An actin-related protein from *Dictyostelium discoideum* is developmentally regulated and associated with mitochondria

Irene Murgia**, Sutherland K. Maciver, Piero Morandini*,**

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

Received 19 December 1994; revised version received 30 January 1995

Abstract An actin-related protein (ACLA) has been identified in the cellular slime mould *Dictyostelium discoideum*. The complete cDNA sequence indicates that within the actin superfamily it belongs to the ARP3 family of actin-related proteins together with *Arp66B* from *Drosophila melanogaster*, *Actin2* from *Bos taurus*, *act2* from *Schizosaccharomyces pombe* and possibly *act2* from *Caenorhabditis elegans*. The ACLA mRNA is regulated during development, showing a maximum between 2 and 4 h after starvation. The protein has been expressed in *E. coli* and antibodies raised against it. Immunofluorescence microscopy shows that ACLA protein co-localises with mitochondria; the protein co-purifies with *Dictyostelium* mitochondria.

Key words: Actin-like protein; cDNA sequence; Northern blot analysis; Immunofluorescence; ARP3; Mitochondrion

1. Introduction

Actin is a very abundant, highly conserved protein which polymerises to form filaments [36]. A great number of accessory proteins (ABP) interact either with filamentous or with monomeric actin (for a review see [1]). Severing, capping, crosslinking, bundling, nucleating and generation of mechanical force are some of the functions performed by these proteins. Because it plays a central role in cell organisation it is not surprising that actin is very well conserved among the eukaryotic organisms: any pair of actins is 70–100% identical with little variation in length (374–376 residues). The high degree of conservation is reflected at the functional level: rabbit actin and *Tetrahymena* actin can co-polymerise to form functional filaments [2].

However, an increasing number of proteins are being discovered with a loose similarity to actin (30–50% identity) and much more variability in length [3–12]. These proteins have been termed ARPs (actin related proteins). Several distinct ARPs exist within one species, so ARPs have recently been classified into three groups [12]. From the sequences available to date, it seems that specific ARPs are more conserved among different species than actin and ARPs within the same species (e.g. canine and human centractin are more than 92% identical while human actin and centractin are only 53% identical [7]). Rather than being true actins, that is functionally performing all the roles of actin, and because of their restricted localisation (e.g. centractin) or expression, they are regarded as proteins with novel functions.

Here we report a new member of the ARP3 family, its cellular localisation and developmental regulation in the cellular slime mould *Dictyostelium discoideum*.

2. Materials and methods

2.1. General

Genomic DNA from strain AX2 was prepared essentially as described [13]; the cDNA library (strain AX3 early aggregation stage) was purchased from Clontech (Palo Alto, CA, USA) and amplified once before use. Standard cloning techniques were as described [14].

2.2. PCR amplifications

Amplifications were performed using a PCR II machine (Techne, Cambridge, UK) equipped with a water cooling unit. *Taq* polymerase was purchased from Promega (Madison, WI, USA) and the primers synthesised by T. Smith (LMB, Cambridge, UK). Reactions contained 1 μM primer, 200 μM dNTPs, manufacturer's buffer at 1 × concentration and 2 U of *Taq* polymerase in a total volume of 50 μl. Samples were heated at 94°C for 5 min, then held at 85°C for 10 min while adding the polymerase ('hot start' technique).

2.2.1. Amplification from genomic DNA. *Dictyostelium discoideum* (strain AX2) genomic DNA (0.3 μg/reaction) was cycled 30 times using primers L2P1 (5′-CGCTGCAGCAIAT(A/T)C(G/T)(A/T)GGICC-3′) and L2P2 (5′-GCGAATTCGGI(A/C)G(A/T)GA(C/T)ACIACIC-3′). Annealing temperature was 53°C except for the first two cycles which were done at 37°C. PCR products were analysed by agarose gel-electrophoresis and bands of interest were gel-purified and re-amplified in order to obtain enough material for subsequent experiments.

2.2.2. Amplification of λgt11 inserts. Positive plaques were touched with a toothpick and adhering phages dispersed in 100 μl of distilled water. Samples were heated at 95°C for 3 min and 5–10 μl used for a PCR reaction. Primers (Catalogue #1218, #1222) were from New England Biolabs (Beverly, MA, USA).

In order to express the protein, suitable sites were engineered into the coding region by PCR amplification primers: 5′-ATGGGATCCAATCCAGCATCAGGTTTACCAGCA-3′, annealing just after the ATG initiation codon of the *aclA* cDNA, and 5′-CCGGTACCAAGCTTACTAATTGATACCACCAATGACAGTGTTAAA-3′, annealing around the stop codon. This PCR product was then ligated into vector pMW172 [37] and expressed in *E. coli* strain BL21(DE3) [16].

