Journal of Cell Science 107, 1671-1678 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

Comparison of human CAP and CAP2, homologs of the yeast adenylyl cyclase-associated proteins

Gang Yu, John Swiston and Dallan Young*

Department of Medical Biochemistry, University of Calgary Health Science Centre, Calgary, Alberta T2N 4N1, Canada

*Author for correspondence

SUMMARY

We previously reported the identification of human CAP, a protein that is related to the *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* adenylyl cyclase-associated CAP proteins. The two yeast CAP proteins have similar functions: the N-terminal domains are required for the normal function of adenylyl cyclase, while loss of the C-terminal domains result in morphological and nutritional defects that are unrelated to the cAMP pathways. We have amplified and cloned cDNAs from a human glioblastoma library that encode a second CAP-related protein, CAP2. The human CAP and CAP2 proteins are 64% identical. Expression of either human CAP or CAP2 in *S. cerevisiae cap*$^-$ strains suppresses phenotypes associated with deletion of the C-terminal domain of CAP, but does not restore hyper-activation of adenylyl cyclase by RAS2^{val19}. Similarly, expression of either human CAP or CAP2 in *S. pombe cap*$^-$ strains suppresses the morphological and temperature-sensitive phenotypes associated with deletion of the C-terminal domain of CAP in this yeast. In addition, expression of human CAP, but not CAP2, suppresses the propensity to sporulate due to deletion of the N-terminal domain of CAP in *S. pombe*. This latter observation suggests that human CAP restores normal adenylyl cyclase activity in *S. pombe cap*$^-$ cells. Thus, functional properties of both N-terminal and C-terminal domains are conserved between the human and *S. pombe* CAP proteins.

Key words: CAP, RAS, adenylyl cyclase, actin

INTRODUCTION

CAP is an intriguing multi-functional protein that has been conserved between yeast and mammals during evolution. CAP was first identified in the budding yeast *Saccharomyces cerevisiae*. Purification of the adenylyl cyclase complex from this yeast revealed the presence of a 70 kDa protein that was tightly associated with the catalytic protein (Field et al., 1988). The *CAP* gene encoding the 70 kDa protein was cloned and found to be allelic with *supC* and *SRV2*, mutations that suppress the heat-shock-sensitive phenotype resulting from the activated *RAS2*^{val19} mutation (Field et al., 1990; Fedor-Chaiken et al., 1990). While CAP is required for the hyper-activation of adenylyl cyclase by RAS2^{val19}, its precise role is not clear. Recent results indicate that purified RAS is capable of activating adenylyl cyclase activity in crude membrane extracts from cells lacking CAP (Wang et al., 1992). Thus, CAP is not required for the interaction of RAS with the adenylyl cyclase complex, but it may be involved in facilitating this interaction in vivo. Deletion analysis revealed that CAP has two distinct functional domains: the N-terminal domain is required for RAS2^{val19} responsiveness of adenylyl cyclase, while the C-terminal domain appears to play a role in nutritional responses and cytoskeletal structure (Gerst et al., 1991; Vojtek et al., 1991). Deletion of the C-terminal region leads to several phenotypes including nutritional and temperature sensitivity, abnormal cell morphology, slow growth, random budding and abnormal actin distribution. Many of these phenotypes are also seen in conditional actin mutants or in yeast that are deficient in profilin, an actin/phosphoinositide-binding protein (Novick and Botstein, 1985; Haarer et al., 1990). Also, overexpression of profilin suppresses these phenotypes in *cap*$^-$ strains, although the normal budding pattern is only partially restored (Vojtek et al., 1991). The ability of mutant forms of profilin to suppress these phenotypes correlates with their ability to bind phosphoinositides. Thus, the observed phenotypes may be a consequence of abnormalities in both actin filament assembly and phosphoinositide metabolism (Vojtek et al., 1991). Overexpression of the yeast SNC1 protein, a homolog of the synaptobrevin family of synaptic vesicle-associated membrane proteins, also suppresses these phenotypes, but the mechanism of suppression by this protein is unclear (Gerst et al., 1992). The relationship between the two functional domains of CAP, and the functional role of the middle region separating these two domains, are not known.

CAP genes have been recently identified in both the fission yeast *S. pombe* (Kawamukai et al., 1992) and mammals (Matviw et al., 1992; Vojtek and Cooper, 1993; Zelicof et al., 1993). The yeast and human proteins are equi-distantly related and share approximately 35% identity with each other. All three domains of CAP are conserved, although homologies among the N-terminal domains are slightly weaker than are the homologies among the middle and C-terminal regions. The functions of *S. cerevisiae* and *S. pombe* CAPs appear to be very

similar: both proteins are associated with adenylyl cyclase, while deletion of the C-terminal domains results in similar morphological and nutritional defects (Kawamukai et al., 1992). Expression of either the *S. pombe* or human CAP proteins in *S. cerevisiae* strains can suppress the abnormal morphological and growth phenotypes associated with deletion of the C-terminal domain of CAP, but fail to restore $RAS2^{val19}$ responsiveness of adenylyl cyclase (Kawamukai et al., 1992; Matviw et al., 1992). Thus, functional properties of the C-terminal domains of CAP proteins have been conserved during evolution, but the N-terminal domains appear to have diverged.

In this report, we present the sequence of a second human CAP protein, CAP2, and show that its expression complements loss of the C-terminal domains, but not the N-terminal domains, of CAP in either *S. cerevisiae* or *S. pombe*. We also show that expression of human CAP complements loss of both the N-terminal and C-terminal domains of CAP in *S. pombe*. Thus, both domains of human and *S. pombe* CAP proteins are functionally conserved.

MATERIALS AND METHODS

Yeast strains and genetic analysis

The *S. cerevisiae* strains SKN32 (*cap*$^-$) and SKN37 (*cap*$^-$ $RAS2^{val19}$) have been described previously (Field et al., 1990). The *S. pombe* strain MK1818d (*cap*$^-$) has been described previously (Kawamukai et al., 1992). *S. cerevisiae* strains were grown in rich (YPD) or synthetic (SC) medium with appropriate auxotrophic supplements (Rose et al., 1990). The *S. pombe* MK1818d strain was grown in rich YEA medium or synthetic PMAA (PMA + 10 mg/l arginine) medium with appropriate auxotrophic supplements (Moreno et al., 1990). The lithium acetate method was used to transform yeast with plasmid DNA (Ito et al., 1983). Tests for heat-shock sensitivity of yeast strains were performed as previously described (Sass et al., 1986).

