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The Intron-Containing Gene for Yeast Profilin (*PFY*) Encodes a Vital Function

VIKTOR MAGDOLEN, ULRICH OECHSNER, GÜNTER MÜLLER, AND WOLFHARD BANDLOW*

Institute for Genetics and Microbiology, Maria-Ward-Strasse 1a, D 8000 Munich 19, Federal Republic of Germany

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The gene coding for profilin (*PFY*), an actin-binding protein, occurs as a single copy in the haploid genome of *Saccharomyces cerevisiae* and is required for spore germination and cell viability. Displacement of one gene copy in a diploid cell by a nonfunctional allele is recessively lethal: tetrad analysis yields only two viable spores per ascus. The *PFY* gene maps on chromosome XV and is linked to the *ADE2* marker. The primary transcript of about 1,000 bases contains an intron of 209 bases and is spliced into a messenger of about 750 bases. The intron was identified by comparison with a cDNA clone, which also revealed the 3′ end of the transcript. The 5′ end of the mRNA was mapped by primer elongation. The gene is transcribed constitutively and has a coding capacity for a protein of 126 amino acids. The deduced molecular weight of 13,340 is in perfect agreement with that determined for the protein product in a sodium dodecyl sulfate gel after in vitro translation of the RNA transcribed from the cDNA by SP6 polymerase.

In nonmuscle cells, actin is involved in multiple functions such as maintenance of cell morphology, cell motility, intracellular transport and secretion, and signal transduction and organization of chromosome segregation and of cytokinesis. In *Saccharomyces cerevisiae*, the single actin gene is essential for cell growth and/or proliferation, as mutations in this gene are lethal or conditionally lethal (for recent reviews see references 13, 25, and 29). The participation in the setup of both, the cytoskeleton and the cell division apparatus, requires that filamentous actin (F-actin) is continuously reorganized in dividing cells. One would expect that these processes of decay and reassembly of the cytoskeletal architecture are controlled by sophisticated mechanisms. Surprisingly, there is no indication for a regulation of the expression of actin. It is thus assumed that the state of actin polymerization is controlled at a posttranslational level by the interplay of mainly two mechanisms. (i) Modulation of the intracellular concentration of calcium triggers the self-assembly and depolymerization of actin fibrils (16, 25). (ii) A wide spectrum of actin-binding proteins controls the polymerization state of actin (29). Some of these accessory proteins cause degradation of F-actin by intercalation and fragmentation (e.g., fragmin and severing proteins). Others prevent polymerization either by blocking the barbed (growing) ends of the polymer (like capping proteins) or by binding to actin monomers, sequestering them from the pool of free globular (G) actin subunits. Profilin was previously thought to belong to the last group and to act exclusively through formation of 1:1 complexes with actin (profilactin) which are not available for polymerization (16). More recently, evidence for a more complex regulation has been obtained. It was suggested that, in addition to controlling actin filament nucleation, profilin is likely to block barbed end growth (T. D. Pollard, Eur. J. Cell Biol. **46**:90, 1988). Profilin was isolated from various sources and was found to be an abundant small soluble protein.

Due to the importance and multiple mutual interactions of actin and actin-binding proteins, the primary structures of actin (10, 31, 32) and of various actin-binding proteins (3, 14, 29), among them profilin (1, 2), have been highly conserved during evolution. On the basis of pronounced domain homology with amino acid sequences published for the proteins from *Acanthamoeba* sp. (2, 4) and cows (1), we have identified and characterized the structural gene for profilin in *Saccharomyces cerevisiae*. It is an essential gene occurring as a single copy in the haploid yeast genome. It contains an intervening sequence of size similar to and at a position comparable to those of most nuclear intron-containing yeast genes. The exons code for a polypeptide of 126 amino acids. Northern (RNA) blot analysis reveals both the intron-containing precursor transcript of about 1,000 bases and the mature mRNA of about 750 bases. Interestingly, the gene is transcribed constitutively, making a regulation of actin polymerization through the modulation of the profilin pool unlikely.

MATERIALS AND METHODS

Strains and growth conditions. For subcloning, the vectors pUC12 and pUC19 were used with the bacterial host JM109 grown in LB or M9 medium (21) in the presence of 100 μg of ampicillin per ml. RNA was isolated from the yeast strains D273-10B (ATCC 25657) (2% lactate, 2% galactose or 6% glucose) and ts368 *MAT***a**, *rna2 ade1 ade2 lys2 tyr1 his7 ura1 gal1*) (18) (0.5% glucose, grown at 23°C with or without a shift to 36°C). For gene disruption experiments the diploid strain AH22 × AH33 *MAT***a**/*MAT***a**, *leu2*/*leu2*, *his4*/*his4*, *ADE*/*ade2* was used. Mapping of the profilin gene was achieved by transverse alternating-field agarose (1%) gel electrophoresis (Geneline, Beckman Instruments, Munich) and chromosome blotting with strain DCO4.

DNA manipulations. For gene disruption the *LEU2* marker gene on a 2,300-base-pair (bp) *Hpa*I fragment was ligated to the single *Pvu*II site near the 5′ end of the *PFY* coding region after partial digestion of the pUC19-derived plasmid with *Pvu*II. The excised construct on a 1,700-bp *Bam*HI-*Hin*dIII fragment, ppfyV, was transformed into *S. cerevisiae* to yield the diploid disruption mutant PFYD0 (*PFY*/*pfy*::*LEU2 leu2*/*leu2 his4*/*his4 ADE*/*ade2*) by using the protoplast method (27). DNA and RNA blotting, hybridization, nick translation, and end labeling of DNA or RNA fragments (21), isolation of total RNA from yeast cells (G. Strobel, V. Magdolen, U. Oechsner, H. S. Huh, and W. Bandlow, Curr.

