Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7376–7380, August 1991
Biochemistry

Yeast has homologs (*CNA1* and *CNA2* gene products) of mammalian calcineurin, a calmodulin-regulated phosphoprotein phosphatase

(phosphoprotein phosphatase type 2B/*Saccharomyces cerevisiae*/null mutations/mating-pheromone response)

MARTHA S. CYERT, RIYO KUNISAWA, DAVID KAIM, AND JEREMY THORNER

Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Communicated by Marc Kirschner, May 6, 1991 (received for review February 12, 1991)

ABSTRACT **Calcineurin, or phosphoprotein phosphatase type 2B (PP2B), is a calmodulin-regulated phosphoprotein phosphatase. We isolated a gene encoding a yeast PP2B homolog (*CNA1*) by screening a yeast genomic DNA library in the expression vector λgt11, first with ^{125}I-labeled yeast calmodulin and then with a human cDNA encoding the catalytic (or A) subunit of calcineurin. The predicted *CNA1* gene product is 54% identical to its mammalian counterpart. Using the polymerase chain reaction (PCR) with oligonucleotide primers based on sequences conserved between *CNA1* and mammalian PP2B genes, we isolated a second gene, *CNA2*. *CNA2* is identical to PP2B$_w$, a partial cDNA clone previously described by others as originating from rabbit brain tissue. Our findings demonstrate that a unicellular eukaryote contains phosphoprotein phosphatases of the 2B class. Haploid cells containing a single *cna1* or *cna2* null mutation, or both mutations, were viable. *MAT*a *cna1 cna2* double mutants were more sensitive than wild-type cells or either single mutant to growth arrest induced by the mating pheromone α factor and failed to resume growth during continuous exposure to α factor. Thus, calcineurin action antagonizes the mating-pheromone response pathway.**

Many cellular processes are regulated by the phosphorylation state of their component proteins, and the role of protein kinases in such control has been well established. It has only recently become clear, however, that phosphoprotein phosphatases also serve regulatory functions *in vivo*. Phosphatases participate in some signal-transduction pathways (1, 2) and control aspects of DNA replication (3), transcription (4), and mitosis (5–9).

Phosphoserine/phosphothreonine-specific phosphoprotein phosphatases can be divided into four classes based on their biochemical properties (10, 11). Phosphoprotein phosphatase types 1, 2A, 2B, and 2C (PP1, PP2A, PP2B, and PP2C) differ in their sensitivity to various inhibitors, as well as in their requirements for divalent cations *in vitro*. PP2B, also called calcineurin, is the only class of phosphatase that requires Ca^{2+} and calmodulin for activity. This phosphatase also exhibits a more restricted substrate specificity *in vitro* than the other phosphatase types. PP2B has been purified from several mammalian tissues, and the holoenzyme has been shown to consist of two polypeptides: the catalytic (A) subunit (60–61 kDa), and a regulatory (B) subunit (19 kDa) (reviewed in refs. 12 and 13). In the presence of Ca^{2+}, calmodulin binds reversibly to the A subunit, forming a fully active, trimeric phosphatase.

Sequences encoding the calcineurin A subunit have been isolated only recently, and only from mammalian brain tissue. Two distinct calcineurin genes (α and β) have been identified in each of three organisms (rats, mice, and humans), and each gene seems to give rise to at least two different mRNAs through alternative splicing (14–17). The α and β forms of calcineurin A are very similar (81% amino acid identity in rats), and differ mostly at their N and C termini (reviewed in ref. 18). How the biochemical properties of these distinct isozymes compare is not known. The PP2B catalytic subunit is also highly conserved between species; for example, the β forms of rat and human are 99% identical. In contrast, a clone obtained from a rabbit brain cDNA library was reported to encode a partial sequence for a third, much more divergent, type of PP2B catalytic subunit, which was designated PP2B$_w$ (19).

Although calcineurin has been characterized extensively *in vitro*, much less is known about its physiological function(s). PP2B appears to control trichocyst exocytosis in *Paramecium* (53), aggregation of pigment granules in fish melanophores (20), and some aspects of mammalian sperm motility (21). Aside from these highly specialized events, the role of PP2B in regulating basic cellular processes remains an open question. In combination with kinases such as protein kinase C and calmodulin-regulated kinases, PP2B is capable of modulating protein phosphorylation in response to changes in Ca^{2+} concentration, but many of its *in vivo* substrates are not known. To address these issues, we undertook an investigation of PP2B in the unicellular eukaryote *Saccharomyces cerevisiae*. No PP2B activity had been conclusively identified in extracts of this yeast, although the presence of types 1, 2A, and 2C phosphatase activities had been detected (22). In contrast, we were able to demonstrate that yeast cell extracts do possess a PP2B-like activity (23), and we have since isolated a yeast gene, *CNB1*, encoding a homolog of the calcineurin regulatory (B) subunit (M.S.C. and J.T., unpublished results). Here we report the isolation of genomic clones for two genes, *CNA1* and *CNA2*, that encode proteins highly related to the catalytic (A) subunit of mammalian calcineurin.*

