

The basic isoform of profilin in pathogenic *Entamoeba histolytica* cDNA cloning, heterologous expression, and actin-binding properties

Marina BINDER¹, Stephan ORTNER^{1,2}, Hertha ERBEN¹, Otto SCHEINER², Gerhard WIEDERMANN¹, Rudolf VALENTA²
and Michael DUCHÊNE^{1,2}

¹ Institute for Specific Prophylaxis and Tropical Medicine, University of Vienna, Austria

² Institute of General and Experimental Pathology, University of Vienna, Austria

(Received 21 June 1995) – EJB 95 1002/1

In the human parasite *Entamoeba histolytica*, components of the cytoskeleton are involved in the pathogenicity by their contribution to immune evasion by antibody capping and shedding. In this study, we focus on profilin as a central regulatory component of the cytoskeleton. Profilin was isolated from trophozoites of the pathogenic *E. histolytica* strain SFL-3, and partial amino acid sequences were used to devise a probe for isolating a profilin cDNA. The deduced complete primary structure was divergent: plant profilins with amino acid sequence identities in the range 33–38% were more closely related than the mammalian profilins with sequence identities 21–28%. The cDNA was expressed as a nonfusion protein in *Escherichia coli*. Isoelectric focussing of the natural profilin isolated from *E. histolytica* showed two isoforms with different isoelectric points; the recombinant profilin migrated with the basic isoform. In a blot overlay experiment, purified ¹²⁵I-labeled recombinant profilin bound not only to plant actin, but also to mammalian actin, demonstrating that cytoskeletal components from distantly related organisms with divergent primary structures can be compatible.

Keywords: *Entamoeba histolytica*; profilin; actin-binding protein; cDNA cloning; cytoskeleton.

The presence of an actin-based cytoskeleton is a hallmark of the eukaryotic cell (Pollard and Quirk, 1994). Such a cytoskeleton allows the cell to maintain or to change its shape, both on the outside and inside. *Entamoeba histolytica*, the causative agent of invasive amoebiasis in humans, is the source of significant morbidity and mortality worldwide (Walsh, 1986). Although it is considered to be a primitive organism (Bakker-Grunwald and Wöstmann, 1993), its cytoskeletal machinery is very efficient. *E. histolytica* is capable of amoeboid motion, it can engulf bacteria and erythrocytes, and it is able to cap and shed antibodies bound to its surface, thus evading the humoral immune response of the host (Calderón et al., 1980).

To understand these mechanisms of amoebic pathogenicity, it is necessary to examine the molecular components involved. Profilin is a ubiquitous component of the cytoskeleton. It was discovered as an actin-binding protein (Carlsson et al., 1976) that is involved in dynamic processes like the acrosomal process (Tilney et al., 1983). Depending on the conditions, profilin is able to inhibit or to promote actin filament formation (Theriot and Mitchison, 1993; Sohn and Goldschmidt-Clermont, 1994). By binding to phosphatidylinositol 4,5-bisphosphate, profilin inhibits the production of the second messengers inositol trisphosphate and diacylglycerol by unphosphorylated phospholipase C- γ 1 (Goldschmidt-Clermont et al., 1990, 1991). Profilin was detected several years ago in *E. histolytica* (Sabanero-Lopez and

Segura-Nieto, 1990). In this study, the isolation and cDNA cloning of *E. histolytica* profilin are reported. Although the molecule had a divergent primary structure, the recombinant molecule was able to interact with plant and mammalian actins. Isoelectric focussing showed that there were at least two profilin isoforms with different isoelectric points.

EXPERIMENTAL PROCEDURES

Reagents. Chemicals were obtained from Merck, restriction enzymes and T4 polynucleotide kinase were from Boehringer, plasmids were isolated using Qiagen columns (Diagen), and DNA was sequenced with T7 DNA polymerase (Pharmacia).

Strains and culture conditions. Trophozoites of the axenic pathogenic *E. histolytica* strain SFL-3 (zymodeme II) were grown in TYI-S-33 medium (Diamond et al., 1978). For standard molecular biological work, *Escherichia coli* XL1-Blue (Stratagene) was used; *E. coli* CJ236 (Kunkel et al., 1987) was used for production of uridine-labeled single-strand phagemid and *E. coli* BL21(DE3) pLysS (Novagene) was used for the expression of recombinant profilin. This strain expresses T7 RNA polymerase under the control of a *lacUV5* promoter, and a low level of T7 lysozyme.

