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Identification of a Human cDNA Encoding a Protein That Is Structurally and Functionally Related to the Yeast Adenylyl Cyclase-Associated CAP Proteins

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The adenylyl cyclases of both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are associated with related proteins named CAP. In *S. cerevisiae*, CAP is required for cellular responses mediated by the RAS/cyclic AMP pathway. Both yeast CAPs appear to be bifunctional proteins: the N-terminal domains are required for the proper function of adenylyl cyclase, while loss of the C-terminal domains results in morphological and nutritional defects that appear to be unrelated to the cAMP pathways. Expression of either yeast CAP in the heterologous yeast suppresses phenotypes associated with loss of the C-terminal domain of the endogenous CAP but does not suppress loss of the N-terminal domain. On the basis of the homology between the two yeast CAP proteins, we have designed degenerate oligonucleotides that we used to detect, by the polymerase chain reaction method, a human cDNA fragment encoding a CAP-related peptide. Using the polymerase chain reaction fragment as a probe, we isolated a human cDNA clone encoding a 475-amino-acid protein that is homologous to the yeast CAP proteins. Expression of the human CAP protein in *S. cerevisiae* suppresses the phenotypes associated with loss of the C-terminal domain of CAP but does not suppress phenotypes associated with loss of the N-terminal domain. Thus, CAP proteins have been structurally and, to some extent, functionally conserved in evolution between yeasts and mammals.

The RAS proteins have been highly conserved in eucaryotic organisms. Despite intensive efforts to understand the function of these proteins, an effector pathway of RAS is known only in the yeast *Saccharomyces cerevisiae*. Genetic and biochemical experiments have demonstrated that RAS proteins activate adenylyl cyclase in this yeast (39). Strains that express the activated $RAS2^{Val-19}$ protein have high levels of adenylyl cyclase activity, resulting in various phenotypes that include failure to arrest in the G1 phase of the cell cycle upon nutrient starvation, concomitant inability to sporulate efficiently, and inability to survive nitrogen starvation or heat shock treatment (20, 35, 39). It is not clear whether RAS interacts directly with adenylyl cyclase or whether activation of adenylyl cyclase is mediated by another protein. However, at least one other protein, named CAP, is associated with adenylyl cyclase (8). The gene encoding CAP was recently cloned (6, 9). Deletion of *CAP* results in a loss of the ability of purified RAS to stimulate adenylyl cyclase activity in membrane extracts, and it reverses the heat shock-sensitive phenotype associated with $RAS2^{Val-19}$ (6, 9). Thus, CAP is required for RAS responsiveness of adenylyl cyclase, but its precise role in the regulation and function of adenylyl cyclase is unclear.

In addition to the phenotypes mentioned above, deletion of *CAP* leads to several other phenotypes that appear to be unrelated to the cyclic AMP (cAMP) pathway, including abnormal cellular morphology, random budding, temperature sensitivity for growth, inhibition of growth on rich medium, and failure to survive nitrogen starvation (6, 9, 13, 40). RAS responsiveness of adenylyl cyclase in Δ*cap* cells can be fully restored by overexpression of the N-terminal domain of CAP, while the other phenotypes are suppressed by overexpression of the C-terminal domain (13, 40). Thus, CAP appears to consist of two distinct functional domains. The phenotypes associated with loss of the C-terminal domain of CAP are also suppressed by overexpression of profilin, an actin- and phospholipid-binding protein (40). The basis for this suppression is unclear, but it may reflect a link between growth signals, mediated by RAS, and remodeling of the cellular cytoskeleton (15, 40).

We recently identified a gene, *cap*, that encodes a CAP-related protein from the fission yeast *Schizosaccharomyces pombe* (22). In this yeast, there is a single known *RAS* gene, *ras1*, which does not appear to regulate adenylyl cyclase (11, 29). Nevertheless, the *S. pombe* and *S. cerevisiae* adenylyl cyclases have significant homology, including homology in the leucine-rich repeat region that is required for RAS activation of adenylyl cyclase in *S. cerevisiae* (4, 10, 38, 41, 42). Furthermore, *S. pombe* cap, like *S. cerevisiae* CAP, is associated with adenylyl cyclase and appears to be required for its proper function (21, 22). Thus, the adenylyl cyclase-CAP complex has been highly conserved, while its regulation by RAS does not appear to have been conserved. However, the regulation of adenylyl cyclase in *S. pombe* has not yet been defined, and it is possible that an unknown RAS protein is involved.