MATERIALS AND METHODS

Yeast Strains and Culture Conditions. All yeast strains were derivatives of YPH499 and YPH500 (24). Basic manipulations of yeast, culture conditions, and media were as described (25) except that twice the recommended levels of nutritional supplements were used in the synthetic media. When acetate was the carbon source, 1% (wt/vol) potassium acetate was used. Glycogen levels were assessed qualitatively by staining colonies with iodine vapor for 3–5 min. DNA was introduced into yeast cells by electroporation (26). Diploid strains homozygous for *cna2Δ1*::*HIS3*, or both *cna2Δ1*::*HIS3* and *cna1Δ1*::*URA3*, were isolated after trans-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PP1, PP2A, and PP2B, phosphoprotein phosphatase types 1, 2A, and 2B.

*The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M64839 (*CNA1*) and M64840 (*CNA2*)].

formation of haploids with a plasmid-borne copy of the *HO* gene (27). Pheromone response was measured by a bioassay (28) using synthetic α factor (Peninsula Laboratories). Isolation of yeast genomic DNA was as described (29). Intact yeast chromosomes were separated by pulsed-field gel electrophoresis (30) to determine the location of cloned genes.

Isolation and Characterization of Clones. The λgt11 yeast genomic library was a gift from Robert Hamataki and Akio Sugino (National Institute of Environmental Health Sciences, Research Triangle Park) and was screened with ^{125}I-labeled yeast calmodulin (31). Phage DNA was isolated according to Sambrook *et al.* (32). The plasmid yeast genomic library was that described by Rose *et al.* (33). PCR was performed using reagents and protocols supplied by Perkin–Elmer/Cetus. Library screening and hybridization analyses of DNA and RNA were performed (32) using Hybond-C extra-supported nitrocellulose membranes (Amersham). High-stringency hybridizations were performed in 6× SSPE/5× Denhardt's solution/0.5% SDS with denatured herring sperm DNA (100 μg/ml) at 65°C; for low stringency, 45°C was used. (SSPE is 150 mM NaCl/10 mM Na_2HPO_4/1 mM EDTA, pH 7.4; Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin.) Radioactive probes were made using random primers (Pharmacia) and [α-^{32}P]dCTP (Amersham) (32).

DNA Sequencing. Restriction fragments were cloned into Bluescript vectors (Stratagene), and nested deletions were created according to this manufacturer's suggestions. The sequences of both strands were determined using these deletions and custom-synthesized oligonucleotide primers. Single-stranded DNA was produced and isolated according to Stratagene. Sequenase enzyme and nonradioactive nucleotides were from United States Biochemical, and sequencing reactions were conducted under conditions recommended by this supplier. [α-[^{35}S]thio]dATP was from Amersham.

RESULTS

Isolation of the *CNA1* Gene. To isolate clones for the catalytic subunit of yeast PP2B, we developed a method, described in detail elsewhere (31), that exploited the ability of this protein to bind calmodulin. We screened a yeast genomic DNA expression library in phage λgt11 with ^{125}I-labeled yeast calmodulin in the presence of Ca^{2+} and identified a collection of 11 phage encoding putative calmodulin-binding proteins. These phage were further screened for hybridization at low stringency to a cDNA encoding a human calcineurin A subunit (type II cDNA; see ref. 17). Two of these 11 phage hybridized to the human calcineurin probe and contained overlapping inserts. Partial DNA sequence from these clones confirmed that they contained an open reading frame related to the human phosphatase. Full-length genomic clones were then isolated from a yeast plasmid library, using as the probe a 1.2-kilobase (kb) *Eco*RI fragment from one of the phage inserts. We determined the complete DNA sequence of 1.96 kb within a 4-kb *Xba* I–*Cla* I fragment from the genomic clone. This sequence predicts one continuous open reading frame of 553 amino acids (calculated molecular weight, 63,005), which we designated *CNA1*, for *c*alci*n*eurin *A 1*. The predicted CNA1 protein contains many regions of near identity to mammalian calcineurin A over its entire length (Fig. 1), and several specific structural features are conserved between the two proteins (see *Discussion*). Thus we concluded that *CNA1* encodes a phosphatase of the calcineurin, or PP2B, class. The primary structure of CNA1 differs from mammalian calcineurin A in four regions (Fig. 1). First, the N terminus of CNA1 is longer than that of mammalian calcineurin A. The yeast protein also contains two internal insertions of sequence relative to its mammalian counterpart (amino acids 273–279 and 425–452). Last, there is a stretch of 19 amino acids near the C terminus that is found in the mammalian protein (amino acids 448–466) but is absent from CNA1.