DNA manipulation and analysis

Procedures used for DNA manipulation and analysis (purification, restriction site mapping, electrophoresis, transformation, etc.) have been described previously (Maniatis et al., 1982). The DNA sequences of both strands of sequenced clones were determined using the Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied BioSystems).

Plasmids

The *S. cerevisiae* expression vector pADANS has been described previously (Colicelli et al., 1991). This vector contains the *S. cerevisiae LEU2* gene and 2 micron sequence, and it also contains the *ADH1* promoter and terminator sequences flanking a *Not*I site. pADHCAP contains the *S. cerevisiae CAP* coding sequence cloned into the *Sma*I site of pAD4Δ, a plasmid similar to pADANS (Ballester et al., 1989; Field et al., 1990). pSC2 contains a *S. pombe cap* cDNA in the *Not*I site of pADANS (Kawamukai et al., 1992). pHSC2 contains the human *CAP* coding sequence in the *Not*I site of pADANS (Matviw et al., 1992). pCAP2 contains the human *CAP2* coding sequence in the *Not*I site of pADANS (this study). The *S. pombe* expression vector pAALN is identical to pAAUN (Xu et al., 1990) except that the 1.8 kb *Hin*dIII fragment containing the *ura4* gene has been replaced with the 2.2 *Hin*dIII fragment containing the *S. cerevisiae LEU2* gene. This plasmid contains the *S. pombe ars1* sequence and *adh1* promoter sequence flanking a *Not*I site. pACL1 contains the *S. pombe cap* cDNA in the *Not*I site of pAALN (Kawamukai et al., 1992). pA70L contains the *S. cerevisiae CAP* coding sequence in the *Sma*I site of pART1, a vector similar to pAALN (Kawamukai et al., 1992). pNHCAP contains the human *CAP* coding sequence in the *Not*I site of pAALN (this study). pNHCAP2 contains the human *CAP2* coding sequence in the *Not*I site of pAALN (this study).

cDNA library

The human cDNA library used in this study was kindly provided by John Colicelli. It contains cDNAs derived from the glioblastoma cell line U118-MG cloned into the *Not*I site of pADANS (Colicelli et al., 1991).

Cloning the human *CAP2* cDNA

The 3′ ends of *CAP2* cDNAs were amplified by PCR from a human cDNA library using the oligonucleotides TTGCGGCCGCTTAGGCCATAATTTCTGCAG and TTGGATCC[CTA]CC[CTA]CC[CTA]CCTCCTCC, where brackets enclose nucleotides used at degenerate positions. PCR reactions were performed using Vent Polymerase (New England Biolabs) and cycling conditions as previously described (Matviw et al., 1992). The 5′ ends of *CAP2* cDNAs were amplified from the human cDNA library by PCR using three sets of nested primers. Each set contained one primer derived from the vector pADANS and one primer derived from the sequence of the 3′ end of the *CAP2* cDNA. First, the primers GTTTCCTCGTCATTGTTCTCGTTC and TTGCTTCTCCCTGGTTAAGT were used in a PCR reaction containing the human cDNA library as template. Then the primers ACAATGTCTATCCCAGAAACTCAA and CGTGAAGGAGAAGATTCCTC were used in a second PCR reaction containing 1/50 of the first reaction product as template. To amplify further the 5′ ends of the *CAP2* cDNAs a third PCR reaction containing 1/50 of the second reaction as template and the primers ACAATGTCTATCCCAGAAACTCAA and TTGCGGCCGCTTCATTCTCGAAAAGTGGA was performed. To obtain clones containing the entire coding sequence of CAP2 the primers TTGCGGCCGCTTAGGCCATAATTTCTGCAG and TTGCGGCCGCATGGCCAACATGCAGGGACT, derived from the 5′ and 3′ ends of the *CAP2* cDNA, were used in a PCR reaction. The PCR products were cloned into the *Not*I site of pBluescript II SK− (Stratagene).

Fluorescent staining of cells

Cells were fixed with formaldehyde and stained with either rhodamine-conjugated phalloidin (Molecular Probes, Inc.) or Calcofluor white M2R (Sigma), as previously described (Pringle et al., 1989).

