), and YDH13 (panel C) all exhibited actin staining patterns typical of wild-type cells: cortical actin plaques and attached actin cables were concentrated at presumptive bud sites of unbudded cells and at the apex of small buds, were randomly distributed within the bud (and eventually the mother as well) as the size of the bud increased, and were localized to the mother/bud junction of cells undergoing cytokinesis. At restrictive temperature, the actin-staining pattern of the cka1ts strains, YAR109.1 (panel D) and YAR13 (panel E), was completely nonpolar. Cortical actin plaques were randomly distributed in both unbudded and budded cells of the population, in the latter case in both mother and daughter and regardless of the size of the bud. Actin cables were few and randomly oriented. These results establish that cka1ts strains are defective in the ability to polarize the actin cytoskeleton, a process fundamental to cell polarity in this organism.

At restrictive temperature, the cka2ts strain YDH13 exhibited a delocalized pattern of cortical actin plaques in both the unbudded and large-budded cells of the arrested population (Fig. 7, panel F; data not shown). However, in this case it cannot be concluded that YDH13 has a defect in cell polarity as...
both the unbudded and budded cells are arrested at points in the cell cycle (G1 and late G2 or M, respectively; see Ref. 11) at which cortical actin patches are normally not polarized. In fact, many of the elongated cells in the population exhibit intense apical staining of the elongated bud, indicative of hyperpolarization. We presume that those elongated cells that lack such staining possessed it at one time and have subsequently lost it.

### DISCUSSION

We report the construction of the first conditional alleles of the *S. cerevisiae* CKA1 gene. Analysis of these alleles reveals a role for CKII in cell polarity, a function not previously associated with CKII in this organism. Our results provide an interesting counterpoint to the role of CKII in cell polarity in other systems, notably *S. pombe*, and provide the first indication of functional specialization of the *CKA1* and *CKA2* genes in *S. cerevisiae*.

Although we have not analyzed the expression of the mutant protein, the inviability of *cka1-D3* strains suggests that the N-terminal region of the CKII catalytic subunit is essential in vivo. A similar N-terminal extension is present in cAMP-dependent protein kinase, where it forms a long α-helix that associates with both the large and small lobes of the catalytic subunit (25). Veron et al. (33) have proposed that this N-terminal helix, as well as the corresponding region of CKII, is crucial in stabilizing the hinge region between the two lobes. Our results are consistent with such a crucial structural role. The viability of *cka1-D2* strains suggests that the first con-

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**TABLE II**

Formation of multinucleate cells by *cka1ts* strains.

Strains were inoculated into YPD at an initial concentration of 1 × 10⁵ cells/ml, grown at 25 °C, and shifted to 37 °C at a concentration of 5 × 10⁵ cells/ml. Aliquots of cells were removed at the indicated times, stained with DAPI, and examined by fluorescence microscopy. Percentage of multinucleate cell bodies was determined as described by Adams et al. (27).

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<th>Mutant allele</th>
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**FIG. 5.** Distribution of nuclear chromatin in *cka1ts* and *cka2ts* strains. Yeast strains were grown and shifted to 37 °C as described in the legend of Fig. 4. Aliquots were removed at the indicated times after the shift, and nuclear chromatin was visualized by staining with the DNA-binding dye, DAPI. Strains and times shown are YPH499 (wild-type), 15 h (A); YAR109.1 (*cka1*-Δ2), 15 h (B); YDH13 (*cka2*–13), 15 h (C). Scale bar in B applies to all panels and indicates 10 μm.

**FIG. 6.** Distribution of cell wall chitin in *cka1ts* and *cka2ts* strains. Yeast strains were grown and shifted to 37 °C as described in the legend of Fig. 4. Aliquots were removed at the indicated times after the shift, and chitin was visualized by staining with calcofluor. Strains and times shown are YAR13 (*cka1*–13), 0 h (A); YAR13, 15 h (B); YDH13 (*cka2*–13), 10 h (C); YPH499 (wild-type), 20 h (D). Scale bar in A applies to all panels and indicates 10 μm.
erved motif (SEARVY of Cka1) is not essential for the function of the N-terminal arm. However, we note that the six amino acids immediately upstream of this motif bear a fortuitous similarity to this sequence so that the motif is only partially disrupted in the cka1-Δ2 allele (see Fig. 1). Complete disruption of the motif might reveal that it is in fact essential.