S. pombe cap, like *S. cerevisiae* CAP, also appears to be a bifunctional protein. *S. pombe* cells that express only the N-terminal domain of cap are temperature sensitive, grow slowly in synthetic medium, and have an abnormal morphology, while cells that lack the entire *cap* gene also conjugate and sporulate under conditions in which wild-type cells do not normally conjugate or sporulate (22). The latter phenotypes are also exhibited by *S. pombe* cells that lack adenylyl cyclase (21). Thus, loss of the C-terminal or N-terminal domain of CAP has similar consequences in the two yeasts. Furthermore, expression of *S. pombe* cap in *S. cerevisiae*

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can functionally suppress phenotypes associated with deletion of the C-terminal domain, but not the N-terminal domain, of CAP (22). Similarly, expression of *S. cerevisiae* CAP in *S. pombe* can functionally suppress phenotypes associated with deletion of the C-terminal domain, but not the N-terminal domain, of cap (22). Thus, the functions of the C-terminal domains of the yeast CAP proteins have been highly conserved, while the functions of the N-terminal domains, which are required for proper adenylyl cyclase function in both yeasts, have been only partially conserved. Interestingly, the region between the N-terminal and C-terminal functional domains is the region most highly conserved between the two yeast CAP proteins. Although deletion of this region is phenotypically silent, it may represent a third functional domain.

Since related CAP proteins are found in these two distantly related yeasts, we reasoned that CAP may be conserved in other organisms as well. In an attempt to detect a human CAP, we designed degenerate oligonucleotides to conserved regions between the two yeast CAP proteins. Using these oligonucleotides as primers in polymerase chain reactions (PCRs), we have identified a human cDNA that encodes a homolog of the yeast CAP proteins.

MATERIALS AND METHODS

Yeast strains and genetic analysis. The *S. cerevisiae* strains SKN32 (Δ*cap*) and SKN37 (*RAS2*$^{val-19}$ Δ*cap*) have been previously described (9). *S. cerevisiae* strains were grown in YPD (rich) or SC (synthetic) medium with appropriate auxotrophic supplements (32). The lithium acetate method was used to transform yeasts with plasmid DNA (19). Tests for heat shock sensitivity of yeast strains were performed as previously described (35).

DNA manipulation and analysis. Procedures used for DNA manipulation and analysis (i.e., purification, restriction site mapping, electrophoresis, and transformation, etc.) have been previously described (28). The DNA sequences of both strands of sequenced clones were determined by the dideoxy-chain termination method (3, 34).

Plasmids. pADANS has been previously described (5). This vector contains the *S. cerevisiae LEU2* gene and 2μm sequence, and it also contains the *ADH1* promoter and terminator sequences flanking the *Not*I site. The first 10 amino acid residues of ADH1 are encoded between the *ADH1* promoter and the *Not*I site. Clones that contain cDNAs inserted in the *Not*I site, in the proper orientation and frame, express a fusion protein with the N-terminal peptide MSIPETQKGVIFYEACGR(N/K). pADHCAP contains the coding region of *S. cerevisiae* CAP cloned into the *Sma*I site of pAD4Δ, a plasmid similar to pADANS (2, 9). pSC2 contains an *S. pombe cap* cDNA in the vector pADANS (22). Other plasmids used in this study are described below.

PCR. The PCR method has been previously described (18, 33). PCR mixtures containing 100 pmol of each oligonucleotide primer, 1 μg of the human cDNA library, 50 mM KCl, 10 mM Tris [pH 8.3], 1.5 mM $MgCl_2$, 200 nM (each) dATP, dCTP, dGTP, and dTTP, and 0.5 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) in a total volume of 100 μl were incubated during 30 step cycles (each cycle was for 1 min at 94°C, 2 min at 55°C, and 1 to 2 min at 72°C) and then for 7 min at 72°C.

cDNA library. The construction of the human cDNA library has been previously described (5). It was constructed by using poly(A)$^+$ RNA derived from the human glioblastoma cell line U118-MG. The cDNAs were originally inserted into the *Not*I site of lambda ZAP II (Stratagene, La Jolla, Calif.). *Not*I fragments containing the cDNAs were purified from this library and inserted into the *S. cerevisiae* expression vector pADANS to generate the library used in this study. It was kindly provided to us by John Colicelli.

Cloning the human CAP cDNA. The PCR method was used to amplify DNA fragments from the human cDNA library by using the A1 and B1 subsets of degenerate oligonucleotides as primers (see Fig. 1). The A1 subset consists of the oligonucleotides TTCTGCAG[ATG]GG[AG]TTCTTGTG [ATG]GT, where the brackets enclose bases at degenerate positions. The B1 subset consists of the oligonucleotides TT GGATCC[CTA]CC[CTA]CC[CTA]CCTCCACC. The A1 oligonucleotides contain *Pst*I sites, and the B1 oligonucleotides contain *Bam*HI sites. The product of the PCR was digested with *Pst*I and *Bam*HI, size fractionated and purified by electrophoresis, and cloned into pBluescript II SK$^-$ (Stratagene). One of these clones was named pPCRF1.

pHSC1, which contains the human CAP cDNA, was isolated by screening a human glioblastoma cDNA library by colony-filter hybridization with a ^{32}P-labeled DNA fragment from pPCRF1.