RESULTS

Isolation of a human cDNA encoding CAP2

Comparison of the human *CAP* sequence with the GenBank database revealed that it has homology with a 226 base pair tag-sequence derived from the 3′ end of a human brain cDNA (Adams et al., 1992). A 57 amino acid residue peptide encoded by the cDNA tag-sequence is 72% identical with a region of the C-terminal domain of human CAP. Thus, the tag-sequence appeared to have been derived from a human cDNA encoding a CAP-related protein. We used PCR techniques to isolate cDNA clones encoding the entire CAP-related protein, which we have named CAP2. We reasoned that a highly conserved polyproline stretch located in the middle of the yeast and human CAP proteins may also be conserved in CAP2. Thus, we designed a degenerate set of oligonucleotides that encode a stretch of proline residues. We used these oligonucleotides and an oligonucleotide homologous to a 20 base pair region of the *CAP2* tag-sequence as primers, and a human glioblastoma cDNA library

```
-083          ATTCTTTGGGGAGGCAACTAGGATGGTGTGGCCGACCACGGATTTGCATTGCCGAGGACGGGACCCCAGGGCAGCGAAGCAGA

 001    ATGGCCAACATGCAGGGACTGGTGGAAAGACTGGAACGAGCTGTCAGCCGCCTGGAGTCGCTGTCTGCAGAGTCCCACAGGCCCCCTGGG
 001    MetAlaAsnMetGlnGlyLeuValGluArgLeuGluArgAlaValSerArgLeuGluSerLeuSerAlaGluSerHisArgProProGly

 091    AACTGCGGGGAAGTCAATGGTGTCATTGCAGGTGTGGCACCCTCCGTGGAAGCCTTTGACAAGCTGATGGACAGTATGGTGGCCGAGTTT
 031    AsnCysGlyGluValAsnGlyValIleAlaGlyValAlaProSerValGluAlaPheAspLysLeuMetAspSerMetValAlaGluPhe

 181    TTAAAGAACAGTAGGATCCTTGCTGGGGACGTGGAGACCCATGCAGAAATGGTGCACAGTGCTTTCCAGGCCCAGCGGGCTTTCCTTCTG
 061    LeuLysAsnSerArgIleLeuAlaGlyAspValGluThrHisAlaGluMetValHisSerAlaPheGlnAlaGlnArgAlaPheLeuLeu

 271    ATGGCCTCTCAGTACCAACAACCCCACGAGAATGACGTGGCCGCACTTCTGAAACCCATATCGGAAAAGATTCAGGAAATCCAAACTTTC
 091    MetAlaSerGlnTyrGlnGlnProHisGluAsnAspValAlaAlaLeuLeuLysProIleSerGluLysIleGlnGluIleGlnThrPhe

 361    AGAGAGAGAAACCGGGGGAGTAACATGTTTAATCATCTTTCGGCCGTCAGCGAAAGCATCCCTGCCCTTGGATGGATAGCTGTGTCTCCC
 121    ArgGluArgAsnArgGlySerAsnMetPheAsnHisLeuSerAlaValSerGluSerIleProAlaLeuGlyTrpIleAlaValSerPro

 451    AAACCTGGTCCTTATGTCAAGGAGATGAATGACGCTGCCACCTTTTACACTAACAGGGTCTTAAAGGACTACAAACACAGTGATTTGCGT
 151    LysProGlyProTyrValLysGluMetAsnAspAlaAlaThrPheTyrThrAsnArgValLeuLysAspTyrLysHisSerAspLeuArg

 541    CATGTGGATTGGGTGAAGTCATATTTGAACATTTGGAGTGAACTTCAAGCATACATCAAGGAACACCACACCACGGGCCTCACATGGAGC
 181    HisValAspTrpValLysSerTyrLeuAsnIleTrpSerGluLeuGlnAlaTyrIleLysGluHisHisThrThrGlyLeuThrTrpSer

 631    AAAACAGGTCCTGTAGCATCCACAGTATCAGCGTTTTCTGTCCTCTCCTCTGGGCCTGGCCTTCCTCCACCCCCTCCTCCTCTGCCTCCT
 211    LysThrGlyProValAlaSerThrValSerAlaPheSerValLeuSerSerGlyProGlyLeuProProProProProProLeuProPro

 721    CCAGGGCCACCTCCACTTTTCGAGAATGAAGGCAAAAAAGAGGAATCTTCTCCTTCACGCTCAGCTTTATTTGCCCAACTTAACCAGGGA
 241    ProGlyProProProLeuPheGluAsnGluGlyLysLysGluGluSerSerProSerArgSerAlaLeuPheAlaGlnLeuAsnGlnGly

 811    GAAGCAATTACAAAAGGGCTCCGCCATGTCACAGATGACCAGAAGACATACAAAAATCCCAGCCTGCGGGCTCAAGGAGGGCAAACTCAA
 271    GluAlaIleThrLysGlyLeuArgHisValThrAspAspGlnLysThrTyrLysAsnProSerLeuArgAlaGlnGlyGlyGlnThrGln

 901    TCTCCCACCAAAAGTCACACTCCAAGTCCCACATCTCCTAAATCTTATCCTTCTCAAAAACATGCCCCAGTGTTGGAGTTGGAAGGAAAG
 301    SerProThrLysSerHisThrProSerProThrSerProLysSerTyrProSerGlnLysHisAlaProValLeuGluLeuGluGlyLys

 991    AAATGGAGAGTGGAGTACCAAGAGGACAGGAATGACCTTGTGATTTCAGAGACTGAGCTGAAACAAGTGGCTTACATTTTCAAATGCGAA
 331    LysTrpArgValGluTyrGlnGluAspArgAsnAspLeuValIleSerGluThrGluLeuLysGlnValAlaTyrIlePheLysCysGlu

1081    AAATCAACTATTCAGATAAAAGGGAAAGTAAACTCCATTATAATTGACAACTGTAAGAAACTCGGCCTGGTGTTTGACAATGTGGTGGGC
 361    LysSerThrIleGlnIleLysGlyLysValAsnSerIleIleIleAspAsnCysLysLysLeuGlyLeuValPheAspAsnValValGly

1171    ATTGTGGAAGTGATCAACTCCCAGGACATTCAAATCCAGGTAATGGGGAGAGTGCCAACAATTTCCATTAATAAGACAGAAGGTTGCCAC
 391    IleValGluValIleAsnSerGlnAspIleGlnIleGlnValMetGlyArgValProThrIleSerIleAsnLysThrGluGlyCysHis

1261    ATATACCTCAGTGAAGATGCATTAGACTGTGAGATCGTGAGCGCCAAGTCATCTGAAATGAACATACTTATCCCTCAGGATGGTGATTAT
 421    IleTyrLeuSerGluAspAlaLeuAspCysGluIleValSerAlaLysSerSerGluMetAsnIleLeuIleProGlnAspGlyAspTyr

1351    AGAGAATTTCCCATTCCTGAACAGTTCAAGACAGCATGGGATGGATCCAAGTTAATCACTGAACCTGCAGAAATTATGGCCTAA
 451    ArgGluPheProIleProGluGlnPheLysThrAlaTrpAspGlySerLysLeuIleThrGluProAlaGluIleMetAla***
```

Fig. 1. Sequences of human CAP2 cDNA and encoded protein. The 3′ stop codon is indicated (***). The number of base pairs from the beginning of the cDNA, and the number of amino acid residues from the beginning of the encoded protein are indicated on the left.

as a template in a PCR reaction (see Materials and Methods). We cloned and determined the sequence of the 750 base pair DNA fragments produced from this reaction. These DNA fragments encode a peptide with significant homology and similar length to the C-terminal half of human CAP. To obtain the 5′ sequence of the *CAP2* cDNAs we used sets of nested primers in sequential PCR reactions: each set of primers contained one primer homologous to the cDNA library vector pADANS and one primer homologous to the 3′ *CAP2* cDNA sequence (see Materials and Methods). Several clones of the PCR fragments were isolated and their sequences were determined and found to overlap with the 3′ *CAP2* cDNA sequence. We then amplified the entire coding sequence of CAP2 from the human cDNA library using oligonucleotides designed from the DNA sequences of the 5′ and 3′ ends of the *CAP2* cDNA.

