The α and β Subunits of Nematode Actin Capping Protein Function in Yeast

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We cloned and analyzed two genes, *cap-1* and *cap-2*, which encode the α and β subunits of *Caenorhabditis elegans* capping protein (CP). The nematode CP subunits are 55% (*cap-1*) and 66% (*cap-2*) identical to the chicken CP subunits and 32% (*cap-1*) and 48% (*cap-2*) identical to the yeast CP subunits. Purified nematode CP made by expression of both subunits in yeast is functionally similar to chicken skeletal muscle CP in two different actin polymerization assays. The abnormal cell morphology and disorganized actin cytoskeleton of yeast CP null mutants are restored to wild-type by expression of the nematode CP subunits. Expression of the nematode CP α or β subunit is sufficient to restore viability to yeast *cap1 sac6* or *cap2 sac6* double mutants, respectively. Therefore, despite evolution of the nematode actin cytoskeleton to a state far more complex than that of yeast, one important component can function in both organisms.

INTRODUCTION

Specialized cell types contain actin filaments with dramatically different locations and stability. Therefore, the activity of the actin-binding proteins that specify such states must be regulated by their presence, location within the cell, interaction with tissue-specific molecules, or posttranslational modifications. Of interest is whether the actin-binding proteins of complex eukaryotes have acquired additional functions, through sequence evolution, that allow them to form tissue-specific actin structures not found in unicellular eukaryotes.

Capping protein (CP) is a classic example of a ubiquitous actin-binding protein that associates with many specialized forms of the actin cytoskeleton in a wide variety of cell types. For example, CP localizes to the Z-lines of vertebrate skeletal muscle (Casella *et al.*, 1987), the junctional complexes of chicken epithelial cells (Schafer *et al.*, 1992), and the cortical actin patches of yeast (Amatruda and Cooper, 1992). This small, heterodimeric protein regulates actin polymerization in vitro (Caldwell *et al.*, 1989a) and has been identified in all eukaryotic organisms thus far examined. The molecular analysis of CP sequences from such evolutionarily distant organisms as chicken (Caldwell *et al.*, 1989b; Casella *et al.*, 1989) and yeast (Amatruda *et al.*, 1990, 1992) reveals a moderately conserved primary structure with no similarity to actin or other actin-binding proteins. The in vitro effects of CP on actin poly-

merization offer plausible explanations of how CP might exert control over the actin cytoskeleton (Caldwell *et al.*, 1989a), yet the details of how and to what extent these processes occur in vivo in different cell types are unclear. Genetic studies suggest that the level of CP activity can have profound effects on cell morphology and actin organization in yeast (Amatruda *et al.*, 1992). Therefore, the question of whether different cell types and organisms use qualitatively or quantitatively different mechanisms to regulate CP activity or localization within cells seems central to understanding the function of this protein in vivo.

In this study, we present the cloning and analysis of the two nematode genes encoding the α and β subunits of CP. We show that together, these sequences encode an actin-filament capping activity and that nematode CP can substitute for yeast CP in vivo. These findings imply that the CPs from nematodes and yeast share functional homology in vivo with respect to their regulation of the actin cytoskeleton. Therefore, information about yeast CP function or analysis of the nematode genes in yeast should be applicable to understanding CP function in nematodes and higher eukaryotes.

MATERIALS AND METHODS

General Methods

Standard methods for the preparation of plasmid and phage DNAs, subcloning and screening of phage libraries were used (Maniatis *et*

al., 1982; Helms *et al.*, 1987). A protocol provided with the λ ZAPI cloning kit (Stratagene, La Jolla, CA) for the automatic excision of plasmid inserts was followed. Oligonucleotides were made using an Applied Biosystems Model 380B DNA synthesizer (Foster City, CA). DNA-modifying enzymes were purchased from New England Biolabs (Beverly, MA), Pharmacia (Piscataway, NJ), or Boehringer Mannheim Biochemicals (Indianapolis, IN) and used according to the manufacturers instructions. Unless otherwise noted, chemicals were from Sigma (St. Louis, MO) and materials and solvents from Fisher Scientific (St. Louis, MO).

Nematode and Yeast Strains

The maintenance and handling of nematodes was carried out using standard procedures (Brenner, 1974). The wild-type Bristol strain N2 was used in all experiments except where indicated. Standard nomenclature was used to assign gene names to the nematode CP genes (Horvitz *et al.*, 1979). The yeast strains used in this study are given in Table 1. Methods for the culture and handling of yeast were as described (Rose *et al.*, 1990).

Amplification of Nematode CP Sequences

The following degenerate oligonucleotides were used to amplify CP sequences from uncloned mixed stage nematode cDNA using the polymerase chain reaction (PCR) (Saiki *et al.*, 1988):

cap-1: 5'-GA(A/G)GA(T/C)GG(T/C/A/G)AA(T/C)GT(T/C/A/G)CA-3' and 5'-GC(T/C)TT(A/G)AA(T/C/A/G)GT(T/C/A/G)GT(A/G)TC-3' representing the conserved amino acid stretches EDGNVQ and DTFKA, respectively, common to most CP α subunits (Amatruda *et al.*, 1992).

cap-2: 5'-TG(T/C)GA(C/T)AA(C/T)(A/C)(G/T/C)AGAT-3' and 5'-(C/A)AC(A/G)TG(A/G)AT(T/A/G)(G/C)(A/T)(A/G)TCCCA-3' representing the conserved amino stretches CDYNRD and WDSHIV, respectively, common to all CP β subunits (Amatruda *et al.*, 1990). Nucleotides in parentheses denote degenerate positions. The amplification reactions were carried out in a Perkin Elmer-Cetus thermal cycler (Norwalk, CT). The cycling profile consisted of a 1-min 94°C denaturation step and a 1-min 45°C annealing step, followed by a 2-min 72°C extension step for 30 cycles.

DNA Sequencing and Computer Analysis

Nested deletions of genomic and cDNA clones in pBluescript I SK(−) or KS(+) vectors (Stratagene) were generated using Exonucleases III and VII (Yanisch-Perron *et al.*, 1985). The sequence of double-stranded templates was determined by the dideoxy method (Sanger *et al.*, 1977) using the protocol accompanying the Sequenase kit (United States Biochemicals, Cleveland, OH). Gels were read using a Science Accessories Corporation (Southport, CT) digitizer connected to an IBM (White Plains, NY) XT running the Gel-Read software of BioRad (Richmond, CA). Raw sequence data was then uploaded to a SUN workstation (Sun Microsystems, Mountain View, CA), assembled, edited, and analyzed using the XDAP, XNIP, and XSIP assembly and analysis software (Dear and Staden, 1991). Alignments between the deduced amino acid sequences of CPs from other organisms were done using the programs GAP (Genetics Computer Group, University of Wisconsin, Madison, WI) and PRETTY (Devereux *et al.*, 1984). Comparison of the nematode CP genomic sequences to a nonredundant database was accomplished using BLASTX (Altschul *et al.*, 1990) at the NCBI using the BLAST network service (National Center for Biotechnology Information, Bethesda, MD). The sequence of both strands was determined.

Preparation and Affinity Purification Of Antibodies

To make polyclonal antibodies specific for the *cap-1* and *cap-2* gene products the CP coding sequences were expressed as glutathione-S-transferase (GST) fusion proteins in bacteria. The coding regions for *cap-1* and *cap-2* were cloned as *EcoRI* cassettes into the expression vector pGEX-3X (Smith and Johnson, 1988) using the following primers to amplify from cDNA clones: *cap-1*: The forward primer 5'-

Table 1. Yeast strains used in this study

Strain	Genotype	Source
W303-1A	<i>MATa, ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 CAP1 CAP2</i>	*
YJC169	<i>MATα, ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 cap1-1::TRP1 cap2-1::HIS3</i>	†
YJC171	<i>MATa, ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 CAP1 cap2-1::HIS3</i>	†
YJC172	<i>MATα, ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 cap1-1::TRP1 CAP2</i>	†
YJC240	<i>MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 MATαade2-1 his3-11,15 leu2-3,112 trp1-1 sac6::LEU2 CAP1 cap2-1::HIS3</i>	‡
YJC244	<i>SAC6 CAP1 CAP2 MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 MATαade2-1 his3-11,15 leu2-3,112 trp1-1 sac6::LEU2 cap1-1::TRP-1 CAP2</i>	‡
YJW1	W303-1A containing YCp50	§
YJW2	YJC169 containing YCp50	
YJW4	YJC171 containing pBM272	
YJW5	YJC172 containing pBM272	
YJW9	YJC172 containing pJWY1E1	
YJW11	YJC171 containing pJWY2E1	
YJW22	YJC240 containing pBM272	
YJW23	YJC244 containing pBM272	
YJW24	YJC244 containing pJWY1E1	
YJW25	YJC240 containing pJWY2E1	
YJW43	YJC169 containing pJWY12E4	
YJW73	YJC169 containing pJWY12E7	

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† Amatruda *et al.*, 1992.