The 5′ ends of human CAP cDNAs were amplified by the PCR method using two nested sets of primers. Each set contains one primer derived from the vector pADANS and one primer derived from the cDNA in pHSC1. First, a PCR with the primers GTTTCCTCGTCATTGTTCTCGTTC and TTCAAGTACTCTGCCACAGGACCA was performed, with the human cDNA library used as a template. Then a second PCR with 2 μl of the first PCR product as a template and the primers ACAATGTCTATCCCAGAAACTCAA and TTGCGGCCGCAAGCAGCGAGTCAAATGC was performed. The final product was digested with *Not*I, fractionated and purified by electrophoresis, and cloned in the vector pBluescript II SK$^-$. A total of 24 independent clones was isolated, and one of these was designated pPCRF4-3.

PCR fragments containing the entire coding region of the human *CAP* cDNA were amplified from the human cDNA library with the primers TTGCGGCCGCAGGTGGTCCAT TATGGCT and TTGCGGCCGCTTATCCAGCAATTTC TG. The PCR product was digested with *Not*I, fractionated and purified by electrophoresis, and cloned into the *S. cerevisiae* expression vector pADANS. Three independent clones were isolated and designated pHSC2, pHSC3, and pHSC4.

Nucleotide sequence accession number. The sequence of the human CAP cDNA has been submitted to GenBank (accession no. M98474).

RESULTS

Cloning and sequence of a human cDNA encoding a CAP-related protein. The *S. cerevisiae* CAP and *S. pombe* cap protein sequences are 34% identical overall but have stronger homology between their C-terminal regions (22). The strongest homology is between the 60-amino-acid regions located near the center of each protein (residues 277 through 337 in *S. cerevisiae* and 306 through 364 in *S. pombe*), which are 61% identical (see Fig. 3). We designed degenerate sets of oligonucleotides that encode the two conserved peptide sequences located at the ends of these regions. The first set of oligonucleotides encodes the conserved peptide (THKNP) at the C-terminal end of the conserved domain. The second set encodes the conserved polyproline peptide (PPPPP) at the N-terminal end of the conserved domain. We

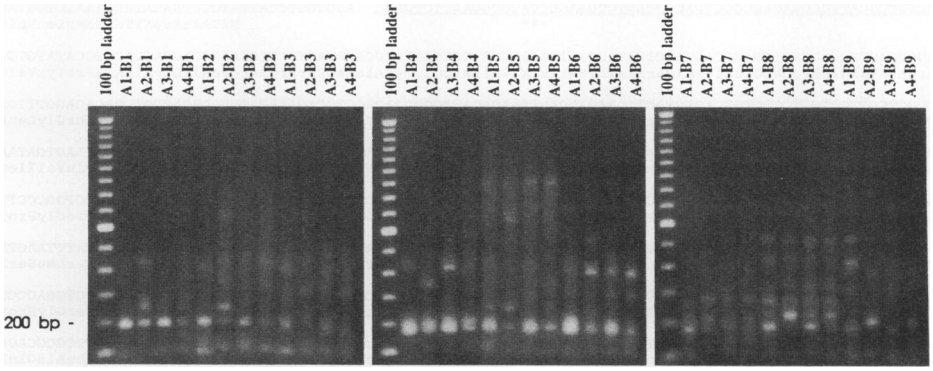

FIG. 1. Detection of human CAP by PCR. PCRs were performed as described in Materials and Methods. The reaction mixtures contained sets of degenerate oligonucleotides as primers (indicated above each lane) and DNA from a human cDNA library as a template (see Materials and Methods). The sets of degenerate oligonucleotides used are listed in Results.

did not use the codons ACG (Thr) and CCG (Pro), which are infrequently used in human genes (26); thus, the degeneracy of the first oligonucleotide set was 72, and the degeneracy of the second set was 253. These two sets of oligonucleotides were used as primers in a PCR mixture containing DNA from a human cDNA library as a template (see Materials and Methods). The cDNA library was derived from the human glioblastoma cell line U118-MG (5). We detected several PCR products that were approximately the length (180 bp) of DNA sequences encoding the conserved yeast CAP regions (data not shown). In an attempt to improve the intensity and resolution of these bands, we reduced the degeneracy of oligonucleotides in our PCRs by dividing each set into subsets. The first set of oligonucleotides was divided into four subsets, designated A1 through A4, each containing 18 different oligonucleotides:

A1: TTCTGCAG[ATG]GG[AG]TTCTTGTG[ATG]GT
A2: TTCTGCAG[ATG]GG[AG]TTCTTATG[ATG]GT
A3: TTCTGCAG[ATG]GG[AG]TTTTTGTG[ATG]GT
A4: TTCTGCAG[ATG]GG[AG]TTTTTATG[ATG]GT

The second set of oligonucleotides was divided into nine subsets, designated B1 through B9, each containing 27 different oligonucleotides:

B1: TTGGATCC[CTA]CC[CTA]CC[CTA]CCTCCACC
B2: TTGGATCC[CTA]CC[CTA]CC[CTA]CCACCACC
B3: TTGGATCC[CTA]CC[CTA]CC[CTA]CCCCCACC
B4: TTGGATCC[CTA]CC[CTA]CC[CTA]CCTCCTCC
B5: TTGGATCC[CTA]CC[CTA]CC[CTA]CCACCTCC
B6: TTGGATCC[CTA]CC[CTA]CC[CTA]CCCCCTCC
B7: TTGGATCC[CAT]CC[CTA]CC[CTA]CCTCCCCC
B8: TTGGATCC[CAT]CC[CTA]CC[CTA]CCACCCCC
B9: TTGGATCC[CTA]CC[CTA]CC[CTA]CCCCCCCC

We performed 36 different PCRs with all possible combinations consisting of one subset from each set of degenerate oligonucleotides. Several of the PCRs produced DNA fragments that were approximately 180 bp (Fig. 1). The 180-bp PCR fragments produced by using the first oligonucleotide subset pair (A1-B1) were cloned, and the DNA sequences of 12 independently derived clones were determined. These clones contain two different types of sequences. Five of the clones contain a DNA sequence that does not contain an open reading frame and were presumably derived from the noncoding region of a cDNA (data not shown). The other seven clones contain a sequence encoding a peptide that has homology with the yeast CAP proteins. One of the latter clones was designated pPCRF1.

We identified a human cDNA clone, pHSC1, by screening the human cDNA library with the cloned PCR fragment in pPCRF1 (see Materials and Methods). The cDNA in pHSC1 encodes a 475-amino-acid protein, beginning from the first ATG, although it lacks an in-frame stop codon 5′ to the first ATG (Fig. 2). Therefore, we did not know whether this cDNA contained the entire coding sequence or lacked only the 5′ untranslated region. To determine the 5′ DNA sequence, we amplified the 5′ ends of cDNAs from a human cDNA library by PCR using two sets of nested primers (see Materials and Methods). Each primer set contained one oligonucleotide derived from the vector pADANS and one oligonucleotide derived from the cDNA in pHSC1 approximately 160 bp from the first ATG. The final PCR product was approximately 300 bp in length, but upon digestion with *Not*I it was reduced to approximately 200 bp. Therefore, we suspect that there is an internal *Not*I site in the 5′ cDNA ends. We purified and cloned the 200-bp fragments containing the amplified 5′ cDNA ends and determined the DNA sequences of 24 independently derived clones. Each of these sequences included, and extended beyond, the 5′ end of the sequence of pHSC1. One clone, pPCRF4-3, contained an additional 58 bp at the 5′ end (underlined in Fig. 2). All of the other sequences are identical to the sequence of pPCRF4-3 except that they lack either the 5′ sequence or other parts of this sequence. Many of the clones lack either the 3-nucleotide sequence GCT at positions 56 to 58 or the 18-nucleotide sequence between positions 38 and 55 or both. The sequences of 10 clones contained the in-frame stop codon (positions 41 to 43) located 20 bp 5′ to the first ATG (positions 71 to 73), indicating that pHSC1 encodes a full-length protein. However, since this stop codon is not present in some 5′ cDNA ends, we cannot rule out the possibility that a second, longer protein is also expressed in humans.

To rule out the possibility that pHSC1 was an anomalous clone, we determined the DNA sequences of three independently isolated clones designated pHSC2, pHSC3, and pHSC4. These clones contain PCR fragments derived from the human cDNA library that encode the entire human CAP protein (see Materials and Methods). The coding sequences of pHSC1, pHSC2, and pHSC3 are identical, while pHSC4 has a single base pair difference at position 693. We suspect that this difference is due to misincorporation of a nucleotide in pHSC4 during PCR.