Comparison of human CAP and CAP2

We determined the entire DNA sequence of four independently isolated clones of the amplified *CAP2* cDNAs. These clones encode a 477 amino acid residue protein that is 64% identical to human CAP (Figs 1, 2). Deletion analyses have previously defined three distinct domains in the yeast CAP proteins corresponding to the N-terminal, middle and C-terminal regions (Gerst et al., 1991; Kawamukai et al., 1992). Homology between human CAP and CAP2 is slightly stronger in the C-terminal domain (71%) than in the middle (63%) or N-terminal domains (57%). Both human proteins are approximately 35% identical to either the *S. cerevisiae* or the *S. pombe* CAP proteins (data not shown).

Expression of human CAP proteins in *S. cerevisiae cap*⁻ strains

To investigate the conservation of functional properties of human CAP and CAP2 we expressed them in *S. cerevisiae* strains in which the yeast *CAP* gene had been replaced with the *ura4* gene. We previously showed that expression of human CAP suppresses some of the phenotypes exhibited by these strains (Matviw et al., 1992). The *S. cerevisiae* strain

```
CAP2    001     MANMQGLVERLERAVSRLESLSAESHRPPGNCGEVNGVIAGVAPSVEAFDKLMDSMVAEFLKNSRILAGDVETHAEMVHS
                ||:|| ||||||||| ||| :|  |:   |         || || |:||| |:   |||:|| |: : |||: ||||||
CAP     001     MADMQNLVERLERAVGRLEAVSHTSDMHRGYADSPS--KAGAAPYVQAFDSLLAGPVAEYLKISKEIGGDVQKHAEMVHT

CAP2    081     AFQAQRAFLLMASQYQQPHENDVAALLKPISEKIQEIQTFRERNRGSNMFNHLSAVSESIPALGWIAVSPKPGPYVKEMN
                 :  :||:|: ||| ||| || :: || |||| | |: ||||:|||| :|||||||||| ||||:|: ||||||||||
CAP     079     GLKLERALLVTASQCQQPAENKLSDLLAPISEQIKEVITFREKNRGSKLFNHLSAVSESIQALGWVAMAPKPGPYVKEMN

CAP2    161     DAATFYTNRVLKDYKHSDLRHVDWVKSYLNIWSELQAYIKEHHTTGLTWSKTGPVASTVSAFSVLSSGPGLPPPPPPLPP
                ||| ||||||||:||: | :|||||| || || |||||||| ||||| ||||||||  :|    | |||     ||| ||
CAP     159     DAAMFYTNRVLKEYKDVDKKHVDWVKAYLSIWTELQAYIKEFHTTGLAWSKTGPVAKELSG---LPSGPSAGSGPPP-PP

CAP2    241     PGPPPLFENEGKKEESSPSRSALFAQLNQGEAITKGLRHVTDDQKTYKNPSLRAQGGQTQSPTKS-HTPSP-TSPKSYPS
                |||||         : | ||||||||:|||| ||  |:|| || || ||| |:|| |   |  |    | | |||
CAP     235     PGPPPPPVSTSSGSDESASRSALFAQINQGESITHALKHVSDDMKTHKNPALKAQSGPVRSGPKPFSAPKPQTSPSPKRA

CAP2    319     QKHAP-VLELEGKKWRVEYQEDRNDLVISETELKQVAYIFKCEKSTIQIKGKVNSIIIDNCKKLGLVFDNVVGIVEVINS
                 |  | |||||||||||| ||:  :||| :|||||||||:||   |:|||||:||| :||||||||||:||||||:|||
CAP     315     TKKEPAVLELEGKKWRVENQENVSNLVIEDTELKQVAYIYKCVNTTLQIKGKINSITVDNCKKLGLVFDDVVGIVEIINS

CAP2    398     QDIQIQVMGRVPTISINKTEGCHIYLSEDALDCEIVSAKSSEMNILIP-QDGDYREFPIPEQFKTAWDGSKLITEPAEIMA
                 |: :||||:|||||||||:||| ||| : |||||||||||||:||| : ||: |||:|||||| |:| ||:|   ||
CAP     395     KDVKVQVMGKVPTISINKTDGCHAYLSKNSLDCEIVSAKSSEMNVLIPTEGGDFNEFPVPEQFKTLWNGQKLVTTVTEIAG
```

Fig. 2. Comparison of the human CAP and CAP2 proteins. Protein sequences were aligned using the FASTA program (Genetics Computer Group, Inc.) (Pearson, 1990). Numbers on the left indicate the positions of amino acid residues. Identical (|) and highly conserved (:) amino acid residues are indicated. Amino acid residues are indicated as highly conserved when both residues fall within one of the following amino acid groups: (D, H, N, Q), (D, E), (H, R), (R, K), (R, W), (M, I, L, V), (F, L), (F, Y).

