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

Received June 1988/Accepted 30 August 1988

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 filaments (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 MATa, rna2 adel adel2 lys2 tyr1 his7 ural gall) (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 MATa/MATa, leu2/leu2, his4/his4, ADE/ade2 was used. Mapping of the profilin gene was achieved by transverse alternating-field agarose (1%) gel electrophoresis (Genecline, 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) Hpa1 fragment was ligated to the single PvuII site near the 5' end of the PFY coding region after partial digestion of the pUC19-derived plasmid with PvuII. The excised construct on a 1,700-bp BamHI-HindIII fragment, ppyFV, was transformed into S. cerevisiae to yield the diploid disruption mutant PFYDO0 (PFY[ppy::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.
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 TaqI fragment (82 bp, position +67 in the cDNA; Fig. 1E) was purified by a Sephadex G-50 spin column (21) and annealed to 15 \( \mu \)g of total yeast RNA in 30 \( \mu \)l 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 \( \mu \)l] 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 SspI 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\(_4\))\(_2\)SO\(_4\) (final concentration, 66%) and centrifuged (10 min, 10,000 \( \times \) 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. \( \Delta G \) 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 SspI 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...
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 selectable LEU2 marker gene into the PvuII 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 SalI or the two HindIII 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 ppfV. 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 HpaI fragment into the PvuII site, the hybridization signal of the profilin-bearing genomic BamHI/HindIII 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 ascii were dissected (data not shown). In a tetrad analysis of the diploid strain PFY-leu2/leu2 his4/his4 ADE2 ade2 PFY/ppfV:LEU2 all seven complete ascii 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 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 open reading frame (Fig. 1E and 5). The 5' donor site (AG GTATG) and the 3' acceptor site for splicing (TAAAAACAG) 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 (AG, −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
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 CYCI transcript in S. cerevisiae, CAA/TGCTTG (12). At 104 bp upstream of this site, the sequence motif TTTTATATGTT (. . . . 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
by two different versions of the transcript, an intron-contain-
ing and a spliced one, or by the utilization of two dif-
ferent 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-contain-
ing 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 regu-
lated 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 β 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. Gall-
witz, 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
centration 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), indicat-
ing a moderately high expression of the PFY gene compar-
able, 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 Acan-
thamoeba 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
7mgG(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-polyacylamide 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 verte-
brate cells (13, 15, 28), so that one would predict the
existence of a similar set of accessory proteins in S. cerevi-
siae as described for higher eucaryotes (25).

We have isolated a gene that has a coding capacity for a
protein of 126 amino acids showing striking sequence homol-
gy 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 Acantha-
moeba castellanii three highly homologous isoforms (more
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.

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

![Image of a gel with bands](image-url)
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 biphosphate (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 aggregation states toward formation of profilactin, with the consequent degeneration 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 biphosphate, it is much less certain at the moment whether it also has phospholipase C and a protein kinase C-mediated system of signal transduction.

ACKNOWLEDGMENTS

The excellent technical assistance by G. Strobel and the secretarial help with the preparation of the manuscript by I. Haberl are gratefully acknowledged. We are deeply indebted to J. Kellermann, Max-Planck-Institut für Biochemie, Martinsried, for his help screening the sequence data bases. Plasmid pYAct1 and strain ts368 rna2 were obtained through the courtesy of H. Domdey, The Gene Center, Martinsried; the BANHI gene bank in S. pombe was a gift from M. Suissa and A. Haid, The Biocenter, Basel, and the cDNA library in pMAC561 from G. L. McKnight, B. L. McConaughy, and B. D. Hall, Seattle, Wash.

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

3. Ampe, C., and J. Vanderkerkhove. 1987. The F-actin capping proteins of Physarum polycephalum: cap42a is very similar, if not identical, to fragmin and is structurally and functionally very homologous to gelosin; cap42b is Physarum actin. EMBO J. 6:1419-1417.