The morphology and budding growth habit of *S. cerevisiae* are mediated by dramatic rearrangements of the actin cytoskeleton and secretory apparatus (34–36). Bud emergence involves the assembly of a ring of cortical actin patches at the presumptive bud site followed by polarized secretion into the region defined by the ring. The actin cytoskeleton, specifically actin cables terminating at the actin patches, is believed to target secretory vehicles to the site of bud growth (37, 38). Both the cortical actin patches and secretion are directed to the apex of the bud early in the budding cycle but become randomly distributed later, the ultimate shape of the bud being determined by the balance between these apical and isotropic modes of growth. These cytoskeletal and secretory rearrangements are themselves dependent upon proper regulation of cell polarity. Two cell division cycle genes *CDC42*, which encodes a Rho-type GTPase, and *CDC24*, which encodes a guanine nucleotide exchange factor, are central to the establishment of cell polarity in *S. cerevisiae* (27, 29). Temperature-sensitive mutants of either gene arrest as spherical, unbudded cells having an isotropic distribution of cortical actin patches as well as cell wall chitin. Cell growth is not halted but occurs isotropically; and the nuclear cycle continues, resulting in a high proportion of cells with two or more nuclei. A similar apolar phenotype is elicited by mutations in several other genes, including the bud emergence genes *BEM1* and *BEM2* (39, 40).

Our analysis of *cka1-Δ2* and a second *cka1*Δ*ts* allele, *cka1-13*, indicates that *cka1*Δ*ts* mutants have a defect in cell polarity. This is in marked contrast to the cell cycle arrest phenotype previously defined for *cka2*Δ*ts* mutants (11). The fact that two molecularly distinct *cka1* alleles yield the one phenotype and five distinct *cka2* alleles (one of which is identical in form to one of the *cka1* alleles) yield the other strongly implies that these phenotypes are specific to the *CKA1* and -2 genes themselves. The terminal phenotype of *cka1*Δ*ts* strains closely resembles that of *cdc24* or *-42* mutants. In both cases, arrested cells display a spherical morphology, continued growth, a nonpolarized actin cytoskeleton, delocalized chitin deposition, and a significant fraction of multinucleate cell bodies. Nevertheless, there exists one important distinction: *cdc24* or *-42* mutants arrest as unbudded cells, consistent with their role in the establishment of cell polarity (27, 29, 34), whereas *cka1*Δ*ts* strains arrest as a mixture of budded as well as unbudded cells. At face value, the latter result implies that CKII is required for the maintenance of cell polarity rather than for its establishment, although a requirement for CKII in both processes would also be consistent with the observations. Although it is formally possible that CKII is required only for establishment of polarity and that both of the alleles we have examined are leaky, this seems unlikely given the distinct nature of the alleles, the absence of any change in the proportion of unbudded cells as arrest occurs (data not shown), and the constancy of the terminal phenotype at different restrictive temperatures.

Several genes that appear to be involved in the maintenance of cell polarity in *S. cerevisiae* have been identified, including *RHO3* and *RHO4*, which encode partially redundant Rho-type GTPases (41, 42), and *BOI1* and *BOI2*, which encode proteins that interact physically and genetically with *BEM1* and genetically with *RHO3* (43, 44). Although the mechanism of action of *Rho3*, 4 and *Boi1*, 2 is unknown, the terminal phenotype of *rho3*Δ*ts* *rho4* or of *boi1* boi2 strains (which are also temperature-sensitive) resembles that of *cka1*Δ*ts* strains, including the loss of cell polarity in budded as well as unbudded cells. We have tested the ability of multicopy *CDC24*, *CDC42*, *BEM1*, *BOI1*, and *BOI2* to suppress *cka1*Δ*ts* as well as *cka2*Δ*ts* alleles and have also constructed *cka1 boi1*, *cka2 boi1*, *cka2 boi2*, as well as *cka1*Δ*ts* boi1 and *cka2*Δ*ts* boi2 double mutants. No genetic inter-

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**Fig. 7. Distribution of filamentous actin in cka1*Δ*ts* and cka2*Δ*ts* strains.** Yeast strains were grown at 29 °C and shifted to 37 °C as described in the legend of Fig. 4. Aliquots were removed at the indicated times after the shift, and filamentous actin was visualized by staining with rhodamine-phalloidin. Strains and times shown are YAR109.1 (*cka1-Δ2*, 0 h (A); YAR13 (*cka1-13*, 0 h (B); YDH13 (*cka2-13*), 0 h (C); YAR109.1, 15 h (D); YAR13, 15 h (E); YDH13, 10 h (F). Scale bar in (E) applies to all panels and indicates 5 μm.
actions were observed in any of these tests. Additional studies will be required to determine whether CKII interacts with any of these functions.