SKN32 (*cap*$^-$) containing the control plasmid pADANS exhibits an abnormal cell morphology characterized by heterogeneous cell size with occasional multiple nuclei and multiple buds (Field et al., 1990) (Fig. 3A). Expression of the *S. cerevisiae* CAP, human CAP or CAP2 in SKN32 (*cap*$^-$) partially restores the normal morphology of these cells (Fig. 3A). Although SKN32 cells expressing either human CAP or CAP2 have a slightly abnormal cell size, they do not exhibit the dramatically enlarged size, multiple nuclei or multiple buds exhibited by some SKN32 (*cap*$^-$) cells containing pADANS. In normal cells buds tend to emerge adjacent to previous bud sites (Freifelder, 1960). A ring of chitin that forms at the bud site remains after cell division (Hayashibe and Katohda, 1973; Cabib and Bowers, 1975). Thus, the pattern of bud scar rings can be seen by staining the chitin with the fluorescent dye Calcofluor. SKN32 (*cap*$^-$) cells containing pADANS exhibit a random budding pattern, as seen by staining with Calcofluor (Vojtek et al., 1991) (Fig. 3B). Expression of *S. cerevisiae* CAP, human CAP or CAP2 in SKN32 (*cap*$^-$) cells suppresses the random budding pattern, although some cells exhibit bipolar bud scars (Fig. 3B). SKN32 (*cap*$^-$) cells containing pADANS also exhibit an abnormal actin distribution, as seen by staining with rhodamine-phalloidin (Vojtek et al., 1991) (Fig. 3C). Normal cells exhibit an asymmetric distribution of actin cables arranged longitudinally along the mother-bud axis, and actin cortical patches in the bud (Adams and Pringle, 1984; Kilmartin and Adams, 1984). In SKN32 (*cap*$^-$) cells containing pADANS actin is present in cortical patches in both mother and bud cells, and the actin cables are absent (Fig. 3C). The normal asymmetric actin distribution is restored in cells expressing *S. cerevisiae* CAP, human CAP or CAP2 (Fig. 3C). Expression of either human CAP or CAP2 in the *S. cerevisiae* strain SKN37 (*cap*$^-$ *RAS2*^{val19}) also suppresses other phenotypes associated with deletion of the C-terminal domain of CAP, including inhibition of growth at 36°C (TS), after nitrogen starvation (NS), or on rich media (YPD) (Fig. 4). Sensitivity to nitrogen starvation in SKN37 (*cap*$^-$ *RAS2*^{val19}) cells is also complemented by expression of the C-terminal domain of *S. cerevisiae* CAP, but because this phenotype also results from the *RAS2*^{val19} mutation it is not complemented by expression of the full-length CAP (Gerst et al., 1991). Thus, expression of human CAP or CAP2 complements all of the observed phenotypes that are associated with the loss of the C-terminal domain of CAP in *S. cerevisiae*. However, the heat-shock-sensitive phenotype of SKN37 (*cap*$^-$ *RAS2*^{val19}) that is lost as a consequence of deletion of the N-terminal domain is not restored by expression of either human CAP or CAP2 (Fig. 4), indicating that neither human protein can restore responsiveness of adenylyl cyclase to the activated RAS protein.

Expression of human CAP proteins in *S. pombe cap*$^-$ cells

To explore further the degree of conservation of CAP proteins we expressed yeast and human CAP proteins in the *S. pombe cap*$^-$ strain MK1818d. Deletion of *cap* in *S. pombe* leads to several phenotypes including temperature sensitivity, abnormal cell morphology and a propensity to sporulate (Kawamukai et al., 1992). MK1818d (*cap*$^-$) cells transformed with the control vector pAALN are temperature-sensitive for growth at 37°C (Fig. 5). They also appear larger and rounder than normal cells and sporulate after growth in liquid synthetic medium for 2 days (Fig. 6). Wild-type cells normally sporulate in response to starvation on synthetic agar plates, but sporulation is not seen after growth in liquid synthetic media. The propensity of MK1818d (*cap*$^-$) to sporulate is due to deletion of the N-terminal domain of CAP and is likely the consequence of attenuation of adenylyl cyclase activity, while the abnormal morphology and temperature-sensitive phenotypes are a consequence of deletion of the C-terminal domain of CAP (Kawamukai et al., 1992). Expression of *S. pombe* CAP in MK1818d (*cap*$^-$) suppresses the temperature-sensitive phenotype and the abnormal morphology and sporulation phenotypes (Figs 5, 6). Expression of *S. cerevisiae* CAP in MK1818d (*cap*$^-$) also suppresses temperature sensitivity and