Using an 830-base-pair (bp) *Eco*RI fragment from the coding region of *CNA1* as a probe, hybridization to poly(A)$^+$ RNA identified a transcript (2.1 kb) of appropriate size to encode CNA1 (data not shown). When all 17 intact yeast chromosomes were subjected to pulsed-field gel electrophoresis and transferred to nitrocellulose, the same *CNA1* probe specifically hybridized to a band representing chromosome II (data not shown). High-stringency hybridization analyses of yeast genomic DNA cut with various restriction enzymes indicated that *CNA1* is a single-copy gene (data not shown).

Construction of a *cna1* Null Mutation. To initiate a genetic analysis of PP2B function in *S. cerevisiae*, a null allele of *CNA1* (*cna1Δ1::URA3*) was constructed *in vitro* and introduced into the yeast genome by DNA-mediated transformation (38). An internal 1-kb *Bsm* I fragment of *CNA1* was replaced with the *URA3* gene flanked by repeats from the *Salmonella hisG* gene (39), and the mutated gene was introduced into a diploid strain, YPH501. Uracil prototrophs were selected, and hybridization analysis confirmed that these transformants were heterozygous for the *cna1Δ1::URA3* allele. Two such diploids were induced to sporulate and the resulting tetrads were dissected. Of 16 asci dissected, 15 yielded tetrads with four viable spores, and Ura$^+$ and Ura$^-$ segregated 2:2 in all 15. Thus, *CNA1* is not essential for viability (data not shown). Furthermore, we were able to detect only one phenotypic consequence of the *cna1Δ1::URA3* mutation in haploid cells (see below).

One explanation for the fact that the *cna1* null mutants had no readily identifiable phenotype is that a second, related gene may exist whose product can also perform the essential function(s) of CNA1. Indeed, we found that a 250-bp *Nde* I–*Bsm* I fragment of *CNA1*, which is deleted in the *cna1Δ1::URA3* allele and contains coding sequence that should be unique to type 2B phosphatases, was able to hybridize to sequences in genomic DNA isolated from *cna1Δ1::URA3* strains (data not shown). This finding suggested that an additional *CNA1*-related gene exists in the yeast genome.

Isolation of the *CNA2* Gene. We created a probe for the *CNA1*-related gene by using PCR. As primers for the reaction, a 256-fold degenerate oligonucleotide that encoded the sequence Met-Cys-Asp-Leu-Leu-Trp (residues 256–261 in CNA1) and a 128-fold degenerate oligonucleotide that encoded the sequence Pro-His-Pro-Tyr-Trp-Leu-Pro (residues 374–380 in CNA1) were used. At 40°C, the PCR product generated with these primers from *cna1Δ1::URA3* DNA was ≈420 bp in size, which was distinctly larger than that produced from *CNA1* itself (370 bp). The 420-bp PCR product hybridized to *CNA1* DNA at low stringency. On a genomic Southern blot, the PCR product hybridized strongly at high stringency to the same bands that hybridized to the *CNA1* probe at low stringency. We used the radiolabeled 420-bp PCR product as a probe to screen a yeast genomic library in a centromeric plasmid vector. Five plasmids were obtained in this way that contained identical DNA inserts. We determined the complete DNA sequence of 2.35 kb within a 4.4-kb *Bgl* II fragment from the plasmid insert. These clones defined one continuous open reading frame of 604 amino acids (calculated molecular weight, 68,503), which we designated *CNA2*. The predicted amino acid sequence of CNA2 is very similar both to that of CNA1 and to mammalian calcineurin A (Fig. 1). Like CNA1, CNA2 exhibits the structural features of a PP2B catalytic subunit (see *Discussion*). Relative to mammalian calcineurin, CNA2 has insertions and deletions at the same positions as those found in CNA1 (Fig. 1). Surprisingly, we found that 1.8 kb of our *CNA2* nucleotide sequence, encoding 505 amino acids of the open reading