‡ Adams *et al.*, 1993.

§ Strain generated in this study.

GCTCGAATTCAATGTCGGAGATCAGTGACGCC-3' containing a synthetic *EcoRI* site followed by 22 nucleotides (nt) matching the *cap-1* cDNA clone from −1 to 21 with respect to the initiator ATG (underlined) was paired with a reverse primer 5'-AGCTCGAATTCGT-TACTTCATTTCTGACCAAT-3' that contains 21 nt matching the last seven codons for *cap-1* including the stop codon followed by an *EcoRI* site. *cap-2*: The forward primer 5'-GCTCGAATTCAATGGGA-GAACAACAAGCTCGAT-3' that contains an *EcoRI* site followed by 22 nt matching the *cap-2* cDNA clone from −1 to 21 with respect to the initiator ATG (underlined) was paired with a reverse primer 5'-AGCTCGAATTCGTTCAGTTCCGCGGTTAGCCAT-3' complementary to the last seven codons for *cap-2* including the stop codon, followed by a synthetic *EcoRI* site. Individual clones expressing a fusion protein of the correct size were sequenced to find those free of missense mutations introduced during the amplification step.

One-liter cultures of these cells produced ~10 mg of >90% pure fusion protein following elution from glutathione-agarose (Smith and Johnson, 1988). The GST carrier was removed by cleavage with Factor Xa (Boehringer Mannheim Biochemicals) according to manufacturer recommendations and separated by electrophoresis on 15% sodium dodecyl sulfate (SDS)-polyacrylamide gels (Laemmli, 1970). The *cap-1* or *cap-2* protein was excised from the unfixed gel, equilibrated in water for 15 min, homogenized in 0.5 ml of phosphate-buffered saline (PBS) and 0.5 ml of Freund's complete (primary injection) or incomplete (boosts) adjuvant, and injected subcutaneously into rabbits. Of the four rabbits injected, none generated an immune response to the GST carrier. The preimmune and immune sera were collected, purified by ammonium sulfate fractionation (Harlow and Lane, 1988), and

frozen at -20°C . Affinity-purified antibodies were made by passing the anti-serum, diluted 1:1 in tris(hydroxymethyl)aminomethane(Tris)-buffered saline (TBS) (150 mM NaCl, 50 mM Tris-HCl, pH 8.0), over a 1 ml 1,1'-carbonyldiimidazole-agarose column (Pierce, Rockford, IL) coupled to 2 mg of bacterial fusion protein. After washing in 5 column volumes of TBS, specific antibodies were eluted in 50 mM Tris, 4 M MgCl₂, pH 6.8, and immediately desalted on a 10-DG desalting column (BioRad) in TBS.

Protein Extracts, Electrophoresis, and Blotting

Total SDS-soluble nematode (Moerman *et al.*, 1988) and yeast (Harlow and Lane, 1988) protein extracts were made as described. Discontinuous SDS-polyacrylamide gel electrophoresis (PAGE) gels were prepared as described (Laemmli, 1970). For immunoblots, proteins were transferred to Immobilon P (Millipore, Bedford, MA) or nitrocellulose according to the manufacturer's instructions. After transfer, blots were blocked in 2% bovine serum albumin, 1% normal goat serum in TTBS (0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 0.1% (vol/vol) Tween-20, and 0.01% NaN₃) for 10 min at 25°C . Primary antibody incubations were done in the same buffer but included 0.5–1.0 $\mu\text{g}/\text{ml}$ of antibody and were agitated for 30–60 min followed by four 2-min washes in TTBS. An alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (TAGO, Burlingame, CA) was used at 1:2500, and the blot was developed as described (Ey and Ashman, 1986).

Actin Polymerization Assays

The actin-capping activity of various protein preparations was measured using falling-ball viscometry (Pollard and Cooper, 1982). Column fractions were mixed with chicken actin (4.6 μM) in Ca²⁺-G Buffer (Hug *et al.*, 1992) and then 10 $\times$ MKEI buffer (1 M KCl, 20 mM MgCl₂, 10 mM ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid [EGTA], 200 mM imidazole-HCl, pH 7.0) was added to 1 $\times$ initiate polymerization. The sample was vortexed 3 sec and immediately drawn into 100 μl glass capillary tubes, plugged with clay, and incubated for 15 min at 25°C in a water bath. The relative viscosities were then measured by determining the time required for a steel ball to fall 8 cm at 45° from horizontal (Pollard and Cooper, 1982).

The depolymerization inhibition activity of various protein preparations was measured using a fluorometric assay with pyrene-labeled actin (Cooper *et al.*, 1983). Pyrene-labeled actin was polymerized at high concentration (20–30 μM) for 30 min at 25°C in MKEI buffer, diluted to 5 μM , mixed, and 55 μl was distributed to separate tubes. Six microliters of a sample to be tested was mixed with the polymerized actin filaments and allowed to equilibrate for 5 min at 25°C . The tube was mixed a final time, and 50 μl was diluted into a fluorometer cuvette containing 2.45 ml of Ca²⁺-G Buffer so that the final actin concentration was below the critical concentration of both the barbed and pointed ends (100 nM). The extent of actin depolymerization was monitored by measuring the decreasing pyrene fluorescence over a 5-min time period.

Expression of Individual Nematode CP Subunits in Yeast

To express the nematode α or β CP subunits in yeast, the coding sequences for the *cap-1* and *cap-2* cDNAs were fused to the yeast *GAL1* promoter at the *Bam*HI (5') and *Sal* I (3') sites of plasmid pBM272 (Johnston and Davis, 1984). The strategies chosen reflect available restriction sites and efforts to minimize the amount of 3' untranslated region (3' UTR) from the CP cDNAs in the final constructs.

cap-1: An *Eco*RI-*Hind*III fragment from a plasmid lacking 70 base pairs (bp) of the 3' UTR of the *cap-1* cDNA (including the vector derived 3' *Eco*RI, *Bam*HI, and *Pst* I sites) was subcloned into pBS-SK(+)(Stratagene) to pick up a *Bam*HI site at the 5' end and a *Sal* I site at the 3' end of *cap-1*. *cap-2*: The *cap-2* cDNA clone was cleaved with *Eco*RI and *Dra* I to remove most of the 3' UTR and the vector-

derived 3' *Eco*RI site. This *Eco*RI-*Dra* I *cap-2* fragment was then subcloned into pBS(-) cleaved with *Eco*RI-*Sma* I and the *Bam*HI site downstream of the *cap-2* coding region was removed by Klenow fill-in and religation. The *Eco*RI-*Hind*III *cap-2* fragment from this construct was then subcloned into pBS II KS(+) (Stratagene) to pick up a unique *Bam*HI site at the 5' end and a *Sal* I site at the 3' end. The *Bam*HI-*Sal* I cassettes of *cap-1* and *cap-2* were ligated into pBM272 to make the expression plasmids pJWY1E1 (*cap-1*) and pJWY2E1 (*cap-2*).