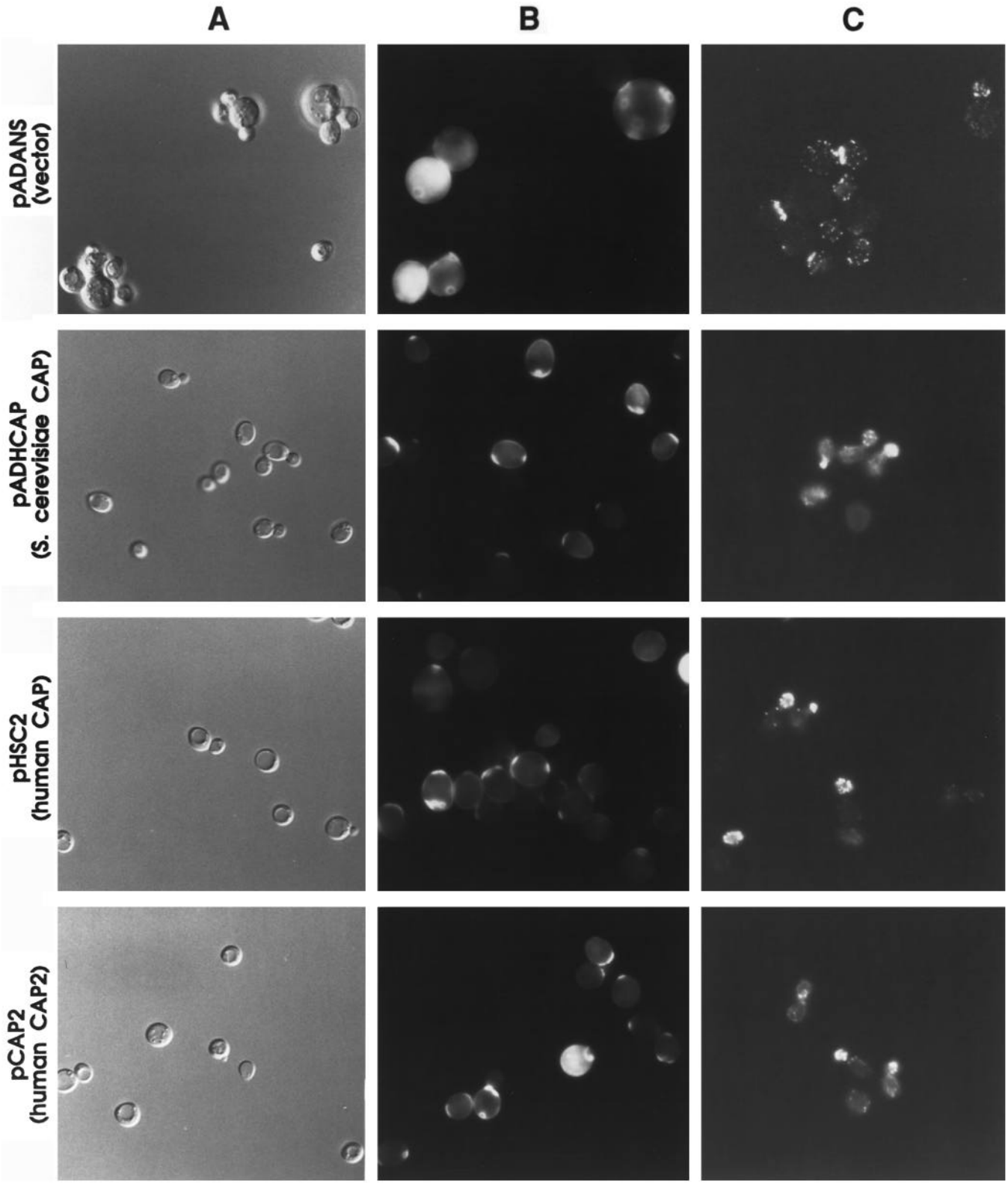

Fig. 3. Suppression of morphological defects in *cap*⁻ *S. cerevisiae*. SKN32 (*cap*⁻) cells were transformed with the control yeast expression vector (pADANS), or with plasmids expressing the *S. cerevisiae* CAP (pADHCAP), human CAP (pHSC2), or human CAP2 (pCAP2) protein. Colonies were picked and grown at 30°C in liquid selective synthetic SC (–Leu) medium for 2 days. (A) Cells were examined by differential interference contrast microscopy (×63). (B) Cells were stained with Calcofluor and examined by fluorescence microscopy (×100). (C) Cells were fixed with formaldehyde, stained with rhodamine-phalloidin and examined by fluorescence microscopy (×100).

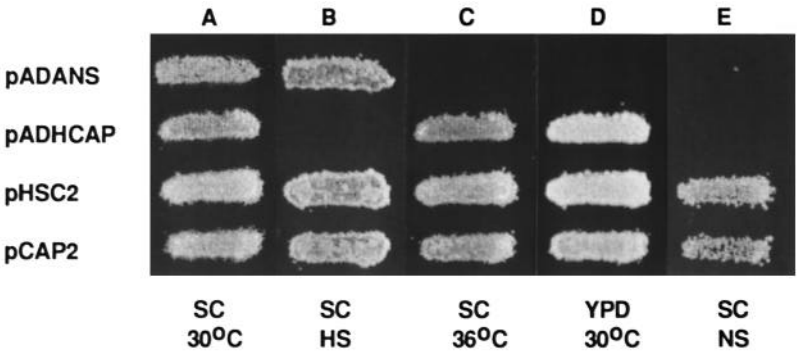

Fig. 4. Suppression of *cap*⁻ phenotypes in *S. cerevisiae*. SKN37 (*cap*⁻ *RAS*^{val19}) was transformed with the control yeast expression vector (pADANS), or with plasmids expressing the *S. cerevisiae* (pADHCAP), human CAP (pHSC2) or human CAP2 (pCAP2) proteins. Patches of cells were grown on a plate containing selective synthetic SC (–Leu) medium at 30°C for 2 days. Replicas of the plate were made on either 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 a SC (–Leu) plate, prewarmed at 55°C, subjected to heat shock treatment at 55°C for 4 minutes, 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 (lacking nitrogen) plate, grown for 9 days at 30°C, replica plated back onto a SC (–Leu) plate, and incubated at 30°C. All replica plates were then grown for 2 days.

partially restores the normal morphology, but cells sporulate at a high frequency after growth in liquid synthetic medium for 2 days (Figs 5, 6). Thus, expression of *S. cerevisiae* CAP complements loss of the C-terminal functional domain of CAP, but does not complement loss of the N-terminal functional domain of CAP in *S. pombe*, as previously reported (Kawamukai et al., 1992). Expression of either human CAP or CAP2 in MK1818d (*cap*⁻) also suppresses the temperature-sensitive phenotype and the abnormal morphology associated with deletion of the C-terminal domain of CAP (Figs 5, 6). Furthermore, expression of human CAP, but not CAP2, suppresses the abnormal sporulation frequency due to deletion of the N-terminal domain of CAP (Fig. 6). Thus, the expression of human CAP fully complements loss of CAP functions in *S. pombe*, while CAP2 only complements loss of the C-terminal functional domain.

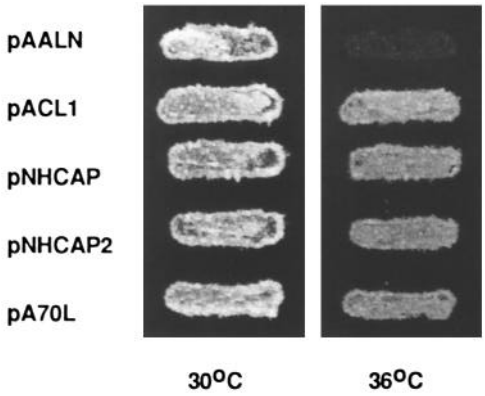

Fig. 5. Suppression of temperature-sensitive phenotype in *cap*⁻ *S. pombe*. MK1818d cells transformed with the control vector (pAALN), or with plasmids expressing *S. pombe* cap (pACL1), *S. cerevisiae* CAP (pA70L), human CAP (pNHCAP) or human CAP2 (pNHCAP2), were grown on synthetic selective PMAA agar plates for 3 days, replica plated onto PMAA plates, and grown at either 30°C or 36°C for 3 days.

DISCUSSION

The conservation of CAP during evolution between yeast and mammals suggests that these proteins have important conserved cellular functions. Our results indicate that some, but not all, of the functional properties of CAP have been conserved during evolution. In *S. cerevisiae*, deletion of the C-terminal domain of CAP leads to multiple phenotypes. It is not clear if these phenotypes are genetically separable, or are the consequence of the loss of a single CAP function. But the genetic and biochemical evidence suggests that at least some of these phenotypes are the consequence of abnormal actin filament assembly. In any event, expression of human CAP or CAP2 can suppress all of these phenotypes, although normal morphology and bud site selection are only partially restored. Similarly, expression of human CAP or CAP2 suppresses the abnormal morphology and temperature-sensitive phenotype associated with deletion of the C-terminal domain of CAP in *S. pombe*. These observations suggest that the role of CAP in regulating nutritional responses and cytoskeletal structure may be conserved between yeast and mammals, and that the two human CAP proteins may be performing similar functions.