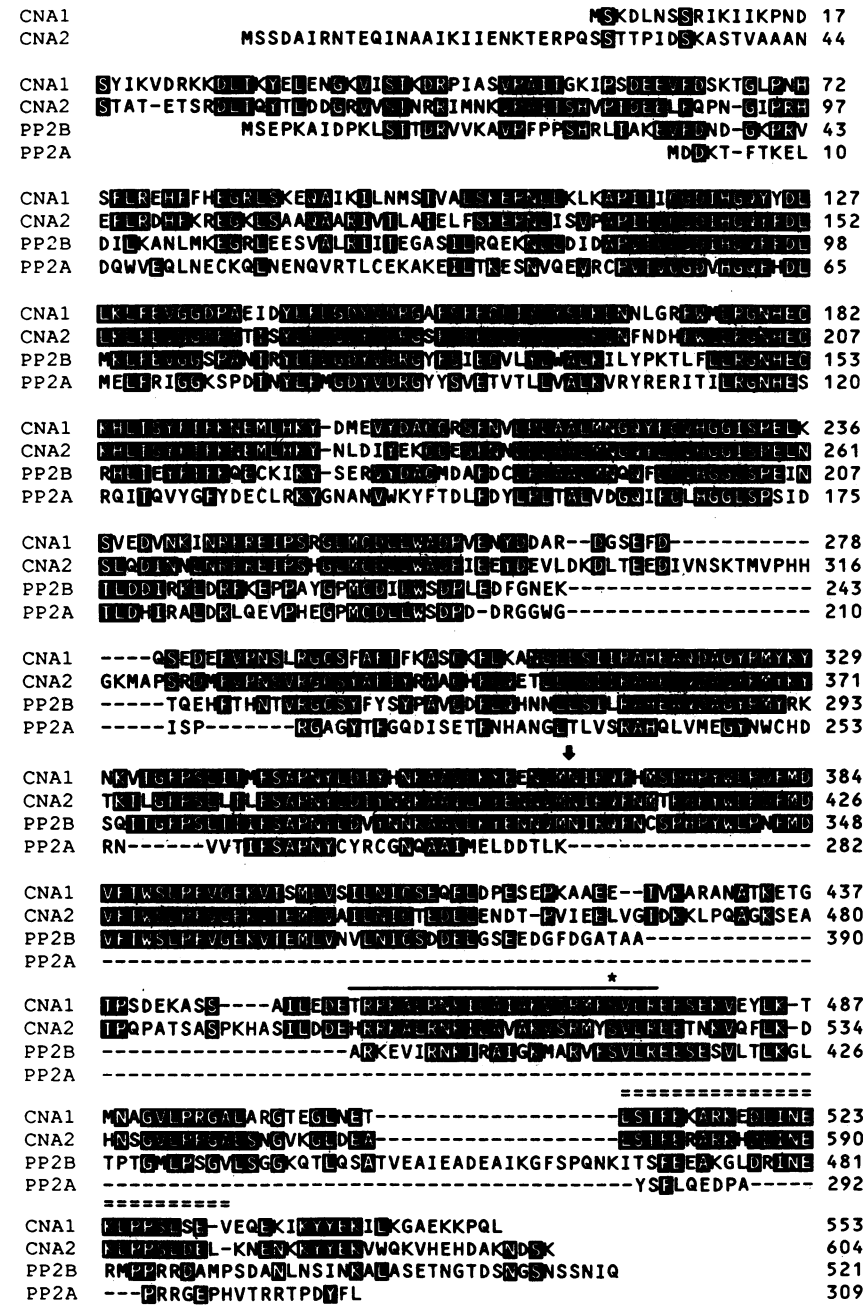

FIG. 1. Alignment of the predicted *CNA1* and *CNA2* gene products with mammalian phosphatases. PP2B is the sequence of mouse brain calcineurin Aα as determined by Kincaid *et al.* (16). PP2A is the sequence of PP2Aα from rabbit skeletal muscle as determined by Berndt *et al.* (34). Amino acid residues that are identical to those encoded in *CNA1* or *CNA2* are represented as white on black. The junction of the catalytic and regulatory domains is denoted by the arrow. The presumptive calmodulin-binding site is overlined. The serine (residue 411 of PP2B) that is phosphorylated *in vitro* by protein kinase C and calmodulin-stimulated kinase II (35, 36) is indicated with an asterisk. A double dashed line denotes the sequence of a synthetic peptide (residues 467–491 of PP2B) found to have inhibitory activity (37).

frame and the 3′ untranslated region of the DNA, was virtually identical to the entire sequence of a clone called $PP2B_w$ (19). $PP2B_w$ was isolated from a cDNA library purportedly derived from rabbit brain tissue. Three observations led us to the conclusion that $PP2B_w$ is actually *CNA2*, an authentic yeast gene. First, at high stringency, an internal probe from *CNA2* (a 660-bp *Bst*XI fragment) hybridized to restriction fragments from the yeast genome whose sizes are those predicted by the sequence of the cloned *CNA2* gene (Fig. 2). Second, this probe also hybridized to a yeast transcript (2.15 kb) of appropriate size to encode the *CNA2* gene product. Third, the same probe hybridized at high stringency to yeast chromosome XVI (Fig. 2). Hence, it appears that the rabbit library used to isolate the $PP2B_w$ clone may have been contaminated by yeast DNA. That the source of $PP2B_w$ was the rabbit genome was not previously assessed by hybridization to either rabbit genomic DNA or rabbit poly(A)$^+$ RNA (19).