Coexpression of the Nematode CP Subunits in Yeast

Initial efforts to coexpress the nematode CP subunits were unsuccessful because little or no protein was made from the *GAL10-cap-2* transcriptional fusion. To eliminate the effects of potential hairpins in this fusion, we used the PCR (see below) to eliminate all 5' and 3' untranslated sequences from the nematode CP cDNAs and replaced the 5' untranslated leaders with the identical sequences from the yeast *CAP1* and *CAP2* genes.

cap-1: An *Eco*RI-*Sal* I *cap-1* cassette was made by amplification of a *cap-1* cDNA with the primers 5'-CGGAATTCGCTCATGTCGGA-GATCAGTGACG-3' (forward) and 5'-TCATGCGTCGCTCGACTTACTT-CATTTCCTGACCAATTC-3' (reverse). The 865-bp product contained a 5' *Eco*RI site, 4 bp of 5' untranslated leader, and the entire *cap-1* coding region immediately flanked by a 3' *Sal* I site.

cap-2: A *Bam*HI-*Sal* I *cap-2* cassette was made with the primers 5'-CGGGATCCCCACCGTAATGGGAGAACACAGCTCG-3' (forward) and 5'-TCATGCGTCGACTCAGTTTCCGCGGTTAGCC-3' (reverse). This 835-bp product has a 5' *Bam*HI site, 9 bp of 5' untranslated sequence, and the entire *cap-2* coding region flanked by a 3' *Sal* I site. The *cap-1* and *cap-2* cassettes were sequenced to ensure that no additional mutations had occurred during the amplification step. These fragments were digested with *Eco*RI and *Sal* I (*cap-1*) or *Bam*HI-*Sal* I (*cap-2*), ligated to the 685-bp *Bam*HI-*Eco*RI *GAL1/GAL10* bi-directional promoter cassette from pBM150 (Johnston and Davis, 1984) and digested with *Sal* I. The 2365-bp *cap-2GAL1/GAL10cap-1 Sal* I fragment was gel-purified and ligated into the *Sal* I site of YCp50 (Rose *et al.*, 1987) or pABZ255 (Bowditch *et al.*, 1991) to create plasmids pJWY12E4 and pJWY12E7, respectively. All expression plasmids were introduced into appropriate yeast strains using lithium acetate (Ito *et al.*, 1983).

To test for galactose-dependent accumulation of the nematode CP in yeast, 10 ml cultures were grown to an OD₆₀₀ of 2.5–3 on synthetic –Ura media with 1% glucose. The cells were derepressed by 10-fold dilution into glucose-free synthetic –Leu or –Ura media and grown for 2 additional h at 30°C . The cultures were induced by addition of galactose to 2% and grown for 12 h before harvesting.

Purification of Nematode CP Expressed in Transgenic Yeast

The purification of nematode CP expressed in transgenic yeast was done using a modification of the procedure used to purify endogenous yeast CP (Amatruda and Cooper, 1992). The column fractions containing nematode CP were identified by falling ball viscometry as described above. Nine liters of a galactose-induced culture of YJW73 was grown to an OD₆₀₀ of 3.6 yielding 100 g of cells after washing and resuspension. All subsequent steps were done at $0-4^{\circ}\text{C}$. The cellulose diethylaminoethyl-cellulose and S200 buffers contained 100 mM NaCl because the nematode CP elutes at a lower concentration of NaCl than yeast CP. The peak of hydroxylapatite-purified CP was concentrated by loading the material onto a MonoQ (Pharmacia, Piscataway, NJ) HR 5/5 column equilibrated in buffer A with 100 mM NaCl as described for the preparative MonoQ column (Amatruda and Cooper, 1992). Protein was eluted with a 15 ml linear 100–300 mM NaCl gradient in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5 mM dithiothreitol, and 0.01% NaN₃ and collected into 1-ml fractions. Nematode CP eluted in 3 ml containing ~1.2 mg of protein. The purity of this preparation is shown in Figure 2 of the RESULTS section. The predicted molar extinction coefficient of nematode CP is 71 600

M⁻¹ cm⁻¹ at 280 nm based on the content of tyrosine and tryptophan and a calculated molecular weight of 62 895 D.

Random Spore Analysis of Yeast

The heterozygous null diploid strains YJW22-YJW25 (Table 1) were sporulated in liquid media (Rose *et al.*, 1990) except that galactose and raffinose replaced glucose as the carbon source. Nonsporulated diploids were killed by an overnight Zymolyase treatment (Ausubel *et al.*, 1989). This haploid suspension was diluted to 10⁴ cells/ml and 10–100 µl was plated onto synthetic –Ura media (to select for the plasmid) containing 2% galactose and 2% raffinose. These primary colonies were then replica plated to either SD-Ura-Leu-Trp (*sac6::LEU2 cap1::TRP1* rescue experiments) or SD-Ura-Leu-His (*sac6::LEU2 cap2::HIS3* rescue experiments) media containing either 2% glucose or 2% galactose-raffinose as the carbon source. Rescued segregants were identified as those clones that grew on galactose (nematode genes induced) but not glucose (nematode genes repressed) after 2–3 d at 30°C. About 10–15% of the primary spores derived from YJW24 and YJW25 were galactose-dependent, glucose-sensitive segregants. No such segregants were detected when ≤20 000 spores from YJW22 and YJW23 were screened. These strains contain the expression vector without an insert. The plasmid-dependence of this rescue was further tested by replica plating these clones to media containing uracil and 5-Fluoro-orotic acid (5-FOA).

Fixation and Rhodamine Phalloidin Staining of Yeast

The morphology and actin organization of yeast strains was examined as described previously (Amatruda and Cooper, 1992) except cells were mounted directly in PBS. To determine the percentage of cells containing phalloidin-detectable actin cables, 200 cells were scored for each strain tested.

RESULTS

Cloning and Sequence Analysis of the Nematode *cap-1* and *cap-2* cDNAs and Genes

We took advantage of the amino acid sequence conservation of the CP subunits among several eukaryotes to design degenerate oligonucleotide primers for use in the PCR (Saiki *et al.*, 1988). Direct amplification of uncloned nematode cDNA generated candidate fragments of the expected size and sequence for both subunits. These fragments were radiolabeled and used to screen a mixed-stage nematode cDNA library (Barstead and Waterston, 1989). The longest clone for each subunit was completely sequenced. The 5' sequence of the smaller clones showed that they were truncated versions of the longest clones.

The lengths of the CP cDNA clones are close to the size of the transcripts (1.2 kilobase [kb], *cap-1*; 1.3 kb,

cap-2) detected by a Northern analysis of total nematode RNA. The 1155 nt *cap-1* cDNA is full length because it contains the last 8 nt of the transspliced leader SL1 found at the 5' end of many *Caenorhabditis elegans* genes (Huang and Hirsh, 1989). The largest *cap-2* cDNA (1223 nt) did not contain a transspliced leader. However, this clone probably lacks some of the 5' untranslated leader sequence because it contains only 4 nt 5' to the inferred initiation codon.

To obtain CP genomic clones for sequence analysis, we used labeled cDNA to probe a λ1059 nematode genomic library (Moerman *et al.*, 1986). For each gene, a single clone containing the entire coding region was restriction mapped and subcloned. The sequence of these two genes, the translation of their proposed exons, and the 5' and 3' boundaries of the cDNA clones described above are shown in Figure 1. The *cap-1* coding sequence is divided into 5 exons, whereas the *cap-2* gene is divided into 8 exons. The *cap-1* and *cap-2* intron-exon boundaries adhere to the consensus donor and acceptor site sequences found in *C. elegans* genes (Emmons, 1988). *cap-1* maps to cosmid C35D7 on chromosome IV between the *deb-1* and *unc-24* genes and *cap-2* maps to cosmid M106 on chromosome II in the *unc-4* to *sqt-1* interval (Sulston and Coulson, personal communication).

A comparison of the deduced amino acid sequences of the nematode CP subunits to published sequences from other organisms is shown in Figure 2. Over the entire sequence, the nematode CP subunits are 55% (*cap-1*) and 66% (*cap-2*) identical to the chicken CP subunits and 32% (*cap-1*) and 48% (*cap-2*) identical to the yeast CP subunits. As is the case with other CPs, the nematode proteins show no similarity to actin or other known actin binding proteins.