In contrast, the functional properties of the N-terminal domains of CAP proteins appear to have diverged, at least somewhat, during evolution. The N-terminal domains are the least structurally conserved regions between human and yeast CAP proteins, and they are also the least conserved regions between the two human CAP proteins. In both *S. cerevisiae* and *S. pombe* the N-terminal domains of CAP proteins are required for the normal regulation of adenylyl cyclase, although expression of either CAP protein in the heterologous yeast cannot substitute for this function (Kawamukai et al., 1992). Similarly, expression of either human CAP or CAP2 cannot replace the function of the N-terminal domain of the *S. cerevisiae* CAP. However, we found that expression of human CAP does complement the loss of the N-terminal domain of cap in *S. pombe*. The implication of this observation is that human CAP is capable of associating with *S. pombe* adenylyl cyclase and permitting its normal function in *cap*⁻ cells. Thus, there may be some similarity in the N-terminal functions of the human and *S. pombe* CAP proteins, and human CAP may be associated with a mammalian protein related to *S. pombe* adenylyl cyclase.

The sequences of the two human CAP proteins are distinct

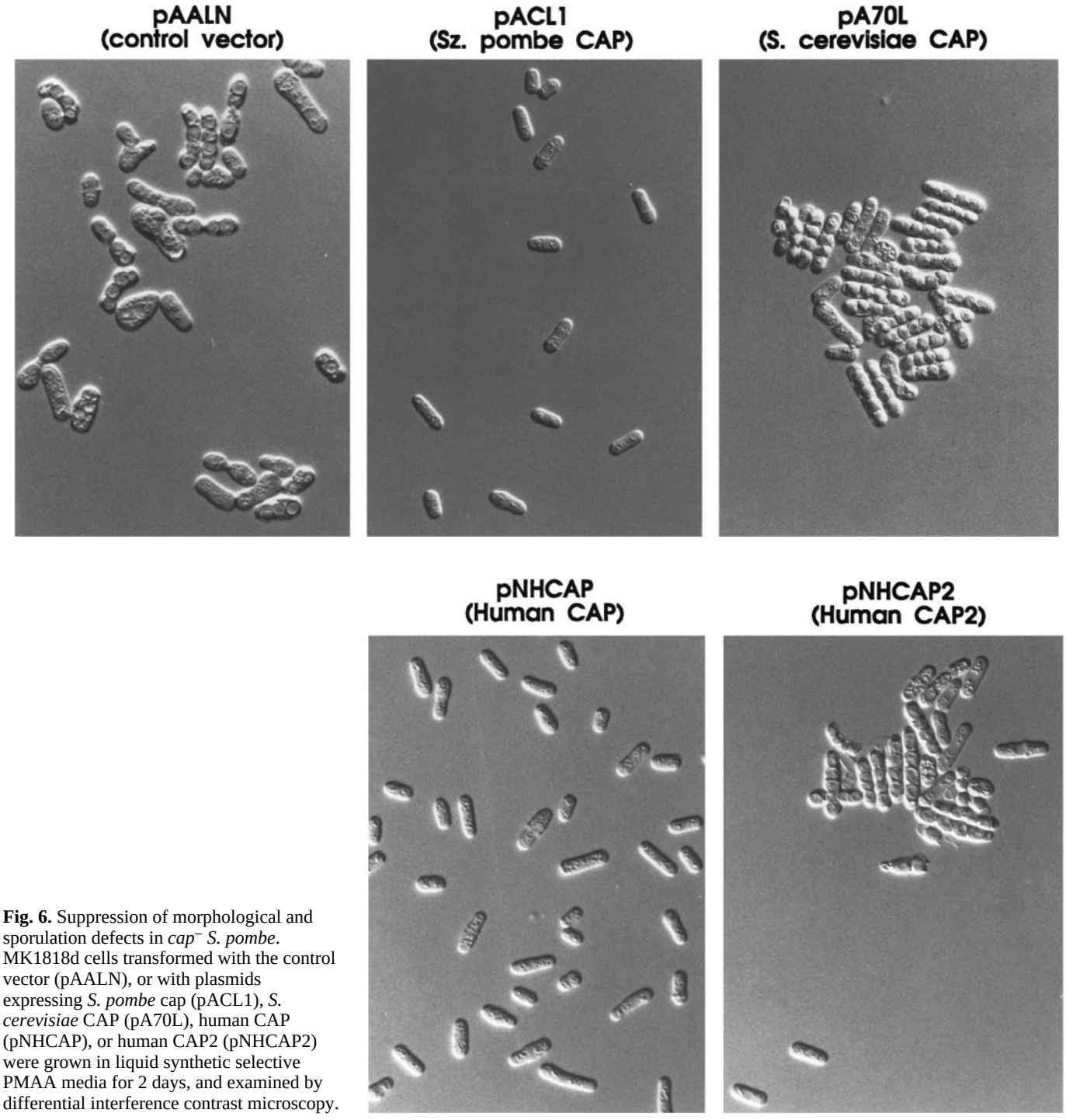

Fig. 6. Suppression of morphological and sporulation defects in *cap*⁻ *S. pombe*. MK1818d cells transformed with the control vector (pAALN), or with plasmids expressing *S. pombe* cap (pACL1), *S. cerevisiae* CAP (pA70L), human CAP (pNHCAP), or human CAP2 (pNHCAP2) were grown in liquid synthetic selective PMAA media for 2 days, and examined by differential interference contrast microscopy.

enough to suggest that their functional roles may be somewhat different. Furthermore, our studies revealed one important difference in the functional properties of the two human CAP proteins based on their abilities to suppress phenotypes in *S. pombe cap*⁻ cells. Thus, these proteins may perform distinct functions in mammalian cells.

The authors thank John Colicelli, Jeffrey Field, Makoto Kawamukai and Michael Wigler for providing plasmids, yeast strains and the human cDNA library. We also thank Randal Johnston for critically reviewing this manuscript. This work was supported by grants from the Alberta Heritage Foundation for Medical Research, the National Cancer Institute of Canada, and the Carole Yates Memorial Endowment Fund for Cancer Research. D. Young is an Alberta Heritage Foundation for Medical Research Scholar. The GenBank accession number for the human *CAP2* cDNA sequence is U02390.