Construction of a *cna2* Null Mutation. To construct a null allele of *CNA2* (*cna2*Δ*1*::*HIS3*), an internal 0.9-kb *Kpn* I–*Sal* I fragment was deleted from the coding region of *CNA2* and replaced with a 1.7-kb fragment from pJJ217 (41) that encodes the *HIS3* gene. DNA-mediated transformation (38) was then used to introduce *cna2*Δ*1*::*HIS3* into the genomes of the diploid strain YPH501, the haploid strain YPH499, and a YPH499 *cna1*Δ*1*::*URA3* mutant. Histidine prototrophs were isolated at high frequency from all three strains, and hybridization analysis confirmed that transplacement of the *CNA2* gene with the *cna2*Δ*1*::*HIS3* allele had occurred. Thus, *CNA2* is not essential for viability, even in a strain containing a *cna1* null mutation.

Characterization of Mutants. Isogenic strains containing *cna1*Δ*1*::*URA3* and/or *cna2*Δ*1*::*HIS3*, were identical to the wild-type parental strain with respect to morphology and growth on media containing glucose, glycerol, or acetate as the carbon source. The mutant strains showed wild-type levels of glycogen accumulation and were neither heat-sensitive nor cold-sensitive for growth. The mutants were able to mate, and diploid strains homozygous for either the *cna1* or the *cna2* null mutation, or for both, were able to

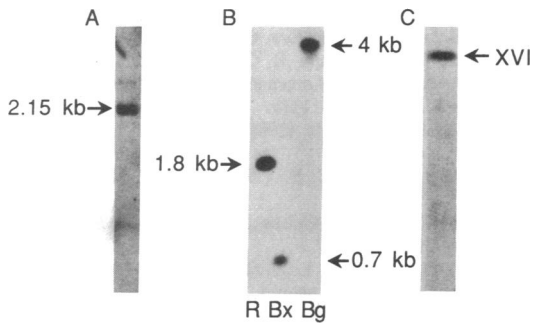

FIG. 2. Hybridization analysis of *CNA2*. (*A*) Poly(A)$^+$ RNA (7 μg) was subjected to agarose gel electrophoresis and hybridized to the 660-bp *Bst*XI fragment of *CNA2* labeled with random primers. Size markers were a mixture of *Eco*RI-, *Hin*fI-, and *Acc* I-digested pBR322 DNA. (*B*) Yeast genomic DNA was digested with *Eco*RI (R), *Bst*XI (Bx), or *Bgl* II (Bg), subjected to agarose gel electrophoresis, and hybridized at high stringency to the same probe as in *A*. (*C*) Chromosomes were separated by pulsed-field gel electrophoresis (30) and hybridized to the same probe as in *A*. Roman numeral refers to chromosome number. Identification of chromosome XVI was verified by hybridization with a probe for *PEP4*, a gene known to reside on chromosome XVI (40).

sporulate. However, the mutant and wild-type strains differed in their response to the mating pheromone, α factor. *MAT***a** strains containing a single *cna* mutation (*cna1* or *cna2*) were twice as sensitive as wild type to α factor-induced growth arrest, and a *cna1 cna2* double mutant was 4 times as sensitive as wild-type (data not shown). Once arrested by α factor, *cna1 cna2* strains failed to resume growth in the continued presence of the pheromone (Fig. 3).

DISCUSSION

We have cloned two genes, *CNA1* and *CNA2*, from the yeast *S. cerevisiae* whose predicted protein products are closely related to each other and to the catalytic subunit of mammalian PP2B. *CNA1* was isolated from a family of 11 λgt11/yeast genomic DNA clones selected for their ability to bind ^{125}I-labeled yeast calmodulin. Our failure to also identify

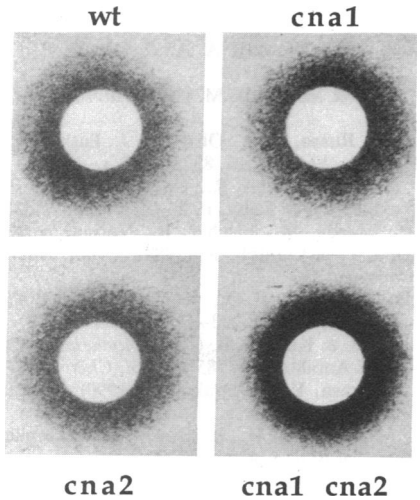

FIG. 3. Recovery of calcineurin mutants from α-factor arrest. Cells were plated in top agar on YPD plates as described (28). Synthetic α factor (10 μg) was pipetted onto sterile filter disks, and these disks were placed on the nascent lawn of cells. Plates were incubated at 30°C for 2 days. (*Upper Left*) Strain YPH499 (wild type, wt). (*Upper Right*) YPH499 containing *cna1*Δ*1*::*URA3*. (*Lower Left*) YPH499 containing *cna2*Δ*1*::*HIS3*. (*Lower Right*) YPH499 containing *cna1*Δ*1*::*URA3* and *cna2*Δ*1*::*HIS3*.