Comparison of human and yeast CAP sequences. Deletion

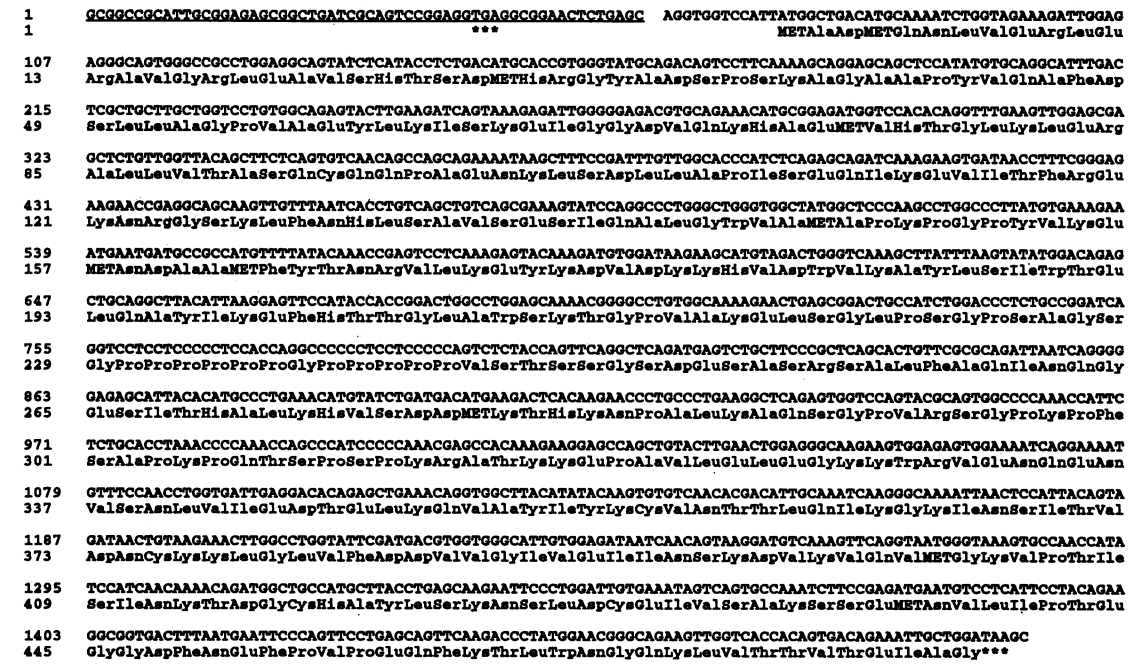

FIG. 2. Sequences of human CAP cDNA and the encoded protein. The underlined 5′ sequence was derived from pPCRF4-3, while the remaining sequence was derived from pHSC1. The in-frame stop codons, located at the 5′ and 3′ ends of the coding sequence, are indicated (***). The cDNA encodes a 475-amino-acid protein. Numbers of base pairs from the *Not*I site and of amino acid residues from the beginning of the encoded protein are indicated on the left.

analyses have defined two distinct functional domains in the yeast CAP proteins that are separated by a third, highly conserved, phenotypically silent domain. The two yeast CAPs are 34% identical overall. Comparison of the human and yeast CAP protein sequences shows that the human protein is 37% identical with *S. cerevisiae* CAP and 35% identical with *S. pombe* cap (Fig. 3). Thus, the human and yeast CAP proteins are equidistantly related. The N-terminal region (residues 1 through 132) of human CAP is 25% identical with the corresponding region of *S. cerevisiae* CAP and 27% identical with *S. pombe* cap. The middle domain (residues 133 through 317) of the human CAP is 43% (*S. cerevisiae*) and 38% (*S. pombe*) identical with the yeast CAPs, while the C-terminal domain (residues 318 through 475) is 38% identical with both yeast CAPs. Thus, the central and C-terminal regions are the most highly conserved while the N-terminal domains are the least highly conserved between the yeast and human CAPs.

The recent partial peptide sequence analysis of a porcine platelet actin-binding protein, ASP-56, revealed that it is related to the yeast CAP proteins (14). Comparison of peptide sequences from ASP-56 with human CAP shows that these two proteins are highly homologous, suggesting that ASP-56 is the porcine CAP protein or a closely related homolog (Fig. 3). However, complete sequence analysis of ASP-56 is required to distinguish these possibilities.

Complementation of Δ*cap* phenotypes in *S. cerevisiae*. Disruption of the N-terminal domain of CAP suppresses the heat shock-sensitive phenotype associated with the $RAS2^{val\text{-}19}$ mutation in *S. cerevisiae*, while loss of the C-terminal domain results in abnormal cellular morphology (round and enlarged cells), random budding, temperature sensitivity, growth inhibition on rich medium (YPD), and failure to survive nitrogen starvation. The functions of the two domains are separable by deletion analysis and appear, on the surface, to be unrelated (13, 40).

To test whether the human and yeast CAP proteins are functionally conserved, we expressed the human and yeast CAPs in the *S. cerevisiae* strains SKN32 (Δ*cap*) and SKN37 ($RAS2^{val\text{-}19}$ Δ*cap*), in which the endogenous *CAP* genes have been deleted. SKN37 also contains the $RAS2^{val\text{-}19}$ mutation that induces heat shock sensitivity in otherwise normal strains but that does not have an effect in this strain because of the absence of CAP. The plasmids pADHCAP (*S. cerevisiae*), pSC2 (*S. pombe*), and pHSC2 (human) contain the different CAP coding sequences under the control of the *S. cerevisiae ADH1* promoter (see Materials and Methods). SKN32 cells that contain pADHCAP, pSC2, or pHSC2 appear to have a relatively normal morphology compared with cells harboring the vector pADANS (Fig. 4). Expression of the human or yeast CAPs also suppressed the other abnormal-growth phenotypes associated with loss of the C-terminal domain of CAP in SKN37 cells. SKN37 cells containing pHSC2, pSC2 or pADHCAP grew normally at 36°C and on rich medium (YPD), whereas cells containing the control plasmid pADANS remained sensitive to temperature and nutrient conditions (Fig. 5). Furthermore, SKN37 cells containing either pSC2 or pHSC2 were resistant to nitrogen starvation (Fig. 5). Sensitivity to nitrogen starvation in SKN37 cells is also complemented by overexpression of the C-terminal domain of the *S. cerevisiae* CAP, but because this phenotype also results from the $RAS2^{val\text{-}19}$ mutation, it is not complemented by expression of the full-length CAP (13).