Isolation and protein sequence analysis of natural *E. histolytica* profilin. *E. histolytica* trophozoites (80×10^6) grown axenically for 48 h were lysed (Lind et al., 1987) in 30 ml buffer A [120 mM Pipes, 50 mM Hepes, 20 mM EGTA, 4 mM MgCl₂, 10 mM glucose, 1.5% (by vol.) Triton X-100, pH 7.2] with added protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 20 mM iodoacetamide), debris was precipitated by centrifugation for 5 min at $2000 \times g$ and 4°C, followed by centrifugation for 90 min at $350\,000 \times g$ and 4°C. The supernatant was incu-

Correspondence to M. Duchêne, Institute for Specific Prophylaxis and Tropical Medicine, Kinderspitalgasse 15, A-1095 Vienna, Austria
Fax: +431 40400 5130.

Enzymes. Trypsin (EC 3.4.21.4); T7 DNA polymerase (EC 2.7.7.7); Reverse transcriptase (EC 2.7.7.49).

Note. The novel nucleotide sequence data published here have been deposited with the EMBL sequence data bank and are available under accession number X90911.

bated with 20 ml poly(L-proline) coupled to CNBr-activated Sepharose 4B (Pharmacia) for 2 h at room temperature and overnight at 4°C. The affinity matrix was washed with 90 ml Tris/NaCl/ATP (20 mM Tris/HCl, 150 mM NaCl, 0.5 mM ATP, pH 7.6), and profilin was eluted with 30 ml Tris/NaCl/ATP containing 2 M urea followed by 90 ml Tris/NaCl/ATP containing 6 M urea. The eluates containing the profilin were dialyzed against H₂O and lyophilized.

Protein sequence analysis was performed by K. Stone at the W. M. Keck Foundation, Biotechnology Resource Laboratory, New Haven, Connecticut. The material was N-terminally blocked. Therefore, tryptic peptides were generated and separated by HPLC. Two peptides were sequenced by Edman degradation (peak 33: VDADEGTAMGK; peak 44: GAEGISYK).

Isolation of a complete profilin cDNA. The construction of the oligo(dT)-primed λ ZAP cDNA library from *E. histolytica* strain SFL-3 has been described (Ortner et al., 1992). To screen the library with a mixed oligonucleotide probe, the peak 33 sequence was reverse translated into probe EHP-1 5'-CCC ATA/T GCA/T GTA/T CCT TCA TCA/T GCA TC-3', complementary to the mRNA and taking into consideration the codon usage of *E. histolytica* (Plaimauer et al., 1993). 20 $\times$ 10⁴ λ ZAP cDNA clones were plated onto *E. coli* strain XL1-Blue (Stratagene), transferred onto nitrocellulose (Schleicher & Schüll), and hybridized (Wallace et al., 1979) with the ³²P-labeled probe EHP-1. Final wash conditions were 6 $\times$ NaCl/Cit (20 $\times$ NaCl/Cit = 3 M NaCl, 0.3 M sodium citrate, pH 7.0), 0.1% SDS at 50°C. 22 positive clones were isolated and plasmids were obtained by *in vivo* excision (Short et al., 1988). Plasmid DNAs were sequenced with the dideoxynucleotide chain-termination method using M13 reverse primer and T7 polymerase (Pharmacia) according to the instructions of the supplier.

To obtain complete profilin cDNAs, two further rounds of screening with unmixed oligonucleotide probes EHP-2 (5'-CAC CCA TAC CTT TTC CAG CT-3') and EHP-3 (5'-GAA TGA ATC AAC ATA TGA TTG C-3') were performed. One of the complete cDNAs (pEHP6) was sequenced with T7 DNA polymerase using M13 and synthetic primers.

RNA methods. Total RNA was isolated (Chirgwin et al., 1979) from trophozoites of *E. histolytica* SFL-3. The RNA was tested on a Northern blot (Sambrook et al., 1989). To examine the 5'-terminal untranslated region of *E. histolytica* profilin mRNA, a primer extension sequencing experiment (Köhler et al., 1989) was carried out using the oligonucleotide EHP-2 (see above) to prime reverse transcriptase on 5 μ g poly(A)-rich RNA/reaction. The products were analyzed on an 8% polyacrylamide sequencing gel (Sambrook et al., 1989).