CNA2 by calmodulin screening may simply reflect a bias in the distribution of DNA fragments present in the library. Alternatively, CNA2 may differ from CNA1 in some way—for example, in its affinity for yeast calmodulin—that prevented detection of *CNA2* under the screening conditions we used. Two of the 11 calmodulin-binding clones identified encoded CNA1, and five others encoded CMK1, a yeast homolog of type II calmodulin-stimulated protein kinases (31). Thus, we believe that this method of screening is of general utility.

The isolation of clones encoding CNA1 and CNA2 provides conclusive evidence that PP2B phosphatases do exist in yeast and presents an opportunity for comparison of calcineurin A sequences from evolutionarily distant organisms. Phosphoprotein phosphatases are among the most highly conserved enzymes known (11). The PP1 and PP2A homologs from *Schizosaccharomyces pombe* and *S. cerevisiae*, for example, are 74–80% identical to the corresponding mammalian proteins (5, 8, 42). The *CNA1* and *CNA2* gene products are each 54% identical to the murine calcineurin Aα isozyme (16) (Fig. 1). This mammalian sequence may represent a brain-specific protein. Because only brain cDNA libraries have been probed, additional calcineurin A genes may exist in mammals that are not expressed in neuronal tissue. Indeed, mouse brain, for example, has 10-fold more PP2Bα mRNA than skeletal muscle by hybridization analysis (16), whereas these two tissues have similar levels of PP2B enzymatic activity (43). Thus, there may be additional mammalian calcineurin genes, as yet uncharacterized, that are more similar to *CNA1* and *CNA2*.

Most of the structural features of the mammalian PP2B catalytic subunit are conserved in CNA1 and CNA2 (Fig. 1). Calcineurin A can be divided into a catalytic and a regulatory domain. The catalytic portion, the N-terminal two-thirds of mammalian calcineurin A (Fig. 1, amino acids 1–329), exhibits significant sequence homology to PP1 and PP2A but contains two small insertions (amino acids 247–253 and 296–301), which may be important for its interaction with the B subunit (17). The sequence of one of these insertions (Thr-Thr-Gly-Phe-Pro-Ser, 296–301) is highly conserved between mammalian PP2B and the two yeast proteins (five of the six residues are identical). The other insertion (residues 247–253) is present in the yeast proteins but is less highly conserved.

The C-terminal third of the PP2B catalytic subunit contains a regulatory domain (not found in PP1 or PP2A) that encodes a calmodulin-binding site, a phosphorylation site, and an inhibitory region. The site of calmodulin binding to mammalian calcineurin seems to be residues 391–414 (44). The corresponding regions of CNA1 (residues 453–476) and CNA2 (residues 500–523) also exhibit the predicted amphipathic α-helical structure common to calmodulin-binding peptides (45–47) and are almost certainly sites of interaction with yeast calmodulin. The isolation of *CNA1* from the λgt11 library by calmodulin screening provides additional evidence that CNA1 does contain a calmodulin-binding site.

In vitro, mammalian calcineurin is phosphorylated by protein kinase C and type II calmodulin-stimulated protein kinase at a site in the calmodulin-binding domain, resulting in a 2-fold increase in its K_m for one substrate, myosin light chain (35, 36). The site of this phosphorylation, Ser-411, is flanked on either side by basic residues that are crucial for recognition by these kinases. This Arg-Xaa-Xaa-Ser-Xaa-Xaa-Arg motif is conserved in the two yeast proteins (Fig. 1). CNA1 and CNA2 may also be phosphorylated by protein kinase C and type II calmodulin-stimulated protein kinase, as yeast cells possess homologs of both of these kinases (31, 48). The physiological relevance of this phosphorylation for any calcineurin, if it does occur *in vivo*, is not known.

Proteolysis of mammalian calcineurin has revealed the presence of an inhibitory domain at the C terminus of the A subunit (49). Removal of this region produces a phosphatase that is fully active in the absence of calmodulin. A synthetic peptide comprising amino acids 467–491 has been shown to inhibit PP2B activity *in vitro*, although the exact nature of this inhibition has not been established (37). We note that this region is moderately well conserved between the yeast and mammalian proteins and is almost identical in CNA1 and CNA2 (Fig. 1).