Nematode CP Purified from Transgenic Yeast Regulates Actin Polymerization In Vitro

Because the *cap-1* and *cap-2* proteins show sequence conservation with CP sequences from other organisms, we tested the nematode gene products for actin-capping activity in vitro. The biochemical properties that define members of the CP family include the ability to bind tightly to the barbed ends of actin filaments preventing the addition and loss of subunits at this end and to nucleate actin polymerization from monomers. These

Figure 1. DNA sequence of *cap-1* and *cap-2* cDNA and genomic clones. Nucleotide positions are numbered to the right of each sequence. Potential SL1 transspliced leader acceptor sites (TTTCAG) in the 5' untranslated regions are underlined. Intron and exon assignments were made by a direct comparison of the cDNA and genomic sequences. Coding sequences are translated into the one letter amino acid code above the first nt of each codon. Amino acids contained in parentheses are composite codons formed at the splice junction. The first 8 nt of the *cap-1* cDNA have been left out because they match the unlinked transspliced leader sequence SL1. The *cap-1* cDNA sequence starts at nt 422 and ends at position 2229 (arrows). The *cap-2* cDNA sequence starts at nt 1480 and ends at 3052 (arrows). The poly A tails and *EcoRI* linker sites of both cDNAs have been omitted. The *cap-1* and *cap-2* genes lack the consensus eukaryotic polyadenylation signal AATAAA but share a 12 nt sequence (underlined) just upstream of the polyadenylation site (3' arrows). All four sequences are available from the EMBL/GenBank databases under accession numbers Z18805 (*cap-1* gene), Z18853 (*cap-1* cDNA), Z18806 (*cap-2* gene), and Z18854 (*cap-2* cDNA).

cap-1

TCTAGAAATGCTCTCACATCTAGTACATATTCTCTCTCCAGCGAATTTTTCACAAATTT 60
 TAAGTGTAAATGAATTAATGTAATCTGAACATGGTCAAAATGCTTTTATTTTATAGAC 120
 TATTATTACTCATTTTATTAATGCTTTTATGCTGCTTTCTTTTCGCTGAAGAAATAA 180
 AAACATTTCAAACTAAATATCTCAAGTAATCTCTATCCACTTTCCGCTGTGGTCTCTCG 240
 AAACCGCAAGCGGCTCTACGGCTAAATCGATCGCTCATTTCTCTGTTTGTGATTCAT 300
 TTTTGTCTACCTTTTATCAATGAATACCACTTTTATTTTGTCAATCTTTATTTCTT 360
 TTAGTTATTGTATAGAAATATATTTTAAATGTTTATAAGCAATCACTTTCTTCGTTCA 420

↓

M S E I S D A E K V R I A S D F I K H A
 GAATGTCGAGATCAGTGACGCCGAAAGGTCGCTCGGATTTTCATTAACACG 480

P P G E F N E
 CGCCACCCGGCGAGTTCAACGGAGTAATTTTTTGTGATTTTATAGGAAATGCTCGTTC 540

AAGCTTGATATTATAGTCAATCAAAAAATTTATATTTTCGCAATTCACCTCAATTTGCAATT 600

V F N S V R M L L E N D D L
 TACCTTTCCATTACAGTATTCAATTCGGTTCGGATGCTTTTGGAAATGATGATCTCTG 660

K N K C V N A I A Q Y N V G Q F V P V K
 AAGAACAATGTGTTAATGCTATCGCTCAATATAACGTTGGACAATTTGTGCCAGTAAAG 720

L D G V A K Q
 TTGGATGGTGTGCCAAGCAGGTATACATTTTTCATTTTTTAACTTTCTTCAAAATATT 780

T L I T P Y N D L G N G R F Y
 AATTATTTTCTCAGACTCTGATCACTCCATACAATGATTGGGTAATGGAGCTTTCTAC 840

D E V S K K V R K F D H V R E A A D L
 GATGAAGTTTCAAGAAAGGTTTCAAAATACGATCAGCTCCGTAAGAGGCCGAGATCTT 900

Q P H P A E S G I T E Q W R O A L Q T O
 CAACCATCTCAGCGGAGTCTGGAATACCGAACAATGGCGTCAAGCTCTTCAAACTCAA 960

L D I Y I D D H Y A K S G T G V V F A R
 CTCGATATATATTGATGATCATTACCGGAAAGTGAACGGGAGTGGTTTTTGTCTGA 1020

N G V F T I C I E S H Q F Q P K N F C N
 AACGGAGTTTTCAGGATTTGCAATCGAATCTCAATTCAGCCAAAAATTTCTGTAAAC 1080

G R W R S E W N V P V G D G K S G S Q E
 GGAAGATGGCGTTTCGGAGTGAATGTCCGGTTGGCGATGGTAAATCAGGATCTCAGGAG 1140

M K G K I L S Q V H Y Y E D G N V Q L F
 ATGAAAGGAAAAATTTGTCGCAAGTTCATTATTACGAAGACGGAACGTCGAGTTGTC 1200

S E K E P V L K P V N V S
 TCTGAGAAAGAGCGGTTCTTAAAGTCAATGTTTCGGTAAATTTTTTTTCCATAAATTT 1260
 ATCCAAATTTAGAGGATAGAAAAAATTTTCAATAAATTTGTAAGAACAAATAAAA 1320
 TCACAGGAATCATTAAATATATTTTTCATAGAGCTATTAAATCGAATAAAAATTTGGC 1380

A D F D K T A K E I I H A I
 ATATAAAGCCCTATTTTCAGGCTGACTTCGACAAACCGCCAAAGAAATATTCATGCCA 1440

S E E T I Y Q N A V Q N A N M S D
 TTTCCGAGGAAGAGACTATATATCAGAATGCTGTACAGAGAAATACGCTAATATGCTG 1500

T T F K A L R R Q L P V T R A K M D W N
 ATACCACTTCAAGCACTTCGTCGCCAATTCGCAAGTACTCGCGCTAAGATGGAGTGA 1560

K
 ATAAGGTATCGTAATTTTCAATATTTTCGGAATGCTTCGGAAGGTGCACAGATTTATT 1620
 CAGATGGGTCTCGGTGTTTCTTATTTTCTGTTAAACAAATGCTTCGAATCAGTTTATAT 1680
 CCCCCTGTCTCAGACACAAATTAATTTGTTGTTTGAAGTAAATTTTAAAGAAAT 1740
 TTTAACAGAAACAGAGATGAAGGCAAAATTTCTTAAATGCGCAAGTAAATTTTGA 1800
 AAAAGATTAAAGCAAGGCTACCAATAAATCTGTGCGCTCTCAAAAGGCATGTCAAAA 1860

A Q T Y R
 TTTTCAGAATTTGATAAATAAAGAAATAAATTTTAAATTTTAAATTCAGGCTCAAACTACCG 1920

I G Q E M K *
 AATGGTTCAGGAATGAAGTAATCACAATATCGTTATCAGATCGCTTTGCTTCAAGTTTCG 1980
 TCTGCTGGTCTTCATCATCTCTCTAATCTCTCCAAAAACCAAAATCTCTCAATTTTCG 2040
 ACCTTTATTTCTCCATTTCTCTTATCTCTTTGGCCGGACACTAGACGCTGTTTACAACA 2100
 TAAATACCTTTCGTTTGTCTCGCTGCTCTTTTTCATGATTGATAATTTGTATCATCTA 2160
 TGTATCTAATTTTATGATGATGATGATTCATTTCATTAATATATTTATTTGTGTGTG 2220