Southern-blot analysis. DNA was isolated from *E. histolytica* trophozoites (Torres-Guerrero et al., 1991). For each lane, 1 μ g DNA was digested with the enzymes *Eco*RI, *Eco*RV, or *Dra*I, electrophoresed on a 1% agarose gel, blotted onto Immobilon N (Millipore), and hybridized with the cDNA insert from clone pEHP6, which was ³²P-labeled using the random hexamer method (Sambrook et al., 1989). The final wash conditions were 1 $\times$ NaCl/Cit, 0.1% SDS, at 60°C.

Expression of the profilin cDNA in *E. coli*. The profilin cDNA insert was excised from the Bluescript pEHP6 plasmid with *Eco*RI and *Xho*I and cloned into the expression vector pET23(+) (Novagene). This phagemid carries a bacteriophage T7 promoter and terminator (Tabor and Richardson, 1985). The pET23(+) phagemid with pEHP6 cDNA insert was transformed into *E. coli* CJ236 (Kunkel et al., 1987), and uridine-labeled single-strand phagemid DNA was produced with the M13 helper phage R408 (Stratagene). To introduce a sequence including the ribosome-binding site at the correct distance upstream of the translation initiation codon, the oligonucleotide EHP-EX 5'-

GAT TGC CAA GAC ATG GTA TAT CTC CTT CTA GAG GGA AAC CG-3' was used to prime the synthesis of mutagenized second strands on the single-stranded uridine-labeled phagemid DNA. Transformation into *E. coli* XL1-Blue (Stratagene) resulted in clones, which were tested by restriction and DNA sequence analysis. The profilin expression plasmid pET-EHP1 with the correct sequence was retransformed into *E. coli* BL21 (DE3) pLysS (Novagene). The *E. coli* cells expressing profilin were grown at 37°C in Luria-Bertani medium to an absorbance of 1.0 at 600 nm, and induced with 0.4 mM isopropyl thio- β -D-galactoside for 3 h. The cells were harvested for 10 min at 2000 $\times$ g and 4°C, suspended in buffer A (see above), and profilin was purified by poly(L-proline) affinity chromatography. The same quantities of reagents as described above were used, but this time the purification was carried out using a column instead of the batch procedure.

Isoelectric focussing. Recombinant or natural profilin (100 ng) was loaded onto a pH 3.0–9.0 isoelectric focussing gel, provided for the Phast system (Pharmacia) and separated according to the instructions of the supplier. After separation, the proteins were silver stained.

Blot overlay. Rabbit muscle actin and bovine serum albumin (Ria grade) were obtained from Sigma. Recombinant actin prepared as described previously (Valenta et al., 1993), was generously provided by Richard B. Meagher, University of Georgia, Athens. On an SDS/polyacrylamide gel, 1 μ g *Arabidopsis* actin, 1 μ g rabbit skeletal muscle actin, and 2 μ g bovine serum albumin were loaded and electrophoresed. Two gels were prepared in the same way. One of the gels was stained with Coomassie brilliant blue R250 and the other gel was blotted onto nitrocellulose. The blot was blocked twice for 5 min and once for 30 min in Tris/NaCl/Tween [90 mM NaCl, 10 mM Tris/HCl, 0.5% (by vol.) Tween 20, pH 7.5] with 1% bovine serum albumin. Recombinant *E. histolytica* profilin, ¹²⁵I-labeled by the chloramine-T method (Sambrook et al., 1989), was added at a concentration of 50 000 cpm/ml, i.e. 1.5 ng/ml Tris/NaCl/Tween, and incubated for 2 h at room temperature. The blot was washed twice for 5 min, once for 30 min in Tris/NaCl/Tween, dried and autoradiographed.