* Corresponding author.

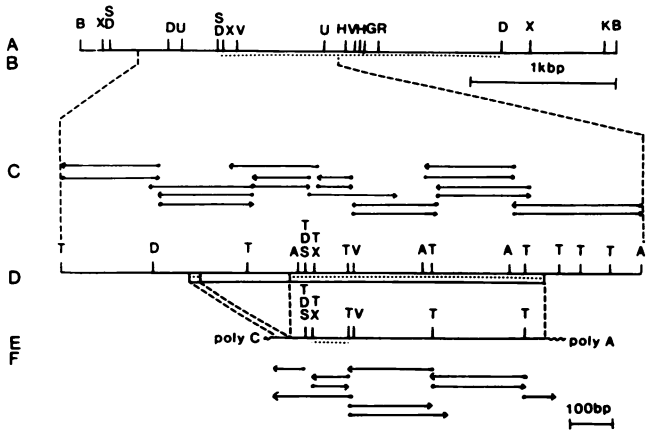

FIG. 1. Restriction map of the genomic and the cDNA clone including sequencing strategies. (A) Restriction map of clone pUHC15-2. Abbreviations: B, *Bam*HI; D, *Hin*dII; G, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; R, *Eco*RV; S, *Sal*I; U, *Pvu*I; V, *Pvu*II; X, *Xho*I. (B) *Hin*dII fragments hybridizing to end-labeled RNA. (C) Sequencing strategy. (D) Coding regions of clone pUHC15-2 and restriction sites used in sequencing. Abbreviations: A, *Hpa*II; T, *Taq*I; others as in panel A. (E) cDNA clone pC15 and 82-bp *Taq*I fragment, used in fragment elongation to map 5′ ends of transcripts. (F) Sequencing strategy for cDNA clone pC15.

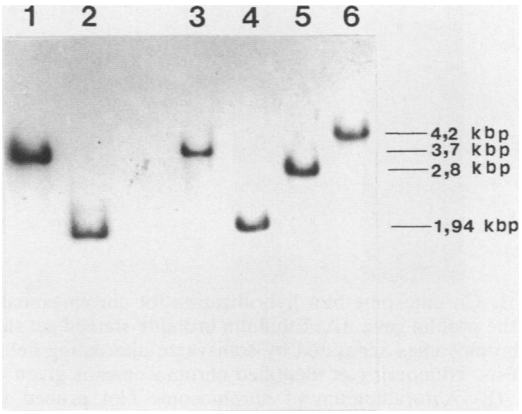

FIG. 2. Autoradiogram of a Southern blot of yeast genomic DNA digested with *Bam*HI (lane 3), *Hin*dII (lane 4), *Hin*dIII (lane 5), or *Bgl*II (lane 6) and control plasmid pUHC15-2 cut with *Bam*HI (lane 1) or *Hin*dII (lane 2). The blot was probed with a nick-translated *Ssp*I fragment (510 bp) from cDNA clone pC15.

Genet., in press), and DNA sequencing (7) were performed according to published standard procedures.

Fragment elongation. The 5′ end of the transcript was mapped by fragment elongation. A 1-pmol sample of 5′ end-labeled *Taq*I fragment (82 bp, position +67 in the cDNA; Fig. 1E) was purified by a Sephadex G-50 spun column (21) and annealed to 15 μg of total yeast RNA in 30 μl of annealing buffer [80% formamide, 40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (pH 6.4), 1 mM EDTA, 400 mM NaCl, 0.5 U of RNasin per μl] at 50°C. After 1 h the incubation mixture was cooled to 32°C within 4 h and ethanol precipitated. After several washes with 80% ethanol, the RNA-DNA hybrid was elongated with murine reverse transcriptase (Pharmacia, Freiburg) as described previously (21).

In vitro translation. A 510-bp *Ssp*I fragment from the cDNA clone pC15 was ligated to the polylinker of pGEM2. A clone with the AUG start codon proximal to the SP6 promoter was selected, linearized 3′ of the insert, and transcribed and translated as described previously (22) by using a nuclease-treated rabbit reticulocyte lysate system. Samples containing these in vitro-synthesized proteins were supplemented with $(NH_4)_2SO_4$ (final concentration, 66%) and centrifuged (10 min, 10,000 × *g*). Polyacrylamide (19%, wt/vol) gel electrophoresis was carried out in the presence of sodium dodecyl sulfate and urea (22). The size of the products was calibrated by comparison with truncated versions of prepromelittin and fusions between the N terminus of the peptide with sequences from mouse dihydrofolate reductase (22).

Computer analysis of sequences. The program ALIGN (5) was used with the mutation data matrix, a penalty for a break of 25 and 100 random runs. The alignment score, given in standard deviation units, is the number of standard deviations by which the maximum score for the real sequence exceeds the average maximum score for random permutations of the sequence. For secondary structure folding of precursor RNA, the program FOLD (33) was applied. ΔG values were calculated as described previously (8).

RESULTS

Characterization of the profilin-encoding clone pUHC15-2. A yeast genomic DNA clone, called pUHC15-2, was isolated as previously described (19; U. Oechsner, V. Magdolen, and W. Bandlow, FEBS Lett., in press). The profilin gene happened to be next to a galactose-controlled gene that was primarily selected by the screening procedure. The entire insert was restriction mapped (Fig. 1A) and hybridized, in a Southern blot, to end-labeled poly(A)$^+$ RNA to detect and roughly map transcribed regions (Fig. 1B). Restriction fragments from the left half of the insert that showed hybridization to RNA were sequenced from overlapping fragments as illustrated in Fig. 1C and 1D. Computer-aided sequence processing and screening of the combined sequence data base, MIPSX (F. Pfeiffer, Max-Planck-Institut für Biochemie, Martinsried, personal communication), revealed an open reading frame with striking homology to the amino acid sequence of the profilins from *Acanthamoeba* sp. (2, 4) and cows (1). A cDNA clone, pC15 (Fig. 1E), was isolated and sequenced (Fig. 1F) (24), providing proof for the expression of the gene in vivo.