Other yeast phosphatases (PP1 and PP2A) are encoded by multiple genes that are closely related in sequence and functionally redundant (5, 8, 42). Our initial characterization of *cna* mutants indicates that CNA1 and CNA2 perform at least one overlapping function in the cell—namely, enabling the cell to resume growth and division after arrest by mating pheromone. Despite this shared role, CNA1 and CNA2 may differ significantly in their regulation, substrate specificity, or localization.

Our findings demonstrate that a Ca^{2+}-regulated enzyme is involved in the recovery of yeast cells from mating-pheromone-induced cell cycle arrest; earlier studies have suggested a role for Ca^{2+} late in the mating pathway (50, 51). The yeast mating response has been the subject of intensive investigation and is known to require the activity of several protein kinases and phosphoproteins (reviewed in ref. 52). Thus, there are multiple points along this pathway where CNA1 and CNA2 could act to attenuate the mating signal and allow cells to resume growth.

Note Added in Proof. Two yeast genes identical to *CNA1* and *CNA2* were isolated by Liu *et al.* (54).

We thank Claude Klee for providing the human calcineurin A cDNA prior to publication and for invaluable advice and encouragement, Mark Pausch for helping to develop calmodulin screening of the λgt11 library and for useful suggestions, Gary L. Anderson for providing the yeast chromosome blot, and Henrik Dohlman and our other colleagues in the Thorner lab for discussion and comments on the manuscript. M.S.C. received Postdoctoral Fellowship PF3118 from the American Cancer Society and is currently a Sterling Research Group Fellow of the Life Sciences Research Foundation. This research was also supported by Research Grant GM21841 (to J.T.) from the National Institutes of Health, by funds provided by the Lucille P. Markey Charitable Trust, and by facilities supplied by the Cancer Research Laboratory of the University of California, Berkeley.