2.3. Sequencing

PCR products were sequenced using the Circumvent kit (New England Biolabs, Beverly, MA, USA) after purification from low melting point agarose. Phagemid ssDNA was purified as described [14] and sequenced using Sequenase (USB, Cleveland, OH, USA). Every base was confirmed by sequencing from at least two different clones derived independently from the library.

2.4. Library screening and Northern analysis

Probe preparation for library screening and northern analysis was as follows: 50 ng of a *Pvu*II–*Eco*RI fragment of the PCR product obtained

*Corresponding author. **Present address*: Dipartimento di Biologia 'L. Gorini', Sezione di Fisiologia e Biochimica delle Piante, Via Celoria 26, 20133 Milano, Italy. Fax: (39) (2) 2660 4399.
E-mail: pm1@imiucca.csi.unimi.it

The sequence reported in this paper has been deposited at EMBL (accession number Z46418).

Abbreviations: ARP, actin related protein; ABP, actin binding protein.

SSDI 0014-5793(95)00111-5

from genomic DNA was incubated in a PCR reaction with a single primer (L2P2) and cycled 20 times for 20 s each at 92, 53 and 72°C. Radiolabelled [α-^{32}P]dATP (5 μl at 800 Ci/mmol in 50 μl total volume) was used instead of the corresponding unlabelled nucleotide. Screening was according to [14]. RNA preparation, gel-electrophoresis and hybridisation were as described [15].

2.5. Cell growth and development

Dictyostelium cells were grown and developed as described [46]. Strain AX2 was used throughout this study.

2.6. Protein expression and purification

The coding region was amplified with two primers (see above), annealing, respectively, to the initiation and the stop codon, bearing adequate sites for cloning, restricted and inserted into pMW172 vector [37]. Expression in *E. coli* was induced with IPTG and the protein was found in inclusion bodies, and purified as in [39]. This material was used to raise antisera in rabbits.

2.7. Isolation of mitochondria

Dictyostelium mitochondria were isolated by the method as described in [44] with modifications [43]. Briefly, AX2 cells were grown in 1 l flask [46], collected by centrifugation, washed in chilled deionised water and resuspended as a slurry in 0.25 M mannitol, 20 mM Tris-HCl, pH 7.6, and 4 mM EDTA with protease inhibitors. The cell pellet was thoroughly homogenised on ice, diluted in the same buffer, and centrifuged at 3000 × *g* for 2 min at 4°C. The supernatant was saved and the pellet re-homogenised and centrifuged again. The two supernatants were pooled and centrifuged at 750 × *g* for 10 min. This pellet was discarded. Supernatant was then centrifuged at 7700 × *g* for 15 min. This mitochondrial pellet was washed in the same buffer and then treated with digitonin as previously described [43].

2.8. Immunostaining

Dictyostelium amoebae were settled at 22°C on coverslips coated with a 2 mg/ml solution of poly-L-lysine for 15 min. Adherent cells were then fixed in methanol/ethanol (1:1) at 4°C for 10 min, ethanol/acetic acid (19:1) at −20°C for 10 min, and finally washed in methanol at −20°C for 10 min. Non-specific sites were blocked with 1% BSA (fraction V) in PBS for 15 min and washed in PBS. The cells were incubated with rabbit anti-ACLA serum (diluted 1:200) for 1 h at room temperature. Coverslips were then washed and incubated with goat anti-rabbit FITC at 1:40 dilution. The specificity of the antibody was also checked on a Western blot.

3. Results

3.1. aclA cloning

*Dictyostelium discoideum acl*A was cloned serendipitously. We were interested in cloning a hydroxylating enzyme from *Dictyostelium*, the biochemical properties of which were very reminiscent of P-450s. To this purpose, several primers were designed to amplify genes with homology to cytochrome P-450s.

Two of these primers, specific for family 52, generated a prominent band of the expected size (around 500 bp) when used with *Dictyostelium* genomic DNA. Direct sequencing of the PCR product revealed an open reading frame with no homology to P-450s but some similarity to actin; moreover, the reading frame was in a direction opposite to what we expected from primer design.

Dictyostelium is known to possess at least 17 actin genes, even though not all of them are equally transcriptionally active. Out of 15 sequenced so far, all but three encode the same protein [17], which is the only one detected in vegetative amoebae [18]. The other three are at least 88% similar. The amplified fragment (nucleotides 495–930 of Fig. 1) had roughly the same degree of identity (30%) to several actin genes, from *Dictyostelium* to maize, suggesting that it belonged to a new, highly divergent, actin rather than one of the several *Dictyostelium* genes for 'canonical' actin.

In order to obtain the full sequence of the gene, we screened a λgt11 cDNA library and found several positive clones. The inserts from seven of them were amplified: insert size was variable (0.6–1.4 kb), but the restriction pattern with *Hin*fI suggested that they had common fragments (not shown). Cloning and sequencing of five inserts confirmed that they were clones deriving from the same gene. The whole sequence is presented in Fig. 1. A long ORF spanning from nucleotide 17 to 1270 was found. The first ATG matched closely the consensus sequence for *Dictyostelium*. Poly(A) tails of variable length were found after nucleotide 1362 in all the clones examined.

Database search using the whole cDNA sequence showed similarity to all actins (Fig. 2) but the highest similarities were to bovine ACT2 (69%), *Drosophila melanogaster* actr66B (68%), *act2* from *S. pombe* (64% identity), and an actin-related protein fragment from *Caenorhabditis elegans*. All other actins gave lower scores (37–39%) regardless of the organism. Also the newly discovered centractin (Actin RPV) was no more similar to ACLA than muscle actin.

Not only the overall similarity but also the pattern of insertions suggest more relatedness among these first 5 actin-related proteins (see Fig. 2, insertions at residues 50, 147, 232, 323 and the C-terminus).

We have compared several ARPs with *acl*A using Clustal W [42] (Fig. 3). The analysis indicates that the ACLA protein belongs to the ARP3 group [12], and that proteins of this group, although being clearly related, are not as conserved across phyla as those of the actin group. Our results are in good agreement with a previous analysis [12].

3.2. Developmental regulation

A Northern blot analysis was performed to investigate mRNA levels during development: single amoeba aggregate to form a multicellular mass that undergoes morphogenesis and differentiation, eventually producing a fruiting body. This is composed of a dead stalk supporting a droplet of spores. Several genes encoding cytoskeletal proteins are regulated during development and some of them are strongly induced soon after starvation (e.g. actin [19], α-actinin [20], ponticulin [21]). The result of the analysis is presented in Fig. 4: *acl*A message level increases soon after starvation (the signal initiating development), reaching a maximum between 2 and 4 h, and steadily decreasing thereafter. *Dictyostelium* actin message behaves similarly [19]. Possibly a second, smaller peak is observed at the end of development. The blot was also hybridised with two probes corresponding to the developmentally regulated genes *ecm*A and *ecm*B [15]. They were used as markers to determine the size of the *acl*A message, estimated at around 1.5–1.6 kb, which is very close to the size of the longest cDNA clone (1.36 kb). The poly(A) tail and a slightly longer 5′ untranslated region could account for the difference.

3.3. Cellular localisation

In order to investigate further the function and the localisation of this protein, we first expressed the cDNA in *E. coli*. Using a pET based vector [16], pMW172 [46], we obtained high levels of expression (up to 50 mg/l of culture). Using the purified protein produced in *E. coli*, we immunised rabbits to obtain antisera. Staining of vegetative amoebae (see Fig. 5), shows a

```
                M  N  P  A  S  G  L  P  A  V  V  I  D  N  G  T  G  Y  T  K  M  G  Y  A  G  N  N  D  P  S  F  I  I  P  T
GCAAGACAAATACAAAATGAATCCAGCATCAGGTTTACCAGCAGTCGTTATCGATAATGGTACAGGTTACACTAAAATGGGTTACGCAGGTAATAATGACCCAAGTTTCATTATTCCAAC
      10        20        30        40        50        60        70        80        90       100       110       120

   T  I  A  T  Q  S  S  K  G  K  Q  T  A  A  S  Q  K  K  G  V  E  D  L  D  F  F  I  G  D  E  A  I  A  N  S  K  T  Y  D  M
CACAATTGCAACTCAATCATCAAAAGGTAAACAAACAGCAGCCTCACAAAAGAAAGGTGTTGAAGATTTAGATTTCTTTATTGGTGATGAAGCTATTGCCAACTCTAAAACTTATGATAT
     130       140       150       160       170       180       190       200       210       220       230       240

   T  N  P  V  K  H  G  Q  I  E  N  W  T  H  M  E  Q  Y  W  E  H  C  V  F  K  Y  L  R  C  E  P  E  D  H  Y  F  L  L  T  E
GACCAATCCAGTCAAACATGGTCAAATTGAAAATTGGACTCATATGGAACAATATTGGGAACATTGCGTATTTAAATATTTACGTTGTGAACCAGAAGATCACTACTTCTTATTGACTGA
     250       260       270       280       290       300       310       320       330       340       350       360

   P  P  L  N  A  P  E  N  R  E  F  T  A  E  I  M  F  E  T  F  N  V  P  G  L  Y  I  A  V  Q  A  V  L  A  L  A  A  S  W  T
ACCACCACTTAATGCACCAGAAAATCGTGAATTTACAGCAGAAATTATGTTTGAAACATTTAATGTACCAGGTTTATATATTGCCGTTCAAGCAGTATTAGCTCTTGCAGCCTCATGGAC
     370       380       390       400       410       420       430       440       450       460       470       480

   S  K  N  A  E  K  T  L  T  G  T  V  I  D  S  G  D  G  V  T  H  V  I  P  I  S  E  G  Y  V  I  G  S  S  I  K  H  I  P  I
CAGTAAGAATGCAGAAAAGACCTTAACTGGTACAGTTATTGATAGTGGTGATGGTGTAACTCATGTTATTCCAATTTCTGAAGGTTATGTTATTGGTAGTTCAATTAAACATATTCCAAT
     490       500       510       520       530       540       550       560       570       580       590       600

   A  G  R  D  I  S  S  Y  V  Q  Q  I  M  R  E  R  E  P  N  I  P  P  A  E  S  L  E  I  A  K  R  V  K  E  Q  Y  S  Y  V  C
TGCCGGTAGAGATATTTCATCATACGTTCAACAAATCATGAGAGAACGTGAACCAAACATTCCACCAGCTGAATCATTGGAAATCGCCAAACGTGTCAAAGAACAATACAGTTACGTTTG
     610       620       630       640       650       660       670       680       690       700       710       720

   P  D  I  V  K  E  F  G  K  Y  D  S  E  P  D  K  W  I  K  T  I  N  A  Q  D  S  V  T  K  K  P  F  S  Y  D  V  G  Y  E  R
TCCAGATATTGTCAAAGAGTTTGGTAAATACGATAGTGAACCAGACAAATGGATTAAAACCATCAACGCTCAAGACTCTGTCACCAAGAAACCATTCAGCTACGATGTTGGTTACGAACG
     730       740       750       760       770       780       790       800       810       820       830       840

   F  L  G  P  E  L  F  F  N  P  E  I  A  S  S  D  Y  L  T  P  L  P  K  V  V  D  D  T  I  Q  S  C  P  I  D  C  R  R  G  L
TTTCTTAGGCCCAGAATTATTCTTCAATCCAGAGATTGCCTCCTCCGACTACCTCACCCCATTACCAAAGGTTGTCGACGATACCATTCAATCCTGTCCAATCGATTGTCGTCGTGGTCT
     850       860       870       880       890       900       910       920       930       940       950       960

   Y  K  N  I  V  L  S  G  G  S  T  M  F  K  D  F  G  K  R  L  Q  R  D  V  K  R  S  V  D  Y  R  I  K  R  S  E  E  L  S  G
CTACAAGAATATCGTCTTATCTGGTGGTTCAACCATGTTTAAAGATTTTGGTAAACGTCTTCAACGTGATGTCAAACGTTCTGTCGATTACCGTATCAAACGTTCCGAAGAATTATCTGG
     970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080

   G  K  I  K  A  V  P  L  A  V  N  V  I  S  H  N  M  Q  R  Y  A  V  W  F  G  G  S  M  L  A  S  T  P  E  F  Y  N  V  C  H
TGGTAAAATTAAAGCTGTCCCATTGGCCGTCAACGTTATCTCTCACAATATGCAACGTTATGCCGTATGGTTTGGTGGTTCTATGTTGGCTTCAACTCCAGAGTTTTACAATGTCTGTCA
     1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200

   T  K  A  Q  Y  D  E  I  G  P  S  I  C  R  F  N  T  V  I  G  G  I  N  *
TACCAAAGCTCAATATGATGAAATCGGTCCATCCATTTGCAGATTTAACACTGTCATTGGTGGTATCAATTAAATAGGTTTTTCTATAAATAATCAAAAAAATATTTTTCCGATTGAAAT
     1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320

AATTAATACTCTAACTTCATTATTAGTTTATTATTACCTATC
     1330      1340      1350      1360
```

Fig. 1. Nucleotide and predicted amino acid sequence of *acl*A.

punctate together with some diffuse cytoplasmic staining. The punctate foci are likely to correspond to mitochondria, because of the dimension and number: counterstaining with DAPI supports this hypothesis. Moreover, a mitochondrial pellet prepared from vegetative amoebae contained ACLA, as judged by Western blot. Some cytosolic ACLA was also detected (not shown).

4. Discussion

We present the cloning and the initial characterisation of an actin-related protein from *Dictyostelium discoideum*. A growing number of such proteins are being discovered in diverse organisms, so that now, together with actin, they are collectively known as the actin superfamily. Analysis of the amino acid sequence of the ACLA protein indicates that it belongs to the ARP3 family (Fig. 3), a distinct group within the actin superfamily [12], because of the comparatively high similarity among these proteins [3,6,9]. There are, however, differences within this group beyond single amino acid changes (size of the insertions at residue 50, a potential protein kinase C phosphorylation site present only in the protein from bovine [6]; see Fig. 2). Further studies will be required to ascertain whether they perform similar (or exchangeable) roles in vivo.

Codon usage indicates that the *Dictyostelium* gene is poorly expressed, since it uses many rare codons [22]. This has been noted already for the fission yeast homologue; the bovine and the *Drosophila* homologues also seem to be poorly expressed.

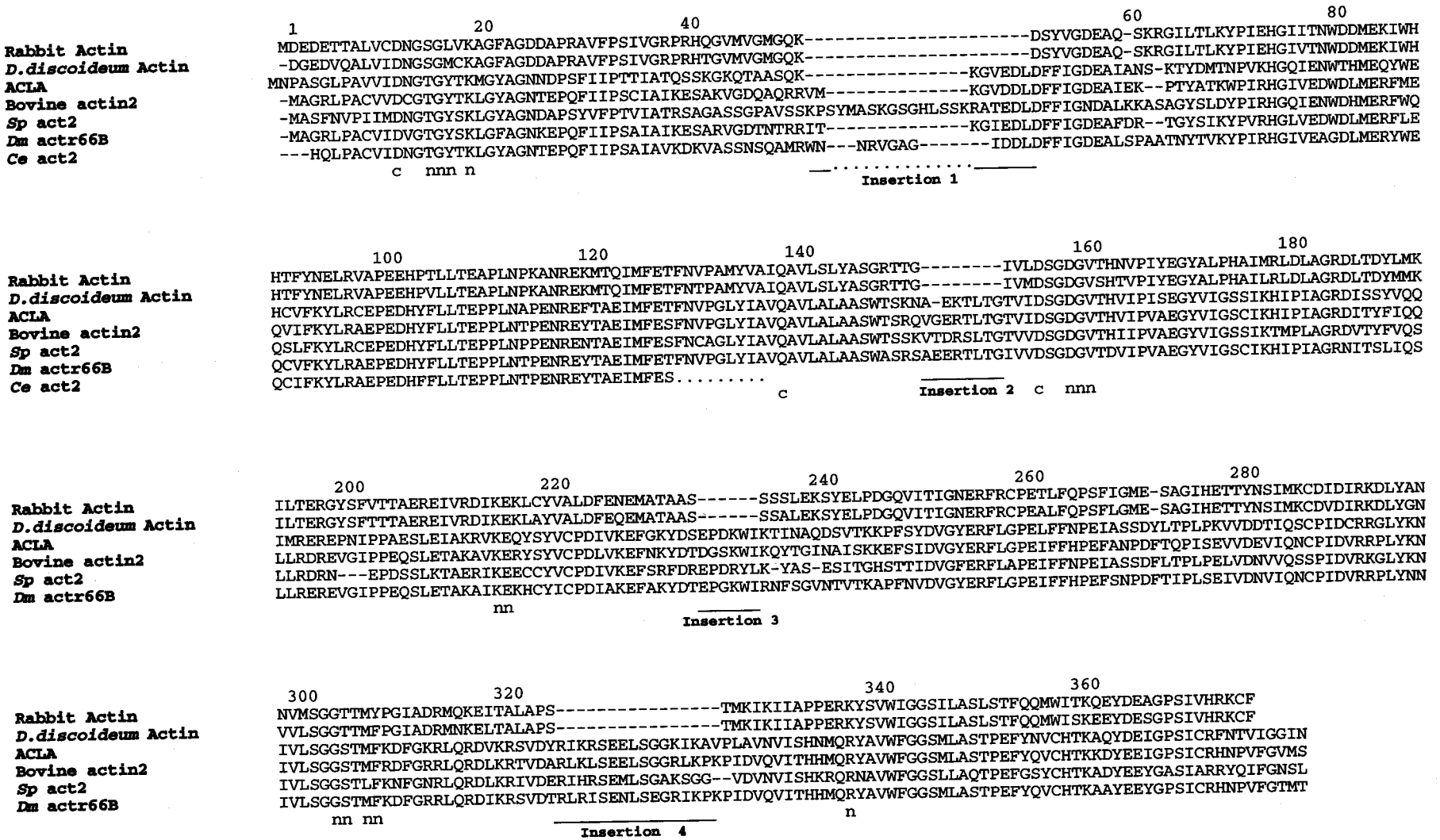

Fig. 2. Comparision of the predicted ACLA amino acid sequence with other ARPs and some 'canonical' actins. Numbering is according to rabbit muscle actin as in Fig. 1 of [23]. Sequences were aligned with the program Clustal W [42]. Residues important for Ca^{2+} or for nucleotide binding are indicated above the *acl*A sequence with 'c' and 'n' respectively. Sequences are from GenBank: rabbit actin [23] (P02568); *Dictyostelium discoideum* (P02577); ACLA (Z46418); bovine actin2 (D12816); Sp act2, *Schizosaccharomyces pombe act2* (M81068); Dm actr66B, *Drosophila act2* (X71789); Ce act2, *Caenorhabditis elegans act2* fragment (CE2011).

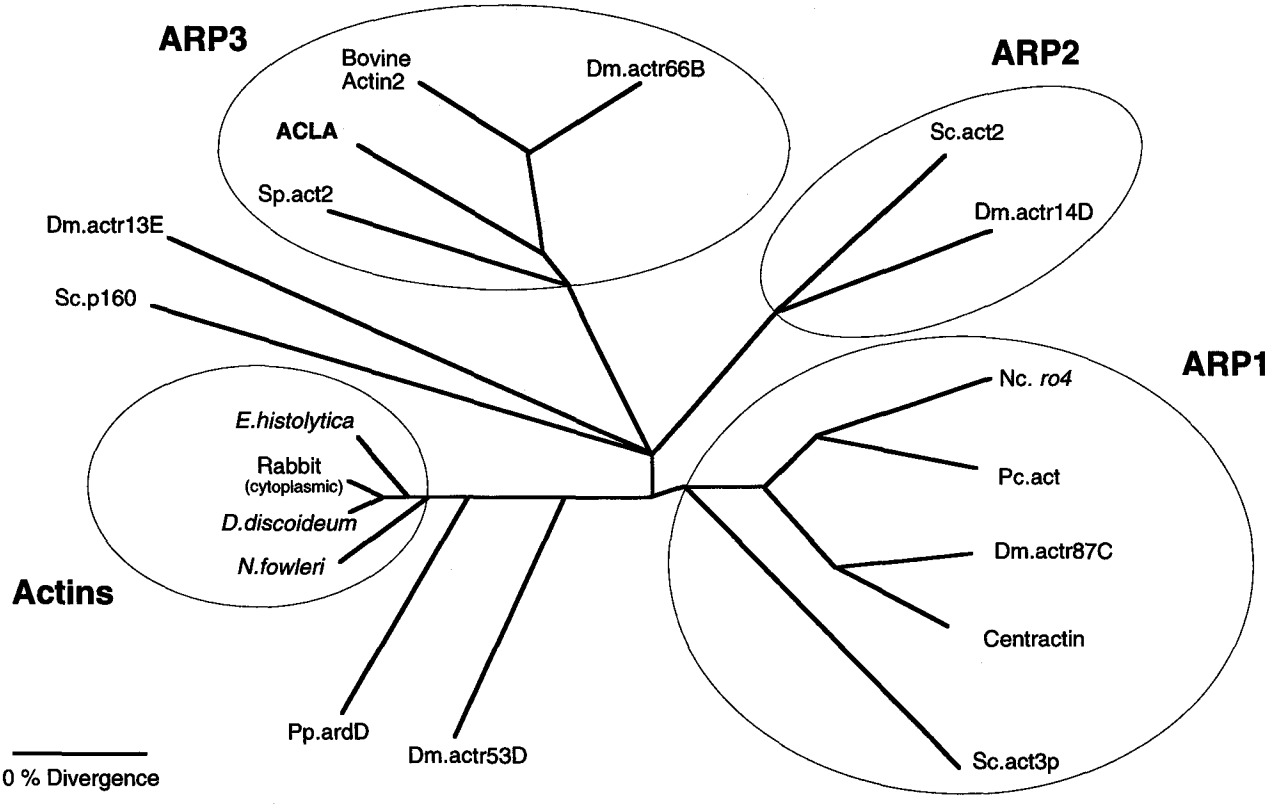

Fig. 3. Non-rooted phylogenetic 'tree' comparing the amino acid sequences of actin-related proteins and a number of actins from diverse phyla. The tree was generated by the computer program Clustal W [42]. Sequences are from GenBank: rabbit non-muscle γ actin (X60733), *Entamoeba histolytica* actin (M16339), *Naegleria fowleri* actin (M90311), Pp. ardD *Physarum polcephalum* (M59234), Centractin from dog (S45367), Pc.act *Pneumocystis carinii* actin II (L21184), Nc. *ro4 Neurospora crassa* centractin (L31505). Sequences beginning with 'Sc' are from *Saccharomyces cerevisiae*: Sc.act2 (X61502), Sc.act3p (X79811), Sc.p160 (X75317). Sequences beginning with 'Dm' are from *Drosophila melanogaster*: Dm.actr13E (L25314), Dm.actr14D (X78486), Dm.actr53D (X78487), Dm.actr87C (X78488). Other sequences are as in Fig. 2.

It is supposed that the folding pattern of ARPs is similar to that of rabbit muscle actin [40], the structure of which has been recently solved [23]. Indeed, almost all the residues involved in binding Ca^{2+} and ATP/ADP, which are located in a deep cleft inside the protein, seem to be retained in ACLA and in the other ARP3 proteins (see Fig 2, residues labelled with 'c' and 'n'), with only 5 residues being replaced by similar amino acids: $Ser^{14}Thr$, $Leu^{16}Tyr$, $Thr^{303}Ser$, $Tyr^{306}Phe$ and $Lys^{336}Arg$ [23]. Moreover, many of the differences in the sequence, and especially insertions, are in regions that should be on the surface (insertions at residue 50, 147, 232, 323 and the C-terminus; changes at the N-terminus, at residues 37–50, 59–66, 87–93, 197–206) and primarily in loops connecting β-strands or α-helices, i.e. in regions exposed to the solvent and less organised. Residues facing the inner cleft appear to be much more conserved, regardless of whether they occur in α-helices, β-strands or connecting loops. One can thus argue that these actin-related proteins have an 'actin' core retaining Ca^{2+} and nucleotide binding, but a surface which interacts with a set of proteins (at least in part) different from that of actin; for instance, residues important for filament formation [24–26] are not very conserved; if any are conserved, the interacting counterpart is not, suggesting that ACLA should not be able to polymerize either alone or together with actin. Our immunofluorescence picture does not show any evidence for filament formation, nor could we detect filament formation in purified recombinant protein preparations (data not shown), although such evidence is not sufficient to settle the question.

Conservation of sites for specific actin binding proteins is also weak: for example, the apolar patch of actin that binds gelsolin is partially retained, while most of the hydrogen bonds bordering this apolar patch are lost [27]. Stretches thought to be important for interaction with myosin [23–25,28] are heterogeneous, also within the ARP3 family, with some very conserved areas with respect to actin and some interrupted by insertions. The region that binds proteins of the profilin family [29,30] is fairly conserved, even though it is exposed to the solvent. Recently, a complex containing an ARP from *Acanthamoeba castellanii* has been shown to bind profilin [31]. The limited peptide sequence available indicate that it may belong to the ARP3 family, yet it has been localised to the cortex and filopodia of the amoebae. Interestingly, antibodies raised to this ARP recognised proteins of similar size in *Dictyostelium* [31].

A clue about function is suggested by the immunofluorescence: ACLA may be involved in moving or anchoring mitochondria. Another ARP (centractin) has been shown to be part of the dynactin complex, responsible for activation of dynein-mediated organelle transport [7,32], and shows both punctate (centrosome) and diffuse cytoplasmic staining [7]. If ACLA has a redundant function in organelle movement in *Dictyostelium*, it might be difficult to understand contribution of specific proteins to the process, as occurs for the cytoskeletal dynamics

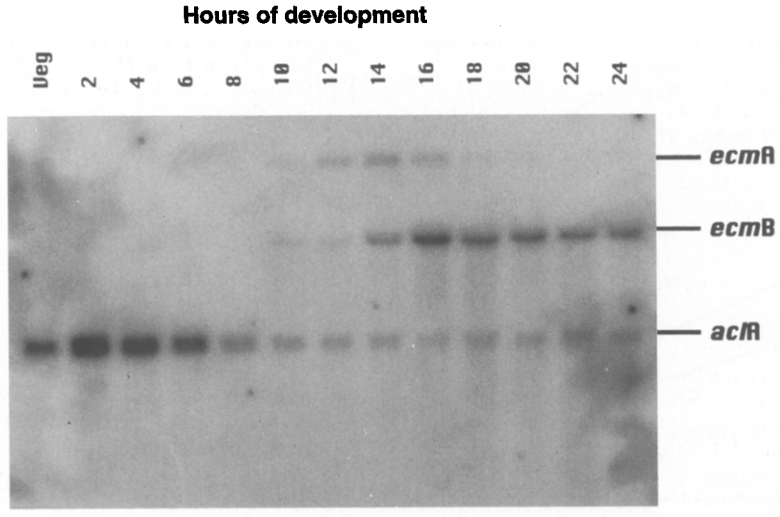

Fig. 4. Northern blot analysis of *acl*A. *Dictyostelium* amoebae were starved and developed on buffered agar. Aliquots of cells were taken every 2 h until completion of development, and frozen on dry ice. RNA extraction was performed after all the samples had been collected. Hybridization of the blotted RNA was done first with an *acl*A probe (see section 2) and subsequently with the combined *ecm*A and *ecm*B probe.

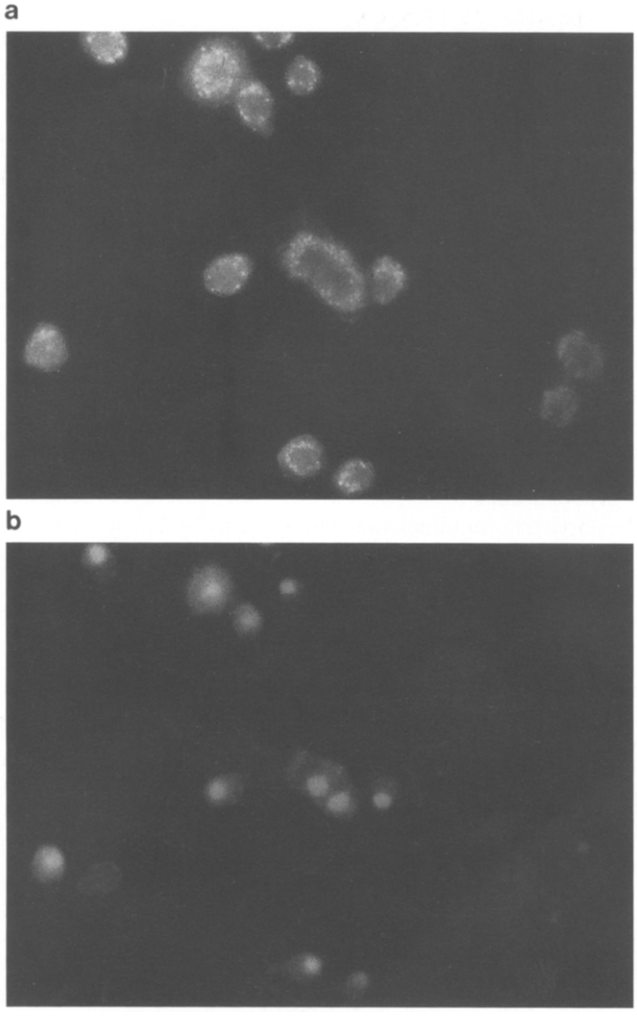

Fig. 5. Localization of ACLA. Immunofluorescence (a) and DAPI staining (b) of vegetative amoebae. Pre-immune serum did not give staining.

[33,34]. The staining pattern observed with the anti-ACLA, however, differed from that observed for *Dictyostelium* porin. Porin was visualised in a 'doughnut'-like pattern, consistent with its outer membrane localisation [41]. ACLA may be an intra-mitochondrial protein (with functions unrelated to movement or anchorage) rather than a mitochondrial surface protein. In this connection, we note that an actin-related polypeptide has been detected in mammalian cells, the expression of which is inhibited by treatment with 'nonactin', a potassium ionophore which collapses energy-dependent import of cytoplasmically transcribed proteins into mitochondria [45]. It is possible therefore that this 60-kDa actin-related protein is a mammalian homologue of ACLA.

Extragenic suppression of lethal actin alleles [25] together with biochemical work might help to assign a function to specific residues or groups of residues: their conservation in *acl*A will offer clues about those ABPs still able to interact. *Dictyostelium*, together with baker's yeast, is the only organism were purification of mutant actins for biochemical analysis is possible [35]. Identification of this ARP and development of a purification procedure should help the understanding of its function.

Acknowledgements: We thank Rob R. Kay and Alan Weeds for continuous support and criticism, Terry Smith for synthesising the oligos, and Paul J. McLaughlin for help and advice on the actin structure and many hours spent at the computer. Many thanks also to Alberto Vianelli and Roberto Colombo for careful reading of the manuscript, and last but not least to all the people in our labs for their help and patience.

References

[1] Maciver, S.K. (1994) Encylcopedia of Molecular Biology, Blackwells, Oxford.
[2] Hirono, M., Tanaka, R. and Watanabe, Y. (1990) J. Biochem. (Tokyo) 107, 32–36.
[3] Lees-Miller, J.P., Henry, G. and Helfman, D.M. (1992) Proc. Natl. Acad. Sci. USA 89, 80–83.
[4] Lees-Miller, J.P., Helfman, D.M. and Schroer, T.A. (1992) Nature 359, 244–246.
[5] Schwob, E. and Martin, R.P. (1992) Nature 355, 179–182.

[6] Tanaka, T., Shibasaki, F., Ishikawa, M., Hirano, N., Sakai, R., Nishida, J., Takenawa, T. and Hirai, H. (1992) Biochem. Biophys. Res. Commun. 187, 1022–1028.
[7] Clark, S. and Meyer, D.I. (1992) Nature 359, 246–250.
[8] Frankel, S., Heintzelman, M.B., Artavanis-Tsakonas, S. and Mooseker