Profilin gene encodes a vital function. To characterize the gene further, we examined whether the sequence homologous to that of profilin exists as a single copy in the yeast genome. Genomic DNA from haploid cells was digested with restriction endonucleases that do not cut sequences coding for profilin. After Southern transfer, the blot was probed with a 510-bp *Ssp*I fragment from the cDNA clone pC15. The stringency of the hybridization (60°C, 5 × SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]) was such that gene copies of profilin isoforms, with up to about 20% protein sequence divergence (as observed in the case of the three *Acanthamoeba* profilins [2] and the two respective histone H2A and H2B isoforms), would have been detected safely. The exclusive occurrence of a single hybridization signal in each lane proves the presence of only one genomic copy of the *PFY* gene (Fig. 2).

Chromosomal blotting and hybridization to nick-translated fragments from *PFY* and *HIS3* (mapping on chromosome XV) marked the lower one of a chromosomal double band containing chromosomes VII and XV, whereas a DNA probe, adjacent to centromere 7, labeled the upper band (Fig. 3). Thus, the gene for profilin can be attributed to

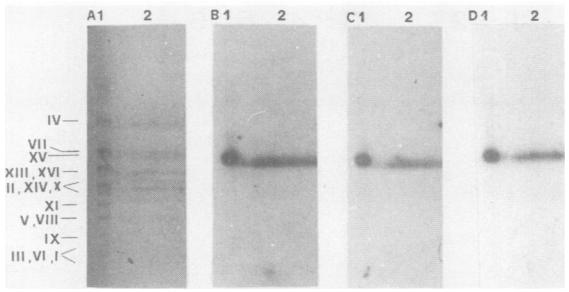

FIG. 3. Chromosome blot hybridization for chromosomal mapping of the profilin gene. (A) Ethidium bromide-stained gel showing yeast chromosomes separated by transverse alternating field electrophoresis. Numbering of identified chromosomes is given on the margin. (B) Autoradiogram of chromosome blot probed with a profilin-specific probe. (C) Autoradiogram of the same blot hybridized with a *HIS3*-specific DNA probe (*HIS3* maps on chromosome XV). (D) As in C, but hybridized with a *CEN7*-derived probe. Lanes: 1, chromosomes from strain AB972 (reference); 2, chromosomes from strain DCO4 (allowing separation of chromosomes VII and XV).

chromosome XV. Mapping studies confirmed this conclusion as they revealed genetic linkage of the *PFY* gene with the *ADE2* marker, which is on chromosome XV (see below).

We further tested whether the encoded gene was essential for cell viability and spore germination. The gene was disrupted by insertion of the selective *LEU2* marker gene into the *Pvu*II site within the N-terminal coding region of the *PFY* gene (Fig. 1A), and the linear construct was excised and transformed into yeast cells as described in Materials and Methods. No viable haploid transformants could be obtained with either this or two similar constructs having the upstream *Sal*I or the two *Hin*dII fragments (Fig. 1A) replaced by the *LEU2* marker. These results argue that an intact *PFY* gene may be indispensable for cell viability. *LEU* prototrophic transformants could, however, be obtained when a *leu2*/*leu2* auxotrophic diploid strain was transformed with construct ppfyV. The correct displacement of one of the two profilin alleles by the disrupted copy was assayed by genomic Southern blotting (Fig. 4). By insertion of the *LEU2* marker on a 2,300-bp *Hpa*I fragment into the *Pvu*II site, the hybridization signal of the profilin-bearing genomic *Bam*HI/*Hin*dIII fragment moved from the wild-type position (1,700 bp, lane 2 in Fig. 4A) to the one expected for the disruption (4,000 bp, lane 1) when we probed with a DNA containing only coding sequences from the profilin gene. The signal for the undisrupted allele stayed at the wild-type position (1,700 bp, lanes 1 and 2). After probing with a *LEU2*-specific fragment (Fig. 4B), one signal was identical with that of the disrupted allele probed with profilin sequences (4,000 bp, lane 1 in Fig. 4B). In addition, the fragment harboring the parental *leu2* alleles is marked (5,000 bp, lanes 1 and 2).

To test directly the requirement of *PFY* for germination and/or cell viability, strains having the desired genomic configuration were sporulated and the asci were dissected (data not shown). In a tetrad analysis of the diploid strain PFYD0 (*leu2*/*leu2 his4*/*his4 ADE*/*ade2 PFY*/*pfy*::*LEU2*) all seven complete asci contained only two viable spores. The residual two spores of each tetrad were unable to germinate or perform a single cell division. None of the viable spores harbored the *LEU2* marker, indicative of the absence of the disrupted *PFY* allele. Of 14 viable spores, all had the genotype *leu2 his4 PFY ADE*. None showed recombination between *PFY* and *ADE2*, confirming that *PFY* maps on