In contrast to the abilities of the human and *S. pombe* CAPs to complement loss of C-terminal CAP functions in *S. cerevisiae*, the heat shock-resistant phenotype resulting from loss of the N-terminal portion of CAP in SKN37 cells

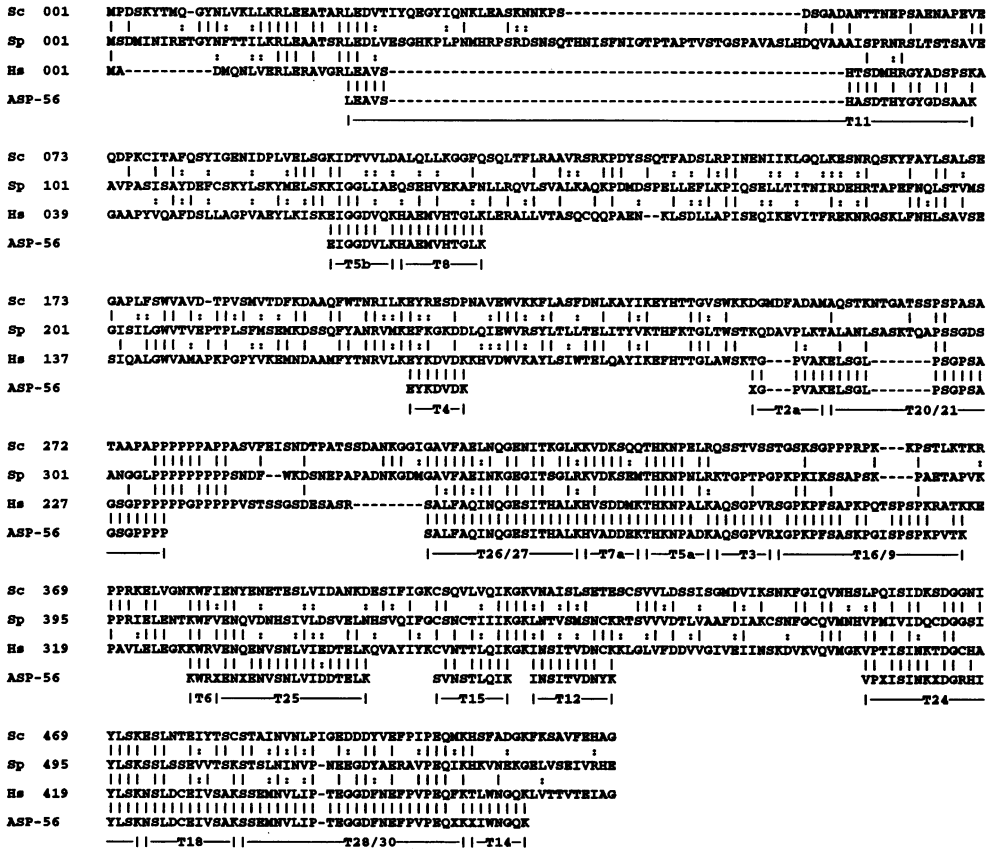

FIG. 3. Comparison of human and yeast CAP proteins. The sequences of the *S. cerevisiae* (Sc), *S. pombe* (Sp), and human (Hs) CAP proteins were aligned by using the Multiple Alignment Construction and Analysis Workbench (MACAW) program (36). Numbers on the left indicate the position of the next amino acid residue in the protein sequence. Identical and conserved amino acid residues are connected by vertical lines and colons, respectively. Previously derived peptide sequences from the porcine platelet actin-binding protein ASP-56 are also aligned below the human CAP protein sequence (14). The peptide names and positions are indicated below each peptide.

was not suppressed by expression of the human or *S. pombe* CAP (Fig. 5). SKN37 cells transformed with pHSC2, pSC2, or pADANS remained heat shock resistant, whereas SKN37 cells transformed with pADHCAP, which express the *S. cerevisiae* CAP protein, became heat shock sensitive. Thus, expression of human or *S. pombe* CAP appears to be insufficient to restore RAS responsiveness in *S. cerevisiae* but can complement the other phenotypes associated with loss of CAP.

DISCUSSION

S. cerevisiae CAP and *S. pombe* cap are related proteins that are associated with the adenylyl cyclases of these organisms. Deletion analyses have defined two distinct functional domains in each yeast CAP. The N-terminal domains appear to be required for the proper function of adenylyl cyclase, while deletion of the C-terminal domains results in other distinct phenotypes that do not appear to be associated with the cAMP pathways of these organisms. In *S. cerevisiae*, CAP is required for full cellular responsiveness to RAS. Thus, it is possible that CAP mediates activation of adenylyl cyclase by RAS in this yeast. The relationship of the different domains of CAP and whether RAS is involved in regulating some or all of CAP functions are not clear.