RESULTS

Isolation and characterization of a complete *E. histolytica* profilin cDNA. Since the sequence similarity between the known profilins was rather low, DNA probes derived from other profilins were not expected to detect the *E. histolytica* cDNA. Therefore, amino acid sequence information was obtained from isolated natural *E. histolytica* profilin. Two sequences were obtained by analysis of tryptic peptides (Fig. 1), and the sequence DADEGTAMG was reverse translated into a mixed oligonucleotide probe. Screening the cDNA library with this probe gave positive clones with significant sequence similarity to profilins. Two further rounds of screening were needed to obtain two complete cDNA clones pEHP6 and pEHP29. Both strands of the insert of clone pEHP6 were sequenced.

The 5'-end of the profilin mRNA was examined by a primer-extension sequencing experiment (data not shown) using oligonucleotide EHP-2 as primer. The sequence information is included in Fig. 1. Since the first 5–6 bases at the transcriptional start could not be identified unambiguously by primer-extension sequencing, the first nucleotide displayed corresponds to nucleotide 6 or 7 of the mRNA. Clone pEHP29 starts at base 8, and pEHP6 at base 13.

With the short 5'- and 3'-untranslated regions, typical for *E. histolytica*, the size of the profilin mRNA was calculated as 443

[illegible]

Fig. 1. Sequence of the *E. histolytica* profilin cDNA, deduced amino acid sequence, and comparison with *Physarum polycephalum* profilin. The amino acid sequences of the internal tryptic peptides in peak 33 and peak 44 are underlined in the deduced amino acid sequence. The *E. histolytica* sequence is compared with the most similar profilin sequence from *Physarum polycephalum*. Eh, *E. histolytica* profilin; Pp1, *P. polycephalum* profilin I (profilin A, basic isoform); identical residues are marked (|); gaps that were introduced for optimal alignment are indicated (·).

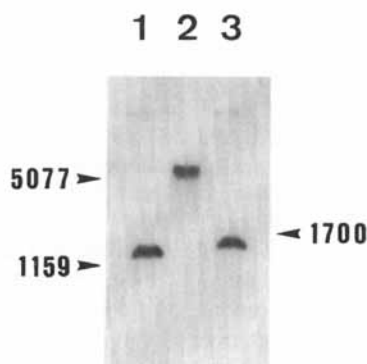


Fig. 2. Southern blot of *E. histolytica* DNA probed with profilin cDNA. For each lane, 1 µg *E. histolytica* genomic DNA was cleaved with the following: lane 1, *Eco*RI; lane 2, *Eco*RV; lane 3, *Dra*I. The samples were transferred onto Immobilon N, and hybridized with the ³²P-labeled cDNA insert of clone pEHP6. The sizes of representative marker fragments (bp) are indicated at the right and left sides.

nucleotides plus the poly(A) tail. A strong signal compatible with this size was observed in a Northern (RNA) blot of total and poly(A)-rich RNA from *E. histolytica* (data not shown).

To test whether there were further closely related profilin genes in *E. histolytica*, a Southern-blot experiment was performed. *E. histolytica* genomic DNA was digested with the restriction enzymes *Eco*RI, *Eco*RV, and *Dra*I, which do not cleave the cDNA pEHP6. The digests were electrophoresed and blotted, and the blot was hybridized with the ³²P-labeled pEHP6 cDNA. The blot was washed with intermediate stringency (1×NaCl/Cit, 0.1% SDS, at 60°C). The results are shown in Fig. 2. All three digests gave only single bands, indicating the absence of further closely related profilin genes.

Analysis of the deduced protein sequence. The sequence obtained from clone pEHP6 (Fig. 1) contained an open reading frame coding for a polypeptide with 130 amino acid residues and a deduced molecular mass of 13.2 kDa. Both tryptic peptide sequences were part of the open reading frame (Fig. 1), and both were preceded by basic amino acid residues as expected for trypt-