↓

GTGATTAAGAAATTTTCAGATATTTTCTGATTTTGAAGAGTTCGGCACCTTTTGTATATA 2280
 TATTTAAACGAATTAGCTAAATAGTAAATATGTTTCTGTTTAAATGAAGAAATTCAGAT 2340
 GCAAAACCTCAGTGAAGTTCTCAATGCGACTTTATTGAGCAACTTGTACAGATTTGGTATC 2400
 ATCGAAGTGAAGTATCAACAGCAGCAGTACAATACCAAGCAGGAAATTCATTTGAC 2460
 TAGTAAGCTTTTCAAGAACATATTTCTAAGATTACACATTTTTCAGCATCTTTTCCCTCA 2520
 AGTGATCATACACTGCAACGCTGATCGCTTCATTTCTTGGTATGTTTGGAGTAGCA 2580
 TGTGCTCTCATTCGGAATTTGTGATAGCCATAAGAAAAAGTGAATCTCTTTCGATTT 2640
 TAAACATTTATTTCTCAATTTTAGGAGAGCTGAATTAATCGGATCAGCAGATGGAGAA 2700
 TTTCTGCTTCAAGACAAAGCGCATTTTCGGCTTCTAGTGGCGAATGGGCCAAAAATCTT 2760
 CGCCACTTATCTTCCATGGGCGGTGGAGGGTATATAAGCAATTTATTTTAAATTTAT 2820
 TACAGTTTGAACTTTAAAGAACTATCGCTCAGCAACATCAAGACAAACCGCTGAACT 2880
 CACTGGATCACCGACAGCGGATGCTGCTGATTTGGGAACGACAGTTCTTCGATGATTCG 2940
 AAGCTACAGTACGTTCTTTTAGATTTATAGTCTTCAATTGA 3000

cap-2

GAATTCCTCACTGAAATTTGTGATAAATAAGCAAAAACTGCCAACAAATATTAATAATA 60
 CTCGGTTTTATAGCAAAAACTCAAGAAAGCGGGCAGTAATCTGAAATCAGATAGAA 120
 AGTTCTAGAAAAAGCGGTATAATCTGAACCTCCAGCTCAATAGTGTACCTAGTTTCGATA 180
 TATCACTGGATTACCGTGAACATTTTATTTATTTAAAAAGAAACCCCTCAGGTTACAG 240
 GAAATAGCTTTTTCGCGAAAAAACATGTAATTTGAGCGCGGAAACATATGAGTGGAA 300
 CCCCCCTTGAACAGAAAAAGTGGCATGTGGCTTTAGTGACAACTGATCTTTTGTCTTCA 360
 AGGAAGATATTGTGATTCTAGAGAACTTTGAACATTTTGGAGTCGGAAAAACAGAAATG 420
 AGTTGCGGAGATGCAATAAATGCAATTAATTTCTTTTCTTTATAGTGACGATTTCAG 480
 GGAAAAATTTGTAAGAGCCCTTTGCAGCGATAAAATTAATTAATCAACGAAATTTCAAAAA 540
 AGAAATCCATTAGCAAGCGCAAGTCAAAAGAAAGTCAAGTTCTGACCGTGGAAATG 600
 TGACTGCTCCTGTTGATGTCACCACTTGAACCTGATCTTTTCATAGTATGCTCACTGCT 660
 TTGCAGAGCGGCTTTGTTCTTTTGCAGCTGAAGCTAGGTTATAATGATTGAAGTCAA 720
 TGGAAAGTGGGAATTTTTCGCAATTTATAATGAAACCTCTTAAACGCACTTTTAAAC 780
 GTTGAAATGCTTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCG 840
 AAATGTCGCGAATTTTCACCCAGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCG 900
 ATGGAAGTTTCGCGGAGTGAAGTTTACTCTGACGCTGCTTTCGTTTTCGTTTTCGTTTTCG 960
 GCAATTAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAG 1020
 ATTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCGTTTTCG 1080
 AAAAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAG 1140
 ATTTTCGCGGAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAG 1200
 TGTAAATCAATGAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAG 1260
 AAATATATTTTTCGAAATTTTAACTTATTTTGTGAACTAAAAATCTTATCCCAATCTGT 1320
 TAGAGTCTTTTATGTTTAAATAGGTTGTAACGACTTAAATCTGACATAAAGTTTAT 1380

↓

TGTTTCAGGAGCACACCCACATCCGGCTCGAAATATCAAAATGGTATTTTAAATAAT 1500

G E Q Q L D C A L
 ATTTTAAAAACAATTTATAATAAATATTTACCAGGAGAACACGCTCGATTGTGCCCT 1560

D L M R R L P P Q H C K N L D L D
 CGACTTGATGCGAAGACTTCTCCACAACACTGCGACAGAAGATTGACGGACCTCATCGA 1620

L C P H L V D D L L (S)
 TCTGTGCCCTCATCTTGTGTGATCTTCTCAGTATGTTTCTTTTAACTAATTTAAAA 1680

T I D Q P L K I A A D R E
 AAATTCGTTGATGGTTTATAGCACTATCGATCAACCGCTGAAATTCGCGCGGACCGTG 1740

T G K Q Y L L C D Y N R D G D S Y (R)
 AGACCGCAACACAGTATTGCTTTGTGACTACAATCGAGATGGCGATGCTACAGGTATG 1800

S P W S N T
 AGTCATTTTATTTGAATCTATCGATTTTTCGATAATTCAGATCACCATTGAGTAATA 1860

Y D P P L E D G Q L P S E K R R K M E I
 CCTATCTCACCACCTCGAGGATGGCAATTCGCATCGGAAAGCGAGAAAAATGGA 1920

E A N A F E S Y R D (L)
 TCGAGGCAATGCCGCTTTTGTGACTACCGTGATTTGTAAGTTGACAGAAAAATCATAT 1980

Y F E G G V S S V Y
 ATTTGCTCATAAATAATAAATTTTCCAGATACCTCGAGGAGCGCTCTCATCAGTTTAC 2040

F W D L D N G G F A G I V L I K K E G D
 TTCTGGGACTTGGATAATGGAGGTTTCGCGGAATGTGCTCATCAAGAAAGAGGAGAC 2100

G A K N I T G C W D S I H V I E I T E R
 GGAGCCAAAAATAACAGGATGTTGGGATCTATTTCAGCTTATTGAGATCAGGAAAGA 2160

A R Q
 GTTGGTTCAAAATGTTGAAGAGTTTTTTTTTTAAATTTAATTTTTCAGGACGCA 2220

A H Y K L T S T I M L W L Q T N K S S S
 GGCTCACTCAAGCTCACCCTCAGTATCATGCTGTGGCTGAGACAAACAAAGCTCCAG 2280

G V M N L G G S L T R Q
 CGGTGTGATGAACCTCGGTGGCTCCCTTACAGACAGGTTTGTGTTTCGAGTTGTGAATA 2340

H E M D A P I N D O N T H
 TATTTAAAAAGAAATATTATAGCATGAGTGGATGACCAATCAACGATCAGAACACACA 2400

L A N M G R M I E D Q E S K M R L T I N
 CTGCTAATATGGGAGCAATGATTGAGGATCAGGAGTGGAAATGCGTCTGACGATCAA 2460

E I Y F G K T K K
 TGAGATATCTTTGAAAGACGAAGGTAAGCAAAATGCAAAATTTATATATGAAAAA 2520

V M S D L
 AGTTTCATTTTTAAAAAGTACAATTTTCAAAAAATATTTTCCAGGTGATGTCGGATCT 2580

R S T E K Q S E L E K O D E I V R E L N
 CCGTCAACAGAGAGAGAGAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAG 2640

N A M A N R G N *
 CAACGCCATGGCTAACCGGGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAGTGAAG 2700
 TCATTTTAAATGCTTTTATTTGCGAGTTTGTATATTTATCTACCAATAATACAGCTGTTT 2760
 CTCTTCTTAAACCTTTTTCATTTGCTTTTCTCGAGTGAAGTGTATTTTCTAGAATA 2820
 TTAATACTCTTTTCCCAAAATTTCTCTCGCTCCAAAGTGTATGTTGCTGATGGCTTTCC 2880
 ACAATTTATACCAAAATGCTTGTGTTCTTCCGAGAGAGCTTGAGCTTTTCTGGA 2940
 CCATTTTCGTTCTAGGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 3000

↓

TTAAGATTACTTTTCAATTAATTTTTCGATGAAGTATTTTATCTTAAATTTA 3060
 AAAAAAGAGAGGGTCTCATCAACCTTTGAGAAATCTCCAAGGCTGCTCTTGA 3120
 TTTAGTTTCTTTCGCGCTTCAAGGGTACATCTTCCATTTTCTTGTCTGTTTATTCAC 3180
 AGCTCGCGGCTCATGCTTCTGCGGCTCCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAG 3240
 AGTTTCTTATGCTTTTATTTGCTAATGAAGTGGCTTCTAGAGCTGAAATAGATA 3300
 TCTTAGTTTAAAAATAGTAATAGAATTAAGAACTTTTCTCAAAATCTCTGCTCT 3360
 CAGCTTGAACCTTCTTCTCAGCTTCTTCAATGATGATCCAAATACTTCAGCTCTCATAA 3420
 TAACAACTGAACCACTCGGAACATCAGTTCCTCTTCAAGTTTCAACTTTCAATCTGCTG 3480
 TGATGTATCTGCTCCCAACAGTCTCCCAAAATTTGTGCTTCTGCTCAAAATCT 3540
 TGCACGGCGGAATGTAATGAAGAAATGTGATCGTGGTGTAGAGCCCTTATTAGCCA 3600
 TCGAGGACACCTCGAGCATCATGGGAGAAACCGGAGATGAATTC 3660