1. Koretzky, G. A., Picus, J., Thomas, M. L. & Weiss, A. (1990) *Nature (London)* **346**, 66–68.
2. Dent, P., Lavionne, A., Nakielny, S., Caudwell, F. B., Watt, P. & Cohen, P. (1990) *Nature (London)* **348**, 302–307.
3. Virshup, D. M. & Kelly, T. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3584–3588.
4. Arndt, K. T., Styles, C. A. & Fink, G. R. (1989) *Cell* **56**, 527–537.
5. Ohkura, H., Kinoshita, N., Miyatani, S., Toda, T. & Yanagida, M. (1989) *Cell* **57**, 997–1007.
6. Doonan, J. H. & Morris, N. R. (1989) *Cell* **57**, 987–996.
7. Felix, M. A., Cohen, P. & Karsenti, E. (1990) *EMBO J.* **9**, 675–683.
8. Kinoshita, N., Ohkura, H. & Yanagida, M. (1990) *Cell* **63**, 405–415.
9. Cyert, M. S. & Thorner, J. (1989) *Cell* **57**, 891–893.
10. Cohen, P. (1989) *Annu. Rev. Biochem.* **58**, 453–508.
11. Cohen, P. T. W., Brewis, N. D., Hughes, V. & Mann, D. J. (1990) *FEBS Lett.* **268**, 355–359.
12. Klee, C. B. & Cohen, P. (1988) *Mol. Aspects Cell. Regul.* **5**, 225–248.
13. Klee, C. B., Draetta, G. F. & Hubbard, M. J. (1988) *Adv. Enzymol.* **61**, 149–200.
14. Ito, A., Hashimoto, T., Hirai, M., Takeda, T., Shuntoh, H., Kuno, T. & Tanaka, C. (1989) *Biochem. Biophys. Res. Commun.* **163**, 1492–1497.
15. Kuno, T., Takeda, T., Hirai, M., Ito, A., Mukai, H. & Tanaka, C. (1989) *Biochem. Biophys. Res. Commun.* **165**, 1352–1358.
16. Kincaid, R. L., Ploavarapu, R. G., Higuchi, S., Tamura, J., Dixon, S. C., Marietta, C. A., Amorese, D. A. & Martin, B. M. (1990) *J. Biol. Chem.* **265**, 11312–11319.
17. Guerini, D. & Klee, C. B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9183–9187.
18. Guerini, D. & Klee, C. B. (1991) *Adv. Prot. Phosphatases* **6**, 391–410.
19. da Cruz e Silva, E. F. & Cohen, P. T. W. (1989) *Biochim. Biophys. Acta* **1009**, 293–296.
20. Thaler, C. D. & Haimo, L. T. (1990) *J. Cell Biol.* **111**, 1939–1948.
21. Tash, J. S., Krinks, M., Patel, J., Means, R. L., Klee, C. B. & Means, A. R. (1988) *J. Cell Biol.* **106**, 1625–1633.
22. Cohen, P., Schelling, D. L. & Stark, M. J. R. (1989) *FEBS Lett.* **250**, 601–606.
23. Cyert, M. S. & Thorner, J. (1989) *J. Cell Biol.* **107**, 841 (abstr.).
24. Sikorski, R. S. & Heiter, P. (1989) *Genetics* **122**, 19–27.
25. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
26. Becker, D. M. & Guarente, L. (1991) *Methods Enzymol.* **194**, 182–187.
27. Herskowitz, I. & Jensen, R. E. (1991) *Methods Enzymol.* **194**, 132–146.
28. Reneke, J. E., Blumer, K. J., Courchesne, W. E. & Thorner, J. (1988) *Cell* **55**, 221–234.
29. Hoffman, C. S. & Winston, F. (1987) *Gene* **57**, 267–272.
30. Chu, G., Vollrath, D. & Davis, R. W. (1986) *Science* **234**, 1582–1585.
31. Pausch, M., Kaim, D., Kunisawa, R., Admon, A. & Thorner, J. (1991) *EMBO J.* **10**, 1511–1522.
32. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
33. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D. & Fink, G. R. (1987) *Gene* **60**, 237–243.
34. Berndt, N., Campbell, D. G., Caudwell, F. B., Cohen, P., da Cruz e Silva, E. F., da Cruz e Silva, O. B. & Cohen, P. T. W. (1987) *FEBS Lett.* **223**, 340–346.
35. Hashimoto, Y., King, M. M. & Soderling, T. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7001–7005.
36. Hashimoto, Y. & Soderling, T. R. (1989) *J. Biol. Chem.* **264**, 16524–16529.
37. Hashimoto, Y., Perrino, B. A. & Soderling, T. R. (1990) *J. Biol. Chem.* **265**, 1924–1927.
38. Rothstein, R. (1983) *Methods Enzymol.* **101**, 202–211.
39. Alani, E., Cao, L. & Kleckner, N. (1987) *Genetics* **116**, 541–545.
40. Mortimer, R. K., Schild, D., Contopoulou, C. R. & Kans, J. A. (1989) *Yeast* **5**, 321–403.
41. Jones, J. S. & Prakash, L. (1990) *Yeast* **6**, 363–366.
42. Sneddon, A. A., Cohen, P. T. W. & Stark, M. J. R. (1991) *EMBO J.* **9**, 4339–4346.
43. Ingebritsen, T. S., Stewart, A. A. & Cohen, P. (1983) *Eur. J. Biochem.* **132**, 297–307.
44. Kincaid, R. L. & Martin, B. M. (1989) *Adv. Exp. Med. Biol.* **255**, 347–358.
45. Guerriero, V., Russo, M. A., Olson, N. J., Putkey, J. A. & Means, A. R. (1986) *Biochemistry* **25**, 8372–8381.
46. Hanley, R. M., Means, A. R., Ono, T., Kemp, B. E., Burgin, K. E., Waxham, N. & Kelly, P. T. (1987) *Science* **237**, 293–297.
47. James, P., Maeda, M., Fischer, R., Verma, A. K., Krebs, J., Penniston, J. T. & Carafoli, E. (1988) *J. Biol. Chem.* **263**, 2905–2910.
48. Levin, D. E., Fields, F. O., Kunisawa, R., Bishop, J. M. & Thorner, J. (1990) *Cell* **62**, 213–224.
49. Hubbard, M. J. & Klee, C. B. (1989) *Biochemistry* **28**, 1868–1874.
50. Ohsumi, Y. & Anraku, Y. (1985) *J. Biol. Chem.* **260**, 10482–10486.
51. Iida, H., Yagawa, Y. & Anraku, Y. (1990) *J. Biol. Chem.* **265**, 13391–13399.
52. Cross, F., Hartwell, L. H., Jackson, C. & Konopka, J. B. (1988) *Annu. Rev. Cell Biol.* **4**, 429–457.
53. Momayezi, M., Lumpert, C. J., Kersken, H., Gras, U., Plattner, H., Krinks, M. H. & Klee, C. B. (1987) *J. Cell Biol.* **105**, 181–189.
54. Liu, Y., Ishii, S., Yokai, M., Tsutsumi, H., Ohki, O., Akada, R., Tanaka, K., Tsuchiya, E., Fukui, S. & Miyakawa, T. (1991) *Mol. Gen. Genet.* **227**, 52–59.