tic fragments. The deduced polypeptide had end-to-end sequence similarity with profilins in the databases. At the time of the preparation of this manuscript, the Swiss-Prot database (version 31.0⁺) contained 26 profilin sequences from 17 eukaryotic species plus two viruses. All the sequences were aligned with the *E. histolytica* profilin sequence and with each other. Table 1 shows a partial list of amino acid sequence identities of profilins from representative species, *E. histolytica* (Ehis), *Acanthamoeba castellanii* (Ac1a, Ac1b, Ac2) (Pollard and Rimm, 1991), *Dictyostelium discoideum* (Dd1, Dd2) (Haugwitz et al., 1991), *Physarum polycephalum* (Pp1, Pp2) (Binette et al., 1990), *Saccharomyces cerevisiae* (Sce) (Magdolen et al., 1988), *Nicotiana tabacum* (Nta) (Mittermann et al., 1995), and *Homo sapiens* (Hs1) (Kwiatkowski and Bruns, 1988) and (Hs2) (Honoré et al., 1993). The degree of sequence similarity between the *E. histolytica* profilin and profilins from other species was only moderate, the closest relatives were the two *Physarum* sequences with 41% sequence identity. The alignment with the basic isoform of *Physarum* profilins (Pp1), which is also the form preferentially expressed in *Physarum* amoebae is shown in Fig. 1. Remarkably, the plant profilins were as closely related as the other amoeba profilins. For example, 38% identity was observed with tobacco profilin, whereas the mammalian profilins were clearly less related (for example 23% and 28% identity with the human profilins). The profilin isoforms were divergent in several species: only 56% identity in the *Dictyostelium*, 66% in the *Physarum*, and 62% in the human profilin isoforms were found.

Expression of *E. histolytica* profilin in *E. coli*. An expression construct pETEHP1 was generated that contained the insert from pEHP6 inserted between a T7 RNA polymerase promoter and terminator, and containing a bacterial Shine-Dalgarno sequence for efficient translation. The plasmid was retransformed into *E. coli* BL21 (DE3) pLysS. Cells were grown in Luria-Bertani medium, and the production of recombinant protein was induced with 0.4 mM isopropyl thio- β -D-galactoside. The recombinant profilin was purified by poly(L-proline) affinity chromatography using a column procedure.

Isoelectric focussing of natural and recombinant *E. histolytica* profilins. Since *Physarum*, *Dictyostelium*, and *Acantha-*

Table 1. Amino acid sequence identities of representative profilins. Amino acid sequences from *E. histolytica* (Eh), *Acanthamoeba castellanii* (Ac1a, Ac1b, Ac2), *Dictyostelium discoideum* (Dd1, Dd2), *Physarum polycephalum* (Pp1, Pp2), *Saccharomyces cerevisiae* (Sce), *Nicotiana tabacum* (Nta), and *Homo sapiens* (Hs1, Hs2) were aligned. The percentage of identical amino acid residues is shown.

Sequence for comparison	Identical amino acid residues for										
	Ac1a	Ac1b	Ac2	Dd1	Dd2	Pp1	Pp2	Sce	Nta	Hs1	Hs2
	%										
Eh	33	34	36	40	35	41	41	38	38	23	28
Ac1a		94	84	48	47	52	55	41	38	26	25
Ac1b			84	49	49	52	54	42	39	25	26
Ac2				42	48	57	55	41	41	29	29
Dd1					56	52	62	39	48	23	27
Dd2						44	49	39	40	26	28
Pp1							66	51	44	28	28
Pp2								44	40	21	25
Sce									32	31	32
Nta										34	33
Hs1											62



Fig. 3. Isoelectric focussing. Profilins were separated by a pH 3–9 isoelectric focussing gel and silver stained. Lane 1, recombinant profilin produced from *E. coli* BL21(DE3) harboring the plasmid pETEHP1; lane 2, natural profilin isolated from *E. histolytica* SFL-3.

moeba all possess at least two isoforms of profilin, it was surprising to observe that the Southern blot of *E. histolytica* DNA displayed only one set of fragments. Since the profilin isoforms in other organisms had different isoelectric points, the natural isolated profilin preparation was examined using an isoelectric focussing gel, spanning a pI range of pH 3.0–9.0. The recombinant and natural profilins were compared. Fig. 3 shows that the natural profilin preparation contained two isoforms. The third visible band at the bottom was thought to be derived from material left in the slot located in the middle of the gel. The recombinant profilin comigrated with the basic natural profilin isoform. This correlated with an isoelectric point of pH 7.9, predicted from the amino acid sequence.