A Protein Sequence of Capping Protein Alpha Subunits

S.c.	MSSSKFEVINKLINDSPGELREVYDDLIKITSENS...KNTI	50
D.d.	MASQELNOTATNELLNAPPCEFEVVSQVHALLPSESLNLSA	
C.e.	MSEISDAEKVRIASDFIKHAPPGEFNEVFNSVRLLENDLLKNC	
X.l.	NEVFNDVRLLLNNDNLLREGA	
G.g.1	MADFEEDRVSDDEEKVRIAIAKFIHAPPGEFNEVFNDVRLLLNNDNLLREGA	
G.g.2	MADLEEQLSDDEEKVRIAIAKFIHAPPGEFNEVFNDVRLLLNNDNLLREGA	
C.f.	MADLEEQLSDDEEKVRIAIAKFIHAPPGEFNEVFNDVRLLLNNDNLLREGA	
Con.	S PP E EV	
S.c.	LDATENVVONCHIEING...NSVITSKYKES...AKEFDPVNSVIEVNI	100
D.d.	GSTFRBYNTSOM...VSTQSKGSAITKEGEISNEYLDPKNKQVITYDH	
C.e.	VNAIAQVNVQFVPVKIDGVAK...LITEYNDLGNGRFYDEVSKKSFKYDH	
X.l.	AHAFAQYNMDQFTFAKTEGYDDQVLITEHGDLCNSRFLDPRNRIIFKFDH	
G.g.1	AHAFAQYNMDQFTVPVKIEGYDDQVLITEHGDLCNGRFDPNRIISPKFDH	
G.g.2	AHAFAQYNLDQFTVPVKIDGYDDQVLITEHGDLCNGKFLDPNRIISPKFDH	
C.f.	AHAFAQYNLDQFTVPVKIEGYDDQVLITEHGDLCNGKFLDPNRIISPKFDH	
Con.	YN I D H	
S.c.	LERKGLIEIEYFTHAKIEKQOLKELHDKHEVYLLQSFQDVSAVYVVP	150
D.d.	IKQEMTGERSASGIEQDIEQRAAFDEEATKYCNEYYPN...GVSAVYGT	
C.e.	VRKEAPLQPHFBSGIT...EOMFCALCTODIIMDDHYAKSGITGVVE...	
X.l.	LRKEASD...PHPDSDVALKSWRDACTALRAYVKEHYPN...GVCTVYCKT	
G.g.1	LRKEASD...PQPEITESALKQWRDACSALRAYVKDHYPN...GVCTVYCKT	
G.g.2	LRKEATD...PRPHEVENAIESWRVSVETAARAYVKEHYPN...GVCTVYCKT	
C.f.	LRKEATE...PRPHEVENAIESWRVSVETAARAYVKEHYPN...GVCTVYCKT	
Con.	I Y V	
S.c.	.EELSKISITIVSTKYAPNNFWNGHWRSSYYIDLE...TRE...LS...GOIST	200
D.d.	VSEGIKITVICISTCIYKPNDFYSGWRWSVWCTFKPG...SGNVTSNGRQVQ	
C.e.	ARNGV...ETICIESHQFQPKNFNGRWRSEWNVFVGDKSGSOEMKKGKLS	
X.l.	IDGQQTIVSCIESHQFQPKNFNGRWRSEWKFITISCTAQLV...GVLKI	
G.g.1	IDGQQTIIACIESHQFQPKNFNGRWRSEWKFITITPETAQVA...AVLKI	
G.g.2	IDGQQTIIACIESHQFQAKNFNGRWRSEWKFITISPTTQVA...GILKI	
C.f.	IDGQQTIIACIESHQFQAKNFNGRWRSEWKFITITPSTTQVV...GILKI	
Con.	I F G WRS	
S.c.	QVHYEDGNVSFQSGKDINO...SN...VDDVVCITRDIETNFENDL	250
D.d.	NVHYEDGNVQL...NTVTQKQITTSADAAQSTAVNAKKAIGKAENLHTAL	
C.e.	QVHYEDGNVQLSEKEPVLKVNSADEDKTAKELIHAISEBEETHYQNAV	
X.l.	QVHYEDGNVQLVSHKDIQDSLSVSNEAQ...TAKEFIKIEAENEYQTAI	
G.g.1	QVHYEDGNVQLVSHKDIQDSLSVSNEAQ...TAKEFIKIEAENEYQTAI	
G.g.2	QVHYEDGNVQLVSHKDIQDSLSVSNEAQ...TAKEFIKIEAENEYQTAI	
C.f.	QVHYEDGNVQLVSHKDIQDSLSVSNEAQ...TAKEFIKIEAENEYQTAI	
Con.	VHY EDGNV S E	
S.c.	DLSSFDLNEKQFKALRRRLPVTRSKINWGS...AIGSYRLGKNAAEGK	300
D.d.	DNYSSTGDTTFKALRRRLPVTRKINWOK...VKNEKIANELNK...	
C.e.	QENYANMSDITTFKALRRQLPVTRKIDWNKA...QVRIQCEMK...	
X.l.	SENYQTSMDITTFKALRRQLPVTRKIDWNK...ILSYKIGKEMQNA	
G.g.1	SENYQTSMDITTFKALRRQLPVTRKIDWNK...ILSYKIGKEMQNA	
G.g.2	SENYQTSMDITTFKALRRQLPVTRKIDWNK...ILSYKIGKEMQNA	
C.f.	SENYQTSMDITTFKALRRQLPVTRKIDWNK...ILSYKIGKEMQNA	
Con.	FKALRR LP R K W	

B Protein Sequence of Capping Protein Beta Subunits

S.c.	MSDAQFPAALDLRLRLPPTTLOENLNNLIEOPNADCLLSVSVVPLSTO	50
D.d.	MTKQOLSCCLDMRLRLPSPQIEDNLACLDLVPDITEDLLSSIDQPLKVA	
C.e.	MGEQQLDCALDLMRLRLPPOHCDKNLIDLIDLCPLHVDLLSHIDQPLKIA	
G.g.	MSDQQLDCALDLMRLRLPPOQTEKNLSLDLIDLVPSICEDLLSSVDOPLKIA	
Con.	M Q LDL RRL P NL L L P L DLLS D PL	
S.c.	KDSALSNREYLCCDYNRDLDSYRSPWSNTYYPELSPKLDQSPFSAHLR	100
D.d.	YD...AVSKKDYLLCDYNRDLDSYRSPWSNKYDF...PLSGACYPSSKLR	
C.e.	ADR...ETGKDYLLCDYNRDLDSYRSPWSNTYDF...PLEDQGLPSEKRR	
G.g.	RDK...VVGKDYLLCDYNRDLDSYRSPWSNKYDF...PLEDGAMPARLR	
Con.	D YL CDYNRD DS RSPWSN Y P L FS R	
S.c.	KLEILANDSFDVYRDLYEGGCISSVYLWDLNEEDFNGHDFAGVVLKFKNK	150
D.d.	DIEVEANEIFEHIYLLNYFEGGVSSVYCDLL...DGN...FAAVVLYKKTK	
C.e.	KMEIEANAFESYRDLYFEGGVSSVYEWDL...D...NG...GFAGIVLLIKKEG	
G.g.	KLEVEANAFDQYRDLYFEGGVSSVYLWDL...D...HGFAGVILLIKKAG	
Con.	E AN F Y LY EGG SSVY WDL D FA L KK	
S.c.	SDHSN...WDSIHVEVITTSFSSPDSFNVRVTITLIDHDKTKTDQ	200
D.d.	DSKKGQPMRQWDSIHVVEVVKLGKDK...AYKLTSTVMSLSTEDN...DN	
C.e.	DGAKN...ITGCWDSIHVIEITE...RARQ...AHYKLTSTIMLWLTQNKSS	
G.g.	DGSKK...ITGCWDSIHVVEVQEKSSGR...TAHYKLTSTVMSLWLTQNKKG	
Con.	WDSIHV E Y T T L	
S.c.	NSHMULSGLNLTQTEKDIAIDMSRFLDVFTSHVANLGLSLIEDIESOMRN	250
D.d.	TKGNLAGSLTRODEKEYTENEVD...THCVNIGKGMVEDMESKTRQ	
C.e.	GV...NNLGGSLTROHEYDABINDO...NTHIANYGRMIEDQESKMLR	
G.g.	SGTMNLGGLSLTROHEKDEIVSDSE...HIANIGRLVEDMENKIRS	
Con.	L G LTRQ E H N G ED E R	
S.c.	ILETVYFEKTRDIFHOTKNAAIASAEANKDAQAEVI...RGLQSL...	300
D.d.	ILETVYFGKTRVWNILRNATCNSELEKRNLSNOIGSAICNRG...	
C.e.	ILETVYFGKTRKVMSDLRSTEKQSELEKODEIVRELNNAMNRGN...	
G.g.	ILETVYFGKTRDIVNGLRSIDAIPDNQYKIDORELSQVLTQRIYIQPDN	
Con.	YF KT R	