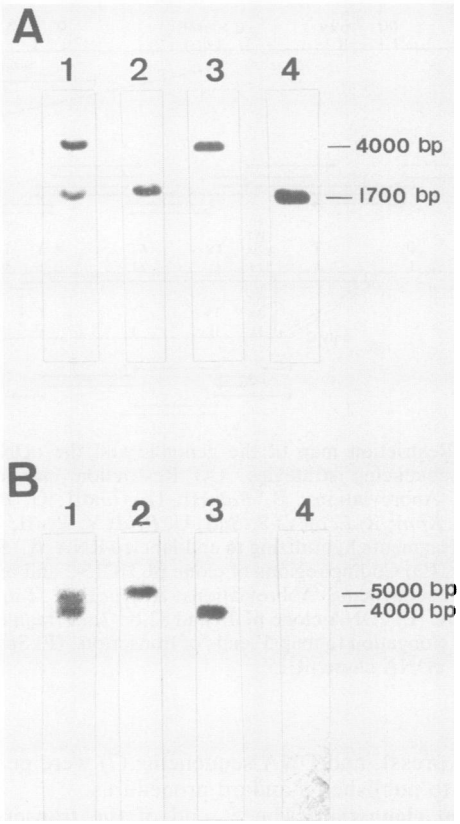

FIG. 4. Autoradiograms of Southern blots of genomic DNA from a strain having the wild-type genomic copy of the *PFY* gene replaced by an allele disrupted by the *LEU2* marker gene. The genomic DNA was cut with *Bam*HI plus *Hin*dIII. Lanes: 1, diploid disruption, PFYD0, one allele having the *Hpa*I fragment from *LEU2* inserted into the single *Pvu*II site of *PFY* (construct ppfyV); 2, AH22×AH33, diploid wild type; 3, control plasmid ppfyV; 4, control plasmid pUHC15-2, containing the undisrupted allele. (A) Hybridization with the 1,700-bp *Bam*HI-*Hin*dIII fragment harboring the profilin gene. (B) Hybridization with the 2,300-bp *Hpa*I fragment from *LEU2*.

chromosome XV. The occurrence of recessive lethality proves (i) that the cloned sequence comprises a gene and (ii) that the function of the encoded protein is indispensable for germination and, possibly, for cell viability and/or proliferation.

Analysis of the gene. Sequence analysis and comparison of the genomic sequences with the cDNA revealed the presence of an intron (Fig. 1E and 5). The 5′ donor site (AG↓GTATGT) and the 3′ acceptor site for splicing (TTTAAACAG↓) are consistent with the consensus for intron-exon boundaries in yeasts (9). The 3′ splice site is constituted by the first AG dinucleotide 3′ of the TACTAAC box. The intervening sequence is unlikely to encode any transacting function, since the longest open reading frame is comprised of only 29 triplets. The introns of both the profilin and the actin precursor RNAs can be folded into an extensive secondary structure (ΔG, −29.5 and −47.6 kcal/mol, respectively), which in the case of the profilin primary transcript leaves the TACTAAC sequence unpaired (data not shown).

Two TATA-like sequences were found in front of the *PFY* gene (Fig. 5). The initiation sites for the cellular profilin-encoding mRNAs were mapped by primer elongation. Three

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                                                                -314
                                                                 TCGA GGACGACGAA GACGAGGATT AGAGGAGACG TTACTTTGTT TATATATATT AGTATGTACA ATCGCAAAGA

-240
 AATGGAGTGA TGACATGTTG TAGTATTTAG TATGAGGTTA CTGTGTGGGA GGTTTTACCA TGATTTTTGG CGAGAACACG CCATGAAATG TCTTTGTACG AAACTCATTA CCCGCATTAA

-120                                                                                                                              5' GGCAAA..
 TATTTTTTTT CTTTTTAAAG CTCAGTTGAC CCTTTCTCAT TCCCTTCTTA AAACAACTGT GTGATCCTTG AGAAAAGATA AATTACATAC ACAACATAAA CCCAACTACG ATCGCAAATT
                                                                                                    •       o o    o  o  o     •  •

+1
   ATG TCT TGG CAA G   GTATGTGAAC GAGACAATTA TCAATTGATT AAGAAAGAAA TGAGTCGGAG GTTAGCTTGT GTGACAATGT TTGGCAATGC CCGATTTTTG TTGATGCGCG
   Met Ser Trp Gln

+114
 TAATTTCGAA GATTAACCAC TCAGAGTAAA TTACTAACTG GAATATCAAA AAACATATGA AATTTCAAAC ATGAATTTCT TTCCGTTTTT TTCTCCTACT TTTAAACAG  CA TAC ACT
                                                                                                                                Ala Tyr Thr

+231
   GAT AAC TTA ATA GGA ACC GGT AAA GTC GAC AAA GCT GTC ATC TAC TCG AGA GCA GGT GAC GCT GTT TGG GCT ACT TCT GGT GGC CTA TCT TTG CAA
   Asp Asn Leu Ile Gly Thr Gly Lys Val Asp Lys Ala Val Ile Tyr Ser Arg Ala Gly Asp Ala Val Trp Ala Thr Ser Gly Gly Leu Ser Leu Gln

+327
   CCA AAC GAA ATT GGT GAA ATT GTT CAA GGC TTC GAC AAT CCA GCT GGT TTG CAA AGC AAT GGT TTG CAT ATT CAA GGC CAA AAG TTC ATG TTG TTG
   Pro Asn Glu Ile Gly Glu Ile Val Gln Gly Phe Asp Asn Pro Ala Gly Leu Gln Ser Asn Gly Leu His Ile Gln Gly Gln Lys Phe Met Leu Leu

+423
   AGA GCT GAC GAT AGA AGT ATC TAC GGT AGA CAT GAT GCT GAG GGT GTT GTT TGT GTA AGA ACT AAG CAA ACC GTT ATT ATT GCT CAT TAT CCA CCA
   Arg Ala Asp Asp Arg Ser Ile Tyr Gly Arg His Asp Ala Glu Gly Val Val Cys Val Arg Thr Lys Gln Thr Val Ile Ile Ala His Tyr Pro Pro