Interaction of recombinant *E. histolytica* profilin with mammalian and plant actins. The actin-binding properties of the cloned profilin were investigated in a blot overlay experiment. Rabbit muscle actin, recombinant actin from *Arabidopsis*, and, as a control, bovine serum albumin, were electrophoresed in parallel on two SDS/polyacrylamide gels. Fig. 4A shows the first gel that was stained with Coomassie brilliant blue. The proteins on the second gel were blotted onto nitrocellulose. Recombinant *E. histolytica* profilin as the possible binding partner was ^{125} I labeled. Fig. 4B shows the autoradiography of a sample of labeled profilin, tested on an SDS/PAGE gel. The same labeled

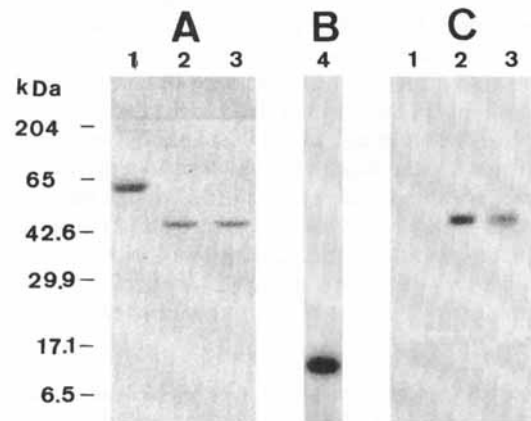


Fig. 4. Actin-binding properties of recombinant *E. histolytica* profilin. Lanes 1, bovine serum albumin; lanes 2, rabbit muscle actin; lanes 3, recombinant *Arabidopsis thaliana* actin; lane 4, recombinant *E. histolytica* profilin. (A) Coomassie-stained SDS/polyacrylamide gel. 1 μ g actin or 2 μ g bovine serum albumin was loaded per lane. (B) SDS polyacrylamide gel. 50 000 cpm ^{125} I-labeled profilin were loaded, electrophoresed, and detected by autoradiography. (C) Blot overlay experiment. The same amounts of protein as in A were loaded onto an SDS/PAGE gel, electrophoresed, and blotted onto nitrocellulose. The blots were incubated with ^{125} I-labeled recombinant *E. histolytica* profilin, and bound protein was detected by autoradiography.

profilin preparation was used to interact with the blot of actins and control protein. Fig. 4C shows the results. Both rabbit and *Arabidopsis* actin clearly bound to *E. histolytica* profilin, whereas no signal was observed for the control protein, bovine serum albumin.

DISCUSSION

In the human parasite *E. histolytica*, a major immune evasion mechanism, the capping and shedding of host antibodies bound to the amoebic surface, is dependent on cytoskeleton-based mechanisms. Profilin is a ubiquitous multifunctional component of the eukaryotic cytoskeleton, therefore we wanted to investigate its role in motility and immune evasion of *E. histolytica*.

A different aspect of profilin is its role as an allergenic protein in plant pollen from grasses, trees, and weeds (Valenta et al., 1991, 1992). Based on the theory that plant allergens may

be related with parasite antigens, which may have been the early IgE antigens, it has been shown that the IgE in serum from profilin-allergic patients (Duchêne et al., 1992) as well as some but not all amoebiasis patients (Binder, M., unpublished results) bound to *E. histolytica* profilin. In this study, a complete profilin cDNA was isolated. The deduced amino acid sequences of *E. histolytica* and plant profilins were rather closely related (Table 1). This unexpected sequence similarity pointed again to a relation of parasite antigens with allergens. So far, however, only few data exist about the relevance of IgE-based humoral immunity in amoebic disease.

When the natural profilin isolated by poly(L-proline) affinity chromatography from an *E. histolytica* lysate was examined by isoelectric focussing, two isoforms were detected, similar to the situation in *Physarum* and *Dictyostelium*. The cloned profilin clearly corresponded to the basic isoform. The acidic and basic isoforms of *Physarum* and *Dictyostelium* profilins were only 66% and 56% identical at the amino acid level. If the situation was similar for *E. histolytica*, this divergence could explain why only one set of signals was observed in the Southern blot. The final proof that the acidic poly(L-proline)-binding protein is also a profilin will require further molecular analysis.

Recombinant *E. histolytica* profilin was able to bind to plant and to mammalian actins and was able to take the place of more closely related plant profilins and more distantly related mammalian profilins. In their crystal structure of the bovine profilin-actin complex, Schutt et al. (1993) described 21 residues of profilin that make the contact to actin. Remarkably, only four of these residues were identical between the bovine and *E. histolytica* profilin sequences, whereas 17 of these 21 residues were conserved among all the mammalian profilins. Therefore, even under conditions of strong sequence divergence, cytoskeletal components from distantly related organisms can interact with each other.