We have identified and cloned a human cDNA encoding a protein that is highly related to the yeast CAP proteins. We first detected the presence of a human CAP-related sequence by the PCR method using degenerate oligonucleotides designed to encode homologous regions of the yeast CAPs. Our observation that several different but closely related primers produced PCR fragments that were approximately the same length suggests that there may be more than one human CAP. The sequence of human CAP is as closely related to each of the yeast proteins as they are to each other. The N-terminal domains of the three different CAPs are the least conserved regions, while the C-terminal and central regions are more highly conserved. It is intriguing that CAP has been conserved in mammals even though the functions of the two domains of CAP appear to be unrelated in yeasts. Thus, there may be some relationship between the two functional domains that requires their presence on the same polypeptide. It is also intriguing that the central region, which has no apparent function in *S. cerevisiae*, is also highly conserved in human CAP. This observation suggests that the central region is important and that it may represent a third functional domain.

Our complementation studies indicate that the C-terminal domains are functionally, as well as structurally, conserved. Expression of either human or *S. pombe* CAP can functionally substitute for the C-terminal domain of CAP in *S. cerevisiae*. Expression of profilin, an actin-binding protein, also suppresses the loss of the C-terminal domain of CAP (40). Furthermore, deletion of profilin results in phenotypes

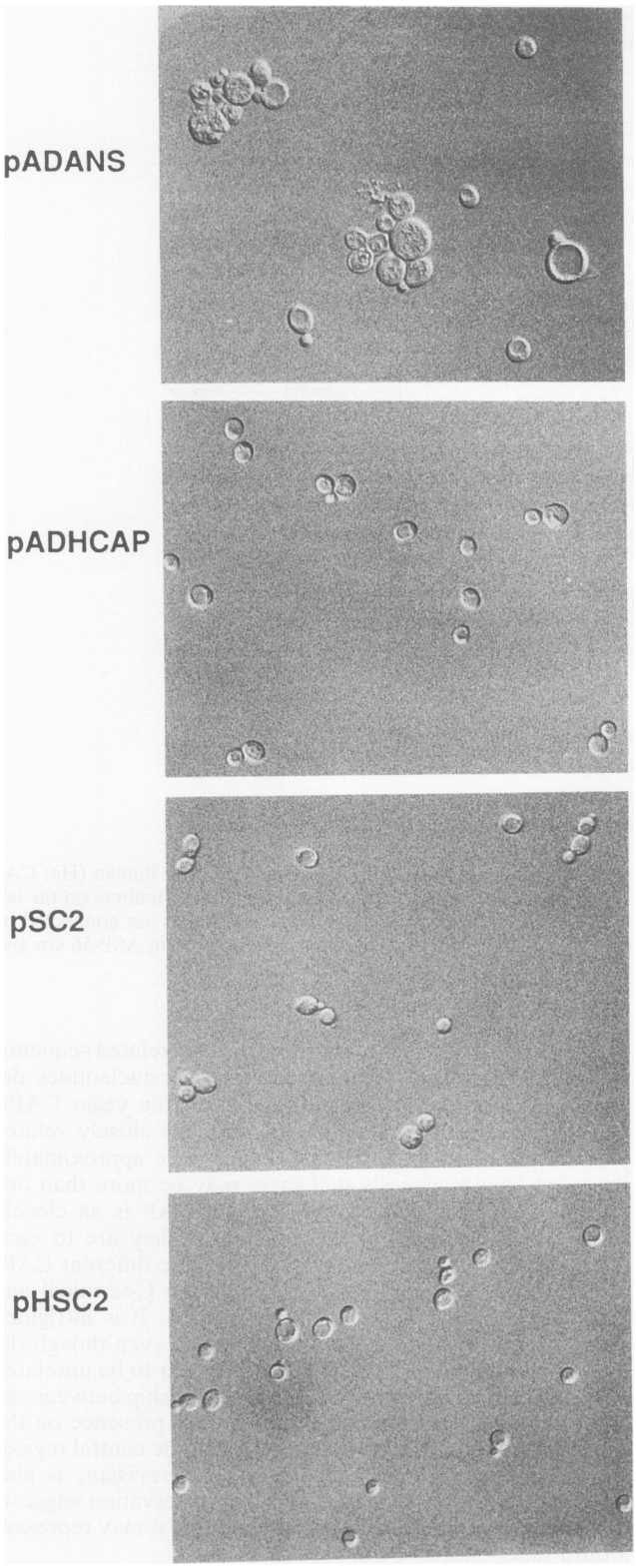

FIG. 4. Suppression of morphological defects in *S. cerevisiae* Δ*cap* strain. *S. cerevisiae* SKN32 (Δ*cap*) was transformed with plasmids expressing the *S. cerevisiae* (pADHCAP), *S. pombe* (pSC2), or human (pHSC2) CAP protein or a control vector (pADANS). Colonies were picked and grown at 30°C in liquid SC −Leu medium (lacking leucine). Cells were examined by differential interference contrast microscopy and photographed after 2 days.