+519
   ACC GTA CAA GCC GGT GAG GCC ACC AAG ATT GTC GAG CAA TTG GCT GAC TAC TTG ATT GGT GTT CAA TAC TAA   TTTATGC AGGTAAAGTT TTCTTGCCTT
   Thr Val Gln Ala Gly Glu Ala Thr Lys Ile Val Glu Gln Leu Ala Asp Tyr Leu Ile Gly Val Gln Tyr ***

+618
 ATACACCACC TATTCTGGCA TCTGCGGGAT TTCGCTTCCT ATTTTACAAA TATTTTATTG ATTGACGCTA ATTATCACTG TAAAAGGCGC ACTTTTTATA TGTAGTCACA TCCGGTATTT

+738                                                                 .. CAATCA 3'
 AACATATTTA CGAAACAGTC TTAAGAATAT CGACATTTGA TATACTTATG TTTAATTTAT CTACATATTA CAATCATACG AGAAACACGC AAAAACAATT ACTTGAATAC TTCGAAAGGA

+858
 GACCAATTTG GATGTACAAC CCTTTTTTCG CCCTTTTCCT TCGATATGTT ATTGATAGCT TCAAAGTCCT CAGTAGACAA AGTAAATATT TTCCTGTTCG TTTTGATTCG ATCGGGATTC

+978
 ACAGATTTTG GCAAGACAAC ATAACCTCTT TGGACGTGCC AGCTAATAAC AACGTGTCCGG
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FIG. 5. Sequence of the profilin gene including 5'- and 3'-flanking regions. The amino acid sequence predicted from the DNA sequence for the *PFY* coding region is given below the nucleotide sequence. The 5' and 3' ends of the cDNA clone pC15 are indicated above the sequence of the genomic clone. Major (●) and minor (○) transcription initiation signals are indicated; presumptive consensus sequences (TATA box, 5' and 3' splice sites, TACTAAC box, and termination signal) are overlined.

major start points of transcription were observed as sites of runoff from the RNA template of the reverse transcriptase (Fig. 7). These sites correlated best with the most upstream TATA box (Fig. 5). Overexposure of the autoradiogram (data not shown) revealed a few more minor signals, which are also indicated in Fig. 5. In addition, an initiation site was noticed about 300 bp upstream (see below).

The 5' end of the isolated cDNA clone maps at position −8 (24). However, we could not detect a transcription start at position −8. Moreover, at this position the genomic sequence is CGC, whereas GGC is found in the cDNA. It is very likely that this discrepancy is due to a reading error of the reverse transcriptase caused by a snap back in the RNA template.

Sequence analysis of the cDNA clone (24) revealed one polyadenylation site (although there could be more). It shows sequence similarity with the termination and polyadenylation site proposed for the *CYC1* transcript in *S. cerevisiae*, CAAT/GCTTTG (12). At 104 bp upstream of this site, the sequence motif TTTTTATATGT(. . . . . TTT) (Fig. 5) is likely to constitute the termination signal as discussed previously (19).

Transcription of the gene. In Northern blot analysis of total yeast RNA, probed with a fragment from the cDNA clone, one transcript of about 750 bases hybridized (Fig. 6). A faint additional RNA band of about 1,000 bases was detected in many preparations. The two signals could be caused either

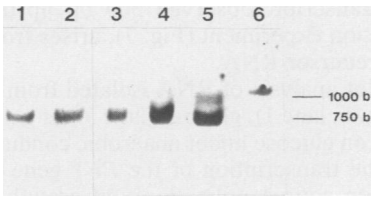

FIG. 6. Autoradiogram of northern blot analysis. Total RNA was isolated from log-phase (lane 1) and stationary-phase (lane 2) cells of lactate-grown wild-type cells, from aerobic (lane 3) and anaerobic (lane 4) glucose-grown wild-type cells, from strain ts368 *rna2* grown at the permissive temperature (23°C) (lane 5), and from the strain ts368 isolated 2 h after a shift to 36°C (lane 6). The blot was probed with a nick-translated 490-bp *Hin*dII fragment from profilin cDNA cloned into pGEM2.

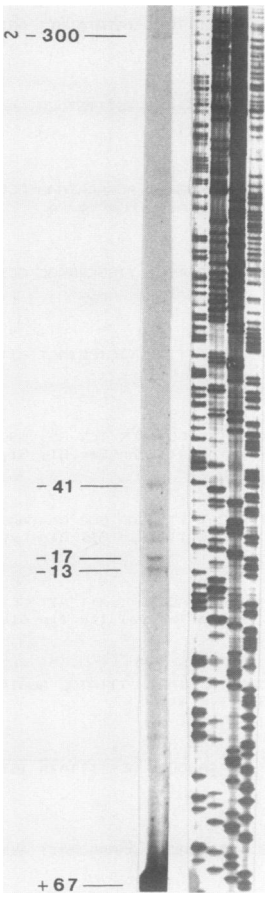

FIG. 7. Fragment elongation to map 5′ ends of *PFY*-specific transcripts. Lanes: 1, end-labeled, denatured 82-bp *Taq*I fragment (Fig. 1E), annealed to yeast wild-type RNA and elongated by murine reverse transcriptase. 2 through 5, ladders of known nucleotide sequences. The 5′ ends of transcripts are indicated.

by two different versions of the transcript, an intron-containing and a spliced one, or by the utilization of two different promoters and two sets of initiation sites. To discriminate between these two possibilities, RNA was isolated and analyzed from strain ts368, carrying the *rna2* mutation. When shifted to the nonpermissive temperature of 36°C, this strain is prevented from splicing intron-containing transcripts (18). Under nonpermissive conditions the longer transcript was accumulated at the expense of the shorter, suggesting that the longer is the unspliced precursor (Fig. 6, lane 6). Simultaneously, these results propose that the signal of the long transcript, observed 300 bp upstream in the primer elongation experiment (Fig. 7), arises from reversely transcribed precursor RNA.