Figure 2. The CP subunit sequences are highly conserved. Alignment of the deduced amino acid sequences of the nematode (*C.e.*) capping protein subunits with those from other organisms. (A) Comparison of the *cap-1* sequences from chicken (*G.g.*) (Casella *et al.*, 1989), *Xenopus laevis* (*X.l.*) (Ankenbauer *et al.*, 1989), *D. discoideum* (*D.d.*) (Hartmann *et al.*, 1989), *S. cerevisiae* (*S.c.*) (Amatruda *et al.*, 1992), and dog (*C.f.*) (Zopf and Walter, unpublished data). (B) *cap-2* sequences from chicken (*G.g.*) (Caldwell, *et al.*, 1989b), *D. discoideum* (*D.d.*) (Hartmann *et al.*, 1989), or *S. cerevisiae* (*S.c.*) (Amatruda *et al.*, 1990). Identities between any two sequences are surrounded in black. The consensus line (Con) shows identities between all sequences. Dots are included to optimize the alignment. Approximate amino acid positions are given at the end of each stretch.

activities can be detected by depolymerization inhibition (Cooper *et al.*, 1983) and low shear falling ball viscometry (Pollard and Cooper, 1982) assays. We coexpressed the nematode CP subunits in a yeast strain that lacks the endogenous CP genes (Amatruda *et al.*, 1992) by making transcriptional fusions between the *cap-1* and *cap-2* coding sequences and the bidirectional GAL1/GAL10 promoter from yeast (Johnston and Davis, 1984) using the high copy number plasmid pABZ255 (Bowditch *et al.*, 1991). Using about 100 g of cells from this coexpression strain (YJW73) as starting material, we purified 1.2 mg of nematode CP to near homogeneity (Figure 3) by pooling the actin-viscosity reducing peak through 5 successive purification steps.

The relative activity of the yeast-produced nematode CP was then compared to chicken skeletal muscle CP

using falling ball viscometry and depolymerization inhibition assays with chicken skeletal muscle actin as substrate. As shown in Figure 4A, the concentration of nematode CP required to reduce the viscosity by 50% is 1.1–1.3 nM compared to 0.5–0.6 nM for chicken CP. This assay is not specific for nucleation and the observed activity, although consistent with nucleation, can also be explained by inhibition of polymerization. In either case, the purified nematode CP behaves similar to chicken skeletal muscle CP in this assay.

In a depolymerization inhibition assay, preincubation of polymerized actin filaments with nematode or chicken CP causes a concentration-dependent inhibition of monomer loss from the barbed end (Figure 4B). A comparison of the relative activities of chicken and nematode CP in this depolymerization assay is pre-

Figure 3. Purification of nematode CP expressed in transgenic yeast. 12% SDS-polyacrylamide gel of samples from successive stages of purification. (Lane 1) 25 000-g extract, (lane 2) pool from DE52 cellulose column, (lane 3) ammonium sulfate $P_{0-65\%}$, (lane 4) pool from Sephacryl S200 column, (lane 5) pool from MonoQ column, (lanes 6 and 7) hydroxylapatite column pool. (Lane 7) is a 10-fold dilution of lane 6 run on a different gel to resolve the subunits. Relative molecular masses shown on the left. All lanes contain $\sim 5 \mu\text{g}$ of protein except lane 7, which contains $0.5 \mu\text{g}$.

sented in Figure 4C. This plot shows the level of inhibition midway through the assay as a function of CP concentration. At low to intermediate concentrations, nematode and chicken CP exert comparable levels of inhibition. At higher concentrations, the absolute level of inhibition by nematode CP plateaus, whereas chicken CP is capable of further inhibition. The reason for the quantitative differences in the activity of these two CPs in these assays is unclear but might result from preferences for actin substrates or defects resulting from production of the nematode CP in a foreign host.

Nematode CP Can Substitute for Yeast CP In Vivo

Overall, the nematode and yeast CP coding sequences are 31% (*cap-1*) and 48% (*cap-2*) identical. In addition, the nematode and yeast conventional actins are 88% identical (Gallwitz and Sures, 1980; Krause *et al.*, 1989). We tested whether the CPs from these organisms showed conservation of function as well as primary structure by transforming yeast null mutants with the nematode genes. We examined these transgenic yeast strains for accumulation of the nematode subunits, cell morphology and actin distribution, and rescue of the lethality of yeast CP-*sac6* (Fimbrin) double mutants (Adams *et al.*, 1993).

Earlier studies showed that the individual α and β CP subunits are unstable in vivo (Amatruda and Cooper, 1992) and inactive in vitro (Hug *et al.*, 1992) when the other subunit is not present. Furthermore, the yeast single subunit null mutants have the same phenotype as the *cap1 cap2* double mutants (Amatruda and Cooper, 1992). Thus, formation of the α/β heterodimer is required for the stability and function of CP in vivo. Consequently, our initial test of conservation of CP function was to determine whether the nematode CP subunits would accumulate when expressed in various yeast strains. Using affinity-purified, anti-nematode CP antibodies (Figure 5) that do not crossreact with the endogenous yeast CP subunits (Figure 6, lane 1), we de-

Figure 4. Relative activity of chicken and nematode CPs in a falling-ball viscometry or depolymerization inhibition assay. (A) Activity measured in a falling-ball viscometric assay starting from actin monomers. Apparent viscosity is plotted against CP concentration. Hatch marks indicate the change in scale to include the viscosity of actin in the absence of added CP. Chicken CP (●) and nematode CP (□). (B) Concentration-dependent inhibition of filament depolymerization by chicken and nematode CPs. These and other curves not shown were used to draw the plots in C. The fluorescence (polymer remaining) midway through assays like those shown in A and B is plotted against the amount of chicken (●) or nematode (□) CP preincubated with the filaments.

tected expression of the appropriate nematode CP subunit in the corresponding transgenic yeast null mutants (Figure 6). This suggests that the nematode subunits can interact with the complementary yeast subunits to form stable heterospecies heterodimers. When both nematode subunits are coexpressed in a yeast CP double mutant (Figure 6, B and C; lane 7), the subunits accumulate to a higher level than when expressed individually in the appropriate yeast single null mutant (Figure 6, lanes 3 and 5 compared to lane 7). This difference is expected because the level of nematode CP subunit accumulation in the single subunit expression strains probably depends on both the amount of and efficiency of interaction with the endogenous yeast CP subunit.