REFERENCES

Adams, A. and Pringle, J. (1984). Localization of actin and tubulin in wild type and morphogenetic-mutant *Saccharomyces cerevisiae*. *J. Cell Biol.* **98**, 934-945.

Adams, M. D., Dubnick, M., Kerlavage, A. R., Moreno, R., Kelley, J. M.,

Utterback, T. R., Nagle, J. W., Fields, C. and Venter, J. C. (1992). Sequence identification of 2,375 human brain genes. *Nature* **355**, 632-634.

Ballester, R., Michaeli, T., Ferguson, K., Xu, H. P., McCormick, F. and Wigler, M. (1989). Genetic analysis of mammalian GAP expressed in yeast. *Cell* **59**, 681-686.

Cabib, E. and Bowers, B. (1975). Timing and function of chitin synthesis in yeast. *J. Bacteriol.* **124**, 1586-1593.

Colicelli, J., Nicolette, C., Birchmeier, C., Rodgers, L., Riggs, M. and Wigler, M. (1991). Expression of three mammalian cDNAs that interfere with RAS function in *Saccharomyces cerevisiae*. *Proc. Nat. Acad. Sci. USA* **88**, 2913-2917.

Fedor-Chaiken, M., Deschenes, R. J. and Broach, J. R. (1990). SRV2, a gene required for RAS activation of adenylate cyclase in yeast. *Cell* **61**, 329-340.

Field, J., Nikawa, J., Broek, D., MacDonald, B., Rodgers, L., Wilson, I. A., Lerner, R. A. and Wigler, M. (1988). Purification of a RAS-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. *Mol. Cell Biol.* **8**, 2159-2165.

Field, J., Vojtek, A., Ballester, R., Bolger, G., Colicelli, J., Ferguson, K., Gerst, J., Kataoka, T., Michaeli, T., Powers, S. et al. (1990). Cloning and characterization of CAP, the *S. cerevisiae* gene encoding the 70 kd adenylyl cyclase-associated protein. *Cell* **61**, 319-327.

Freifelder, D. (1960). Bud position in *Saccharomyces cerevisiae*. *J. Bacteriol.* **80**, 567-568.

Gerst, J. E., Ferguson, K., Vojtek, A., Wigler, M. and Field, J. (1991). CAP is a bifunctional component of the *Saccharomyces cerevisiae* adenylyl cyclase complex. *Mol. Cell Biol.* **11**, 1248-1257.

Gerst, J. E., Rodgers, L., Riggs, M. and Wigler, M. (1992). SNC1, a yeast homolog of the synaptic vesicle-associated membrane protein/synaptobrevin gene family: genetic interactions with the RAS and CAP genes. *Proc. Nat. Acad. Sci. USA* **89**, 4338-4342.

Haarer, B. K., Lillie, S. H., Adams, A. E., Magdolen, V., Bandlow, W. and Brown, S. S. (1990). Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. *J. Cell Biol.* **110**, 105-114.

Hayashibe, M. and Katohda, S. (1973). Initiation of budding and chitin ring. *J. Gen. Appl. Microbiol.* **19**, 23-39.

Ito, H., Fukuda, Y., Murata, K. and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**, 163-168.

Kawamukai, M., Gerst, J., Field, J., Riggs, M., Rodgers, L., Wigler, M. and Young, D. (1992). Genetic and biochemical analysis of the adenylyl cyclase-associated protein, cap, in *Schizosaccharomyces pombe*. *Mol. Biol. Cell* **3**, 167-180.

Kilmartin, J. and Adams, A. (1984). Structural rearrangements of tubulin and actin during cell cycle of the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* **98**, 922-933.

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982). *Molecular Cloning, A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Matviw, H., Yu, G. and Young, D. (1992). Identification of a human cDNA encoding a protein that is structurally and functionally related to the yeast adenylyl cyclase-associated CAP proteins. *Mol. Cell Biol.* **12**, 5033-5040.

Moreno, S., Klar, A. and Nurse, P. (1990). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Meth. Enzymol.* **194**, 795-823.

Novick, P. and Botstein, D. (1985). Phenotypic analysis of temperature-sensitive yeast actin mutants. *Cell* **40**, 405-416.

Pearson, W. R. (1990). Rapid and sensitive sequence comparison with FASTP and FASTA. *Meth. Enzymol.* **183**, 63-98.

Pringle, J., Preston, R., Adams, A., Stearns, T., Drubin, D., Haarer, B. and Jones, E. (1989). Fluorescence microscopy methods for yeast. *Meth. Cell Biol.* **31**, 357-435.

Rose, M. D., Winston, F. and Hieter, P. (1990). *Methods in Yeast Genetics. A Laboratory Course Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sass, P., Field, J., Nikawa, J., Toda, T. and Wigler, M. (1986). Cloning and characterization of the high-affinity cAMP phosphodiesterase of *Saccharomyces cerevisiae*. *Proc. Nat. Acad. Sci. USA* **83**, 9303-9307.

Vojtek, A., Haarer, B., Field, J., Gerst, J., Pollard, T. D., Brown, S. and Wigler, M. (1991). Evidence for a functional link between profilin and CAP in the yeast *S. cerevisiae*. *Cell* **66**, 497-505.

Vojtek, A. and Cooper, J. (1993). Identification and characterization of a cDNA encoding mouse CAP: a homolog of the yeast adenylyl cyclase associated protein. *J. Cell Sci.* **105**, 777-785.

Wang, J., Suzuki, N. and Kataoka, T. (1992). The 70-kilodalton adenylyl cyclase-associated protein is not essential for interaction of *Saccharomyces cerevisiae* adenylyl cyclase with RAS proteins. *Mol. Cell Biol.* **12**, 4937-4945.

Xu, H. P., Wang, Y., Riggs, M., Rodgers, L. and Wigler, M. (1990). Biological activity of the mammalian RAP genes in yeast. *Cell Regul.* **1**, 763-769.

Zelicof, A., Gatica, J. and Gerst, J. E. (1993). Molecular cloning and characterization of a rat homolog of CAP, the adenylyl cyclase-associated protein from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268**, 13448-13453.

(Received 20 December 1993 - Accepted 25 February 1994)