Northern blot analysis of RNA isolated from cells grown on lactate (Fig. 6, lane 1), glucose (lane 3), or galactose (data not shown) or on glucose under anaerobic conditions (lane 4) reveals that the transcription of the *PFY* gene is not regulated by carbon source utilization and growth conditions. The signal with RNA from anaerobic cells was relatively strong. It is unlikely that it is caused by a real regulation phenomenon, because simultaneously a stronger signal was observed in this lane after hybridization of the same blot with an actin-specific probe (data not shown).

Lane 1 of Fig. 6 contained RNA from logarithmically grown cells; the RNA in lane 2 was from stationary-phase cells. No significant difference in the concentration of *PFY*-specific mRNA was observed. Very similar results were obtained when, as a control, the blot was probed with nick-translated DNA from the actin gene, which is regarded to be transcribed constitutively (H. Domdey and D. Gallwitz, personal communications; U. Oechsner, unpublished results). Also, from the assay of β-galactosidase activity in yeast transformants in which the *PFY* promoter had been fused to the *lacZ* structural gene, no indication of an extensive regulation by either carbon source or growth phase could be derived (data not shown). Since the relative concentrations of precursor and mature transcript did not vary under normal conditions (Fig. 6, lanes 1 through 4), it appears that the expression of profilin is not regulated at the level of splicing either. The results suggest that the gene is transcribed and processed constitutively in a manner similar to that of actin.

Deduced amino acid sequence. The coding capacity of the profilin gene is sufficient for a protein of 126 amino acids with a calculated molecular weight of 13,340. The codon usage in the yeast profilin gene reveals a relatively high bias (codon bias index, 0.67) toward frequently used triplets (6), indicating a moderately high expression of the *PFY* gene comparable, for example, to that of histones H2A and H2B but somewhat lower than that of actin and most ribosomal protein genes.

Computer analysis of the primary structure of the protein, deduced from the nucleotide sequence of the gene, revealed a strong homology with profilin; the amino acid sequence of profilin has been established from *Acanthamoeba* sp. (2, 4) (homology, 35.5 standard deviation units) and calf spleen (1) (homology, 6.4 standard deviation units) (Fig. 8). The *Acanthamoeba* protein contains 125 residues; the species from calf contains 142 amino acid residues. The highly conserved parts of the protein are mainly confined to the N terminus and, among *Acanthamoeba* sp. and *S. cerevisiae*, a short region near the carboxyl end.

In vitro translation of profilin. To prove that the observed open reading frame is, in fact, translated and that the encoded protein has the expected size, the cDNA sequence was cloned into a pGEM vector that allows transcription of the succeeding sequence by SP6 polymerase. Using 7mG(5′)ppp(5′)G (caps) for transcription initiation and a reticulocyte lysate for coupled translation of the message, we found one major labeled protein after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 9). The size of the product from the *PFY* gene, calibrated as detailed in Materials and Methods, is in perfect agreement with the expectation (126 amino acids).

DISCUSSION

Actin interacts with a variety of actin-binding proteins, among them profilin, which control the state and location of actin polymerization. It is an extremely highly conserved protein, present in all eucaryotes and in all tissues. It appears to fulfill partly analogous tasks in yeast and vertebrate cells (13, 15, 28), so that one would predict the existence of a similar set of accessory proteins in *S. cerevisiae* as described for higher eucaryotes (25, 29).

We have isolated a gene that has a coding capacity for a protein of 126 amino acids showing striking sequence homology with profilin from *Acanthamoeba* sp. (125 amino acids) (2, 4) and cow (142 amino acids) (1). It is a single-copy gene in *S. cerevisiae*, whereas in the motile organism *Acanthamoeba castellanii* three highly homologous isoforms (more