Given the apparent functional redundancy of CKA1 and -2 (9, 10), the distinct arrest phenotypes displayed by cka1Δ (this work) versus cka2Δ mutants (11) are paradoxical. As one way to resolve this paradox, we propose that Cka1 and -2 are both able to phosphorylate all essential CKII substrates but that, because of differing kinetic constants or different localizations, they do so with different efficiencies. Strains lacking one or the other gene would thus harbor a different spectrum of hypophosphorylated substrates, with the result that different functions initially become limiting when the activity of the remaining subunit is removed. Because a cka1Δ mutant harbors a null allele of cka2 and vice versa, to explain the observed phenotypes it is necessary to postulate that cka1 preferentially phosphorylates substrates required for cell cycle progression and cka2, substrates required for cell polarity, although this is undoubtedly an oversimplification. Cka1 and Cka2 in fact differ at two well conserved positions (Lys-76 and Lys-80 of Cka1) which have recently been implicated in recognition of the +3 position of the protein substrate (45). Regardless of the underlying mechanism, the distinct phenotypes of cka1Δ and cka2Δ mutants provide the first evidence for functional specialization of CKA1 and -2.

The hyperpolarization displayed by cko2Δ mutants relative to the apolar phenotype of cka1Δ strains is also surprising. However, such ambidextrous behavior is not unprecedented, even for a single gene and indeed for a single allele. For example, incubation of cdc24Δ cells at semipermissive temperature (33 °C) results in the stochastic production of cells with an elongated bud (29). Interestingly, such buds stain uniformly for chitin, as do those produced by cka2Δ strains. The explanation for these and similar situations may lie in the complexity of the interactions regulating cell polarity in S. cerevisiae. These interactions include a network of Rho-type GTPases (40) as well as protein-protein interactions mediated by pleckstrin homology and SH3 domains (43). It is possible that there is cross-talk among these functions in various mutant backgrounds. The involvement of CKII in the cell cycle may also be relevant given the intimate connections that exist between cell cycle progression and cell polarity in S. cerevisiae (18, 46).

The ability of cka1Δ strains to proceed through several cell divisions in the apparent absence of CKII activity has one important implication for the function of CKII during the cell cycle (11), namely that the crucial cell cycle targets of CKII are not dephosphorylated in an obligatory manner at each cell cycle. This implies that CKII is not part of the cell cycle oscillator per se. An important caveat of course is that CKII activity is rapidly and fully inactivated in cka1Δ strains.

The phenotype of the cka1Δ alleles described here shares many features in common with that of orb5Δ alleles of S. pombe (17). Both types of mutant undergo several cycles of cell division prior to arrest, arrest is accompanied by a loss of viability, and arrested cells are spherical and have a non-polarized actin cytoskeleton. The tubulin cytoskeleton of orb5Δ strains is disorganized as well. Coupled with the cell-cell aggregation common to CKII mutants in both organisms, these similarities suggest that the physiological role of CKII in S. cerevisiae and S. pombe may be better conserved than was previously apparent. However, the mechanism underlying the development of the apolar phenotype appears to be different in the two cases. Analysis of double mutants in S. pombe indicates that orb5 strains remain polarized but become deficient in the re-establishment of polarized growth following cytokinesis. In this model, the spherical morphology of orb5Δ strains is generated by continued rounds of cell division in the absence of growth, until the cell is approximately spherical and too small to divide again. The observed cytoskeletal disorganization is either illusory, due to the difficulty of identifying a polar distribution in a small spherical cell, or secondary. However, this model cannot, even in principle, explain the spherical morphology of S. cerevisiae cka1Δ mutants, first, because growth is a prerequisite for cell division (budding) and, second, because the absence of growth cannot convert an oval cell into a spherical one. As noted above, our results suggest that the primary defect in cka1Δ strains is a loss of cell polarity and that the spherical morphology is generated by subsequent isotropic growth. We do not at present have an explanation for the apparent difference in mechanism between the two systems. CKII is also implicated in cell polarity in mammalian cells. Treatment of cultured neuroblastoma cells with antisense oligonucleotides directed against the CKII catalytic subunit inhibits serotonin deprivation-induced neuritogenesis, and this inhibition is correlated with the dephosphorylation of the microtubule-associated protein MAP1B at sites that are phosphorylated in vitro by purified CKII (47). The potential involvement of the tubulin cytoskeleton may be relevant to the orb5 phenotype in S. pombe, since disruption of microtubules affects cell morphology in S. pombe, and the microtubule cytoskeleton is perturbed in orb5 strains (17). In contrast, it appears unlikely that such an explanation underlies the loss of cell polarity in cka1Δ strains since polarized growth is predominantly if not exclusively a function of the actin cytoskeleton in budding yeast (37, 38). Thus, although an effect of CKII on polarity is observed in phylogenetically diverse systems, the underlying mechanism may not be identical in all cases.

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Temperature-sensitive Mutations of the CKAI Gene Reveal a Role for Casein Kinase II in Maintenance of Cell Polarity in Saccharomyces cerevisiae
Asokan Rethinaswamy, Mark J. Birnbaum and Claiborne V. C. Glover

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