Yeast CP null mutant cells are larger and rounder than wild-type cells. In addition, these cells no longer restrict the cortical actin dots to the growing bud and lose actin cables in the mother cell (Amatruda *et al.*, 1990; Amatruda and Cooper, 1992). A comparison of the abnormal cell morphology and disorganized actin cytoskeleton of these yeast CP mutants to wild-type cells is shown in the top rows of Figures 7, A and B, respectively. The subsequent panels show the result of introducing either vector control plasmids or constructs containing the nematode CP coding sequences into the yeast CP mutants. As shown in Figure 7A, expression of the nematode CP subunits results in a conversion from the round mutant morphology to an oval-shaped, wild-type morphology (left versus right). Similarly, the actin dots become restricted to the growing bud, and the cables reappear in the mother cell (Figure 7B). Thus, the nematode *cap-1* subunit rescues yeast *cap1* mutants; the nematode *cap-2* subunit rescues yeast *cap2* mutants, and the coexpressed nematode heterodimers rescue the yeast *cap1 cap2* double mutant. This rescue is entirely dependent on transcription of the nematode sequences, because growth of these same strains on glucose, which represses the expression of the nematode subunits from the *GAL1* and *GAL10* promoters, results in mutant cells that are indistinguishable from the vector-transformed control cells. Rescue of the cytoskeletal defects of the yeast CP null mutants by the nematode CP subunits is not complete because mutant cells are detected in the

Figure 5. Specificity of anti-nematode CP antibodies. Total nematode protein extracts separated by SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue (lane 1), blotted and probed with preimmune IgGs (lanes 2 and 4), affinity-purified anti- α (lane 3), or anti- β (lane 5) subunit-specific antibodies. Markers in kDa. The species detected are consistent with the molecular weights predicted by the deduced amino acid sequences of these two proteins.

Figure 6. Accumulation of the nematode CP subunits in yeast. (A) Coomassie stained 12% SDS-polyacrylamide gel of total SDS-soluble protein from transgenic yeast strains. Gels run in parallel were blotted to nitrocellulose and probed with affinity-purified anti-nematode CP α (B) or β (C) subunit specific antibodies. Size standards in kDa. Wild-type *CAP1 CAP2* strain containing *YCp50* vector control plasmid (lane 1), *cap1* Δ *CAP2* strain containing pBM272 vector control plasmid (lane 2), or the nematode CP α -subunit plasmid pJWY1E1 (lane 3). *CAP1 cap2* Δ strain containing pBM272 vector control plasmid (lane 4) or the nematode CP β -subunit plasmid pJWY2E1 (lane 5), *cap1* Δ *cap2* Δ strain containing vector control plasmid *YCp50* (lane 6), or the nematode CP α/β coexpression plasmid pJWY12E4 (lane 7). The amount of material loaded per lane was based on equivalent OD₆₀₀ of the log phase cultures. The nature of the higher molecular weight species detected in lane 3 is unknown but is likely the result of translational readthrough because it is present only in induced cultures containing this plasmid and reacts specifically with the anti-nematode α -subunit specific antibodies.

rescued populations. There are 30% fewer cells with detectable actin cables in *cap1 cap2* strains coexpressing the nematode subunits than in wild-type strains. Similarly, there are 60% fewer cells with detectable actin cables in *cap1* or *cap2* strains rescued by the appropriate nematode CP subunit when compared to wild-type strains.

The yeast *SAC6* gene encodes the actin-bundling protein fimbrin (Adams *et al.*, 1991). *sac6 cap1* and *sac6 cap2* double mutants are inviable (Adams *et al.*, 1993). When *sac6 cap1* and *sac6 cap2* haploid double mutants are generated by random sporulation of a heterozygous null diploid (e.g. *sac6/SAC6 cap/CAP*) carrying a nematode CP α or β subunit expression plasmid, respectively, viability is restored to these segregants under inducing conditions (Figure 8, left). When these rescued segregants are replica-plated onto glucose to repress transcription of the nematode sequences from the *GAL1* promoter, growth is impaired (Fig. 8, middle). However, even this marginal viability is completely dependent on the nematode sequences because these cells cannot grow on 5-FOA-containing media that selects against the *URA3* gene on the expression plasmid. (Figure 8, right).

DISCUSSION

We have identified single genes for the α and β subunits of CP in the nematode. Efforts to find closely related or multiple genes by low stringency hybridization of CP sequences to Southern blots and amplification with degenerate oligonucleotides did not identify additional genes. The primary sequence conservation of the *cap-1* and *cap-2* proteins, their *in vitro* effects on actin polymerization, and their functional substitution for yeast

Figure 7. The nematode CP subunits correct the morphological and cytoskeletal defects of the corresponding yeast null mutants. (A) Differential interference contrast microscopy of haploid yeast CP mutants transformed with the indicated plasmids. Bar, 10 μ m. (B) Haploid yeast CP mutants carrying the indicated plasmids were stained with rhodamine phalloidin to visualize the actin cytoskeleton. Bar, 2.5 μ m.

CP *in vivo* demonstrate that together *cap-1* and *cap-2* encode the *C. elegans* homologue of actin CP. The observation that either nematode CP subunit can interact with the complementary yeast CP subunit and function

in vivo (Figures 6 and 7) implies the sequences required for subunit association are conserved. In addition, the interacting surfaces of the CPs and actin filament barbed ends from chicken, nematode, and yeast are similar because nematode CP can regulate chicken actin polymerization *in vitro* and substitute for yeast CP *in vivo*. Remarkably, this conservation is seen despite dramatic differences in the form of the actin cytoskeleton in these organisms. This suggests that the differences in the actin cytoskeleton between tissues are because of the presence of additional proteins or mechanisms that regulate CP activity or location within a cell.

There are three published reports of functional replacement of an actin cytoskeleton-associated protein in a unicellular eukaryote with a homologue from a multicellular organism. The essential function of the *Schizosaccharomyces pombe* *cdc8⁺* gene, a novel tropomyosin, is rescued by expression of a vertebrate tropomyosin cDNA (Balasubramanian *et al.*, 1992) and, vertebrate cofilin, an actin depolymerizing protein, can restore viability to *Saccharomyces cerevisiae* COF1 null mutants (Iida *et al.*, 1993). Similarly, chicken β -actin can restore viability to *S. cerevisiae* strains lacking the conventional actin gene *ACT1* (Karlsson *et al.*, 1991). These findings, and those reported here, indicate that although the morphology and motility of cells vary widely across the phylogenetic tree, important components of the actin cytoskeleton from these diverse systems have common functions *in vivo*.

Figure 8. The individual nematode CP subunits restore viability to *cap1 sac6* or *cap2 sac6* double mutants. Rescued *cap1 sac6* (top) or *cap2 sac6* (bottom) haploid double mutants and the heterozygous null diploid parents from which they were derived were streaked onto media lacking uracil but containing galactose and raffinose as carbon sources. These primary plates were then replica-plated sequentially to the same media (left), media containing glucose (middle), or media containing galactose, raffinose, uracil, and 5-FOA. The latter is toxic to cells that carry a functional *URA3* gene (e.g., the nematode CP plasmids). Cells that can survive without the plasmid should grow on the latter media.

The ability to test the function of nematode CP *in vitro* and *in vivo* by expression in yeast is particularly useful for determining the primary defects in nematode CP missense mutants identified by future genetic screens. Specifically, such mutations might affect 1) heterodimer formation (i.e., protein stability), 2) *in vitro* actin-capping activity, or 3) phenotypic rescue of the corresponding yeast mutants. Mutations that do not affect processes 1) and 2) but are defective *in vivo* would be excellent substrates for a suppression analysis to identify interacting components other than actin that are required for CP function in cells (Hodgkin *et al.*, 1987). Similarly, the *in vivo* rescue of yeast CP null mutants by the nematode homologues suggests that information about CP function in yeast will apply to nematode and vertebrate CPs. For example, a temperature-sensitive yeast CP mutation will likely be temperature sensitive for nematode CP function if the change is located at a conserved residue. This can be tested directly by introducing this change into the nematode CP coding sequence and scoring for temperature sensitive rescue of *sac6*-CP double mutants in yeast. This class of mutation would be quite useful for determining the function of CP at different stages of nematode development.

Mutational analysis of CP function in the nematode is of interest because the requirement for CP in a wide range of tissues can be assessed genetically. Immunolocalization and biochemical studies of vertebrate CPs imply functional requirements for CP in the assembly or maintenance of specific actin-containing structures (Casella *et al.*, 1987; Schafer *et al.*, 1992). The availability of methods to inhibit gene expression in specific nematode tissues (Fire *et al.*, 1991) and the cloned genes described here should permit testing of these hypotheses.

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