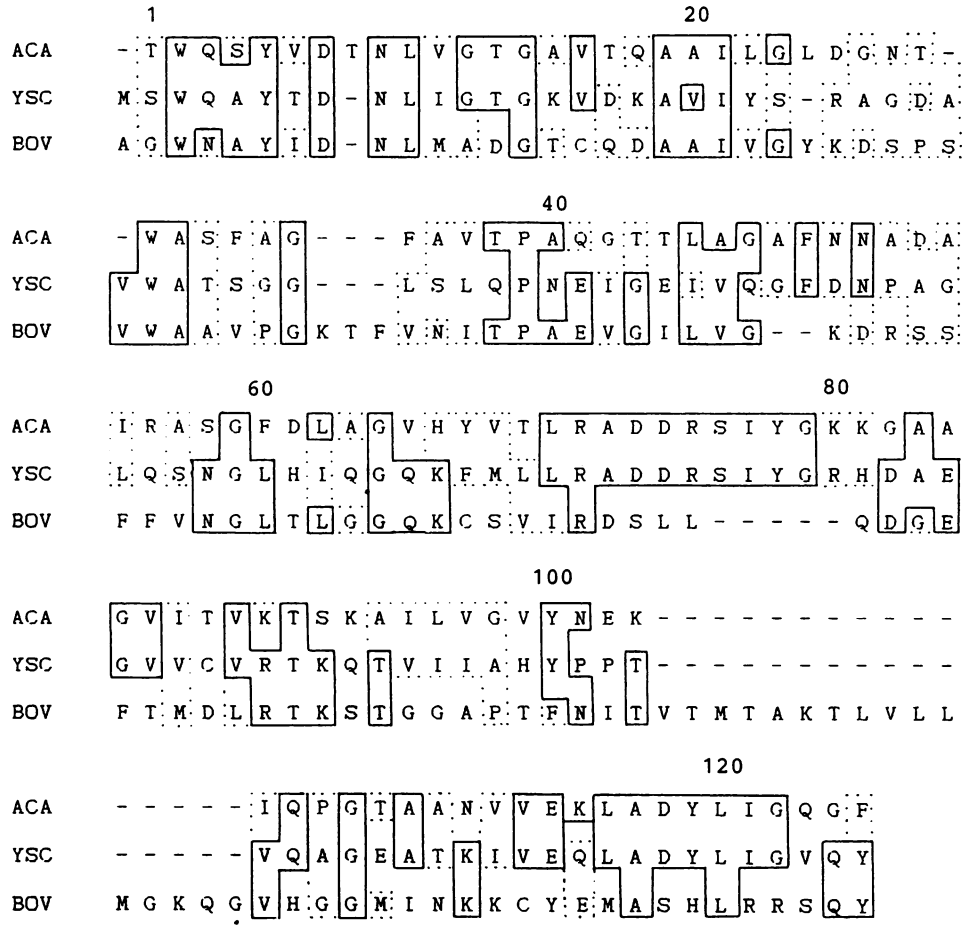

FIG. 8. Alignment of the amino acid sequences of the profilins from *Acanthamoeba* sp. (isoform 1) (ACA), *S. cerevisiae* (YSC), and cow (BOV). Amino acids are given in the one-letter code. Identical amino acids are boxed by solid lines, homologous amino acids by dotted lines. Homologous amino acids are P, A, G, S, and T; L, M, I, and V; H, K, and R; W, Y, and F; Q, N, E, and D. Position numbers refer to the *Acanthamoeba* sequence.

than 82% identity) have been characterized (2). Based on the following five criteria, we have shown that sequences carried by the plasmid pUHC15-2 encode the gene for yeast profilin and that this gene has a vital function. (i) The open reading frame was transcribed and spliced into an mRNA of the appropriate length. (ii) A cDNA clone of similar length (lacking the intron) was isolated. (iii) In an in vitro system the messenger was translated into a protein product of the expected size (deduced molecular weight, 13,340). (iv) The encoded gene product was essential for cell growth and/or proliferation in a way which is reminiscent of actin. (v) The deduced amino acid sequence revealed striking homology with the known primary structures of *Acanthamoeba* profilin and—a little less pronounced—with bovine profilin.

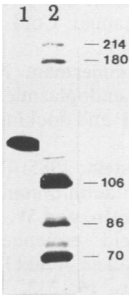

FIG. 9. In vitro translation of profilin. The profilin gene, cloned in a pGEM2 vector, was transcribed by SP6 polymerase, and the mRNA was translated in a nuclease-treated rabbit reticulocyte lysate system. The products were electrophoresed and fluorographed (lane 1). Lane 2 is a control track for size calibration (number of amino acids is given on the right).

Based upon its pronounced conservation, the N-terminal region of profilin was suspected to contain the contact sites with actin (4). However, a first indication, that C-terminal profilin sequences fulfill this task (similar to actin) (20; Pollard, Eur. J. Cell Biol. **46**:90, 1988), can be derived from the fact that this region in profilins from *Acanthamoeba* sp. and *S. cerevisiae*, displays sequence similarities with other actin-binding proteins like cap 42a (fragmin) from *Physarum polycephalum* and gelsolin from macrophages (3). In addition, recent cross-linking studies identified Lys-115 of the *Acanthamoeba* profilin as one residue in proximity to the contact site with actin (Pollard, Eur. J. Cell Biol. **46**:90, 1988).

Previous results with actin mutants and actin-overexpressing transformants had indicated that the manipulation of the actin concentration is harmful to the cell (23, 26, 28). The finding that profilin fulfills an essential function infers that its interaction with actin is required to prevent these

deleterious effects and to provide a means for controlling and fine-tuning actin polymerization. Surprisingly, transformants having the profilin gene on a multicopy vector exhibit no obvious phenotype (Bandlow and Strobel, unpublished results).

F-actin is formed from monomeric G-actin by a self-assembly process which requires the presence of calcium and ATP (11, 30). Since actin, apparently, is expressed constitutively, one would expect the available pool of monomeric actin to be controlled by the concentration of profilin through the formation of profilactin complexes that do not participate in the polymerization of actin. However, the cellular mRNA pool of profilin is not regulated with relation to growth rate or growth phase. It is therefore unlikely that profilin is involved in the control of actin polymerization by a mere variation of its pool size. Rather, the affinity for actin must be the parameter that is regulated. This could be accomplished through modulation of the availability of ligands that have been shown to affect actin polymerization and complex formation with profilin, i.e., ATP, calcium (16, 30), and phosphatidylinositol bisphosphate (17). Most importantly, the latter effector was shown to bind to profilin, presumably at its basic C terminus, preventing profilactin formation, whereas the products of hydrolysis by phospholipase C have no effect (17). Shifting the equilibrium between actin molecules in various aggregational states toward formation of profilactin, with the consecutive degradation of actin filaments, such a mechanism would link depolymerization of actin filaments to the ligand-induced turnover of phosphatidylinositol. This would couple the reorganization of the cytoskeleton to the progression of the cell cycle. Although *S. cerevisiae* was shown to contain phosphatidylinositol bisphosphate, it is much less certain at the moment whether it also has phospholipase C and a protein kinase C-mediated system of signal transduction.

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ADDENDUM

After the submission of this manuscript we received a personal communication from S. H. Lillie and S. S. Brown showing that the isolation of profilin from *S. cerevisiae* has been achieved and that microsequencing data of this protein are in accordance with the nucleotide sequence of the yeast profilin gene, published in this paper.