We thank R. Meagher from the University of Georgia, Athens, for the gift of recombinant *A. thaliana* actin. This study was supported by grant P09256-MED from the Austrian Science Foundation, Vienna, Austria.

REFERENCES

- Bakker-Grunwald, T. & Wöstmann, C. (1993) *Entamoeba histolytica* as a model for the primitive eukaryotic cell, *Parasitol. Today* 9, 27–31.
- Binette, F., Benard, M., Laroche, A., Pierron, G., Lemieux, G. & Pal-lotta, D. (1990) Cell-specific expression of a profilin gene family, *DNA Cell. Biol.* 9, 323–334.
- Calderón, J., de Lourdes Muñoz, M. & Acosta, H. M. (1980) Surface redistribution and release of antibody-induced caps in *Entamoebae*, *J. Exp. Med.* 151, 184–193.
- Carlsson, L., Nyström, L.-E., Lindberg, U., Kannan, K. K., Cid-Dresdner, H., Lövgren, S. & Jörnvall, H. (1976) Crystallization of a non-muscle actin, *J. Mol. Biol.* 105, 353–366.
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease, *Biochemistry* 18, 5294–5299.
- Diamond, L. S., Harlow, D. R. & Cunick, C. C. (1978) A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*, *Trans. R. Soc. Trop. Med. Hyg.* 72, 431–432.
- Duchêne, M., Ortner, S., Wiedermann, G., Kraft, D., Scheiner, O. & Valenta, R. (1992) *Entamoeba histolytica* profilin binds serum IgE from pollen allergic patients, in *Molecular biology and immunology of allergens* (Kraft, D. & Schon, A., eds) 1st edn, pp. 173–175, CRC Press, Boca Raton.
- Goldschmidt-Clermont, P. J., Machesky, L. M., Baldassare, J. J. & Pollard, T. D. (1990) The actin-binding protein profilin binds to PIP₂ and inhibits its hydrolysis by phospholipase C, *Science* 247, 1575–1579.
- Goldschmidt-Clermont, P. J., Kim, J. W., Machesky, L. M., Rhee, S. G. & Pollard, T. D. (1991) Regulation of phospholipase C- γ 1 by profilin and tyrosine phosphorylation, *Science* 251, 1231–1233.
- Haugwitz, M., Noegel, A. A., Rieger, D., Lottspeich, F. & Schleicher, M. (1991) *Dictyostelium discoideum* contains two profilin isoforms that differ in structure and function, *J. Cell Sci.* 100, 481–489.
- Honoré, B., Madsen, P., Andersen, A. H. & Leffers, H. (1993) Cloning and expression of a novel human profilin variant, profilin II, *FEBS Lett.* 330, 151–155.
- Köhler, K., Kutchan, T. M. & Domdey, H. (1989) Specific oligodeoxynucleotide probes obtained through RNA sequencing, *DNA* 8, 143–147.
- Kunkel, T. A., Roberts, J. D. & Zakour, R. A. (1987) Rapid and efficient site-specific mutagenesis without phenotypic selection, *Methods Enzymol.* 154, 367–382.
- Kwiatkowski, D. J. & Bruns, G. A. P. (1988) Human profilin: molecular cloning, sequence comparison, and chromosomal analysis, *J. Biol. Chem.* 263, 5910–5915.
- Lind, S. E., Janmey, P. A., Chaponnier, C., Herbert, T.-J. & Stossel, T. P. (1987) Reversible binding of actin to gelsolin and profilin in human platelet extracts, *J. Cell. Biol.* 105, 833–842.
- Magdolen, V., Oechsner, U., Müller, G. & Bandlow, W. (1988) The intron-containing gene for yeast profilin (PFY) encodes a vital function, *Mol. Cell. Biol.* 8, 5108–5115.
- Mittermann, I., Swoboda, I., Pierson, E., Eller, N., Kraft, D., Valenta, R. & Heberle-Bors, E. (1995) Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): increased profilin expression during pollen maturation, *Plant Mol. Biol.* 27, 137–146.