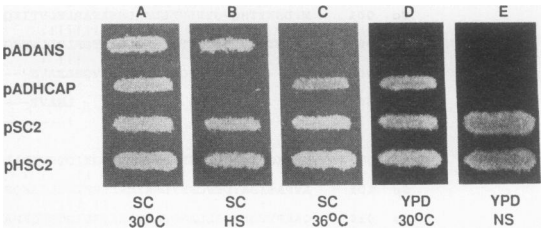

FIG. 5. Complementation of Δ*cap* phenotypes in *S. cerevisiae*. *S. cerevisiae* SKN37 (Δ*cap RAS2*$^{val-19}$) was transformed with plasmids expressing the *S. cerevisiae* (pADHCAP), *S. pombe* (pSC2), or human (pHSC2) CAP protein or a control vector (pADANS). Patches of cells were grown on a plate containing selective synthetic medium (SC −Leu medium) at 30°C for 2 days. Replicas of the plate were made on either synthetic (SC −Leu) or rich (YPD) medium. (A) As a control, a replica was made on an SC −Leu plate and incubated at 30°C. (B) To test for heat shock sensitivity (HS), a replica was made on an SC −Leu plate, prewarmed at 55°C, subjected to heat shock treatment at 55°C for 4 min, and incubated at 30°C. (C) To test for temperature-sensitive growth, a replica was made on an SC −Leu plate and grown at 36°C. (D) To test for growth on rich medium, a replica was made on a YPD plate and incubated at 30°C. (E) To test for sensitivity to nitrogen starvation (NS), a replica was made on a YNB-N plate (lacking nitrogen), grown for 14 days at 30°C, replica plated back onto a YPD plate, and incubated at 30°C. All replica plate cultures were grown for 2 days.

that are similar to those resulting from loss of the C-terminal domain of CAP in *S. cerevisiae* (17, 40). Thus, CAP and profilin may belong to the same or related signalling pathways. Profilin appears to regulate actin filament formation, but its precise role is unclear (17, 30, 37). In addition to binding to monomeric actin, profilin also specifically binds to polyphosphoinositides. This results in the dissociation of profilin and actin (24, 25) and in the protection of PIP_2 from hydrolysis by phospholipase C (16, 27). Thus, profilin may be a component of a signal transduction pathway that modulates the cytoskeletal structure, and it may also be involved in phosphoinositide metabolism. The suppression of C-terminal CAP functions in *S. cerevisiae* by profilin appears to be independent of the interaction between profilin and actin, but it may be related to the interaction of profilin with polyphosphoinositides (40). These observations led to the suggestion that loss of CAP alters the formation of second messengers that result from phosphoinositide metabolism (40). An association between CAP and actin was recently suggested by the recent characterization of ASP-56, a porcine actin-binding protein (14). Comparison of partial peptide sequences from ASP-56 with human CAP suggests that ASP-56 is the porcine CAP protein or a closely related homolog. Exactly how CAP is connected to actin, profilin, and phosphoinositide metabolism is not clear, but the implication of these observations is that CAP is somehow involved in mediating changes in the cellular architecture, perhaps in response to the activation of cellular signalling pathways that regulate growth and morphology.

In contrast to the functional conservation of the C-terminal domains, neither human nor *S. pombe* CAP is capable of functionally substituting for the N-terminal domain of *S. cerevisiae* CAP. It is not surprising that the functions of the N-terminal domains have diverged, since the known mammalian adenylyl cyclases are structurally distinct from the two yeast adenylyl cyclases. While there is minor homology between the mammalian adenylyl cyclases and the catalytic domains of the yeast adenylyl cyclases, there is essentially

no homology outside these regions (1, 7, 12, 23). Furthermore, while the mammalian adenylyl cyclases are regulated by heterotrimeric G proteins, adenylyl cyclase is regulated by RAS in *S. cerevisiae*. In *S. pombe*, adenylyl cyclase does not appear to be regulated by *ras1*, the single known *RAS* gene in this organism (11, 29). Thus, both the regulation of adenylyl cyclase and the role of the N-terminal domain of CAP have diverged in evolution. Nevertheless, there is significant sequence homology between the N-terminal regions of the yeast and human CAPs, suggesting some underlying functional conservation. Perhaps the most intriguing possibility is that CAP proteins may be required to mediate RAS functions in mammals. Although the regulation of adenylyl cyclase by RAS has not been conserved in mammals, perhaps the relationship between RAS, CAP, and the cytoskeleton has been conserved. Further studies must be done to elucidate the function of human CAP and to determine whether it is involved with RAS, adenylyl cyclase, and/or the regulation of actin filament formation.

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