LITERATURE CITED

1. **Ampe, C., F. Markey, U. Linberg, and J. Vandekerckhove.** 1988. The primary structure of human platelet profilin: reinvestigation of the calf spleen profilin sequence. FEBS Lett. **228**:17–21.
2. **Ampe, C., M. Sato, T. D. Pollard, and J. Vandekerckhove.** 1988. The primary structure of the basic isoform of *Acanthamoeba* profilin. Eur. J. Biochem. **170**:597–601.
3. **Ampe, C., and J. Vandekerckhove.** 1987. The F-actin capping proteins of Physarum polycephalum: cap42(a) is very similar, if not identical, to fragmin and is structurally and functionally very homologous to gelsolin; cap42(b) is *Physarum* actin. EMBO J. **6**:4149–4157.
4. **Ampe, C., J. Vandekerckhove, S. L. Brenner, L. Tobacman, and E. D. Korn.** 1985. The amino acid sequence of *Acanthamoeba* profilin. J. Biol. Chem. **260**:834–840.
5. **Barker, W. C., L. T. Hunter, B. C. Orcutt, D. G. Geroge, L. S. Jeh, H. R. Chen, M. C. Blomquist, G. C. Johnson, and M. O. Dayhoff.** 1983. Atlas of protein sequence and structure, protein data base, vol. 7. National Biochemical Research Foundation, Washington, D.C.
6. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
7. **Chen, E., and P. H. Seeburg.** 1985. Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA **4**:165–170.
8. **Freier, S. M., R. Kierzek, J. A. Jaeger, N. Sugimoto, M. H. Caruthers, T. Neilson, and D. H. Turner.** 1986. Improved free-energy parameters for predictions of RNA duplex stability. Proc. Natl. Acad. Sci. USA **83**:9373–9377.
9. **Gallwitz, D., H. Halfter, and P. Mertins.** 1987. Splicing of mRNA precursors in yeast, p. 27–40. *In* J. R. Kinghorn (ed.), Gene structure in eukaryotic microbes. SGM special publication 22. IRL Press, Oxford.
10. **Gallwitz, D., and I. Sures.** 1980. Structure of a split yeast gene: complete nucleotide sequence of the actin gene in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **77**:2546–2550.
11. **Greer, C., and R. Shekman.** 1982. Calcium control of *Saccharomyces cerevisiae* actin assembly. Mol. Cell. Biol. **2**:1279–1286.
12. **Henikoff, S., J. D. Kelly, and E. H. Cohen.** 1983. Transcription terminates in yeast distal to a control sequence. Cell **33**:607–614.
13. **Huffaker, T. C., M. A. Hoyt, and D. Botstein.** 1987. Genetic analysis of the yeast cytoskeleton. Annu. Rev. Genet. **21**:259–284.
14. **Isenberg, G., U. Aebi, and T. D. Pollard.** 1980. An actin-binding protein from *Acanthamoeba* regulates actin filament polymerization and interactions. Nature (London) **288**:455–459.
15. **Kilmartin, J., and A. Adams.** 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces cerevisiae*. J. Cell. Biol. **98**:922–933.
16. **Korn, E. D.** 1982. Actin polymerization and its regulation by proteins from nonmuscle cells. Physiol. Rev. **62**:672–737.
17. **Lassing, I., and U. Lindberg.** 1985. Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin. Nature (London) **314**:472–474.
18. **Lustig, A. J., L. Ren-Jang, and J. Abelson.** 1986. The yeast RNA2 gene products are essential for mRNA splicing in vitro. Cell **47**:953–963.
19. **Magdolen, V., U. Oechsner, and W. Bandlow.** 1987. The complete nucleotide sequence of the gene coding for yeast adenylate kinase. Curr. Genet. **12**:405–411.
20. **Malm, B.,** 1984. Chemical modification of Cys-374 of actin interferes with the formation of the profilactin complex. FEBS Lett. **173**:399–402.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Müller, G., and R. Zimmerman.** 1987. Import of honey bee prepromelittin into the endoplasmic reticulum: structural basis for independence of SRP and docking protein. EMBO J. **6**:2099–2107.
23. **Novick, P., and D. Botstein.** 1985. Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell **40**:405–416.
24. **Oechsner, U., V. Magdolen, and W. Bandlow.** 1987. The cDNA and deduced amino acid sequence of profilin from *Saccharomyces cerevisiae*. Nucleic Acids Res. **15**:9078. Corrigendum, 1988. Nucleic Acids Res. **16**:1232.
25. **Pollard, T. D., and J. A. Cooper.** 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Annu. Rev. Biochem. **55**:987–1035.
26. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A Saccharomyces cerevisiae genomic plasmid bank based on a centromer-containing shuttle vector. Gene **60**:237–

243.
27. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
28. **Shortle, D., J. Haber, and D. Botstein.** 1982. Lethal disruption of the yeast actin gene by integrative DNA transformation. Science **217**:371–373.
29. **Stossel, T. P., C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, and K. S. Zaner.** 1985. Nonmuscle actin-binding proteins. Annu. Rev. Cell Biol. **1**:353–402.
30. **Tobacman, L. S., and E. D. Korn.** 1982. The regulation of actin polymerization and the inhibition of monomeric actin ATPase activity by Acanthamoeba profilin. J. Biol. Chem. **257**:4166–4170.
31. **Vandekerckhove, J., and K. Weber.** 1978. Actin amino acid sequences. Comparison of actins from calf thymus, bovine brain, and SV40-transformed mouse 3T3 cells with the rabbit skeletal muscle actin. Eur. J. Biochem. **90**:451–462.
32. **Vandekerckhove, J., and K. Weber.** 1978. Mammalian cytoplasmic actins are the products of at least two genes and differ in primary structure in at least 25 identified positions from skeletal muscle actins. Proc. Natl. Acad. Sci. USA **75**:1106–1110.
33. **Zuker, M., and P. Stiegler.** 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. Nucleic Acids Res. **9**:133–148.