- Ortner, S., Plaimauer, B., Binder, M., Wiedermann, G., Scheiner, O. & Duchêne, M. (1992) Humoral immune response against a 70-kilodalton heat shock protein of *Entamoeba histolytica* in a group of patients with invasive amoebiasis, *Mol. Biochem. Parasitol.* 54, 175–183.
- Plaimauer, B., Ortner, S., Wiedermann, G., Scheiner, O. & Duchêne, M. (1993) Molecular characterization of the cDNA coding for translation elongation factor-2 of pathogenic *Entamoeba histolytica*, *DNA Cell Biol.* 12, 87–94.
- Pollard, T. D. & Rimm, D. L. (1991) Analysis of cDNA clones for *Acanthamoeba* profilin-I and profilin-II shows end to end homology with vertebrate profilins and a small family of profilin genes, *Cell Motil. Cytoskeleton* 20, 169–177.
- Pollard, T. D. & Quirk, S. (1994) Profilins, ancient actin binding proteins with highly divergent primary structures, *Soc. Gen. Physiol. Ser.* 49, 117–128.
- Sabanero-Lopez, M. & Segura-Nieto, M. (1990) Proteínas asociadas a la actina: La profilina en los trofozoitos de *Entamoeba histolytica*, *Arch. Invest. Med.* 21, 191–195.
- Sambrook, J. F., Maniatis, T. & Fritsch, E. F. (1989) *Molecular cloning: a laboratory manual*, 2nd edn, Cold Spring Harbor Laboratory, Cold Spring Harbor NY.
- Schutt, C. E., Myslik, J. C., Rozycki, M. D., Goonesekere, N. C. W. & Lindberg, U. (1993) The structure of crystalline profilin- β -actin, *Nature* 365, 810–816.
- Short, J. M., Fernandez, J. M., Sorge, J. A. & Huse, W. D. (1988) λ ZAP: a bacteriophage λ expression vector with *in vivo* excision properties, *Nucleic Acids Res.* 16, 7583–7600.
- Sohn, R. H. & Goldschmidt-Clermont, P. J. (1994) Profilin: at the crossroads of signal transduction and the actin cytoskeleton, *Bioessays* 16, 465–472.
- Tabor, S. & Richardson, C. C. (1985) A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes, *Proc. Natl Acad. Sci. USA* 82, 1074–1078.
- Theriot, J. A. & Mitchison, T. J. (1993) The three faces of profilin, *Cell* 75, 835–838.
- Tilney, L. G., Bonder, E. M., Coluccio, L. M. & Mooseker, M. S. (1983) Actin from *Thyone* sperm assembles on only one end of an actin filament: a behaviour regulated by profilin, *J. Cell. Biol.* 97, 112–124.
- Torres-Guerrero, H., Peattie, D. A. & Meza, I. (1991) Chromatin organization in *Entamoeba histolytica*, *Mol. Biochem. Parasitol.* 45, 121–130.
- Valenta, R., Duchêne, M., Pettenburger, K., Sillaber, C., Valent, P., Bettelheim, P., Breitenbach, M., Rumpold, H., Kraft, D. & Scheiner,

- O. (1991) Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals, *Science* 253, 557–560.
- Valenta, R., Duchêne, M., Ebner, C., Valent, P., Sillaber, C., Deviller, P., Ferreira, F., Tejkl, M., Edelman, H., Kraft, D. & Scheiner, O. (1992) Profilins constitute a novel family of plant pan-allergens, *J. Exp. Med.* 175, 377–385.
- Valenta, R., Ferreira, F., Grote, M., Swoboda, I., Vrtala, S., Duchêne, M., Deviller, P., Meagher, R. B., McKinney, E., Heberle-Bors, E., Kraft, D. & Scheiner, O. (1993) Identification of profilin as an actin-binding protein in higher plants, *J. Biol. Chem.* 268, 22777–22781.
- Wallace, R. B., Shaffer, J., Murphy, R. F., Bonner, J., Hirose, T. & Itakura, K. (1979) Hybridization of synthetic oligodeoxyribonucleotides to Φ X174 DNA: the effect of single base pair mismatch, *Nucleic Acids Res.* 6, 3543–3557.
- Walsh, J. A. (1986) Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality, *Rev. Infect. Dis.* 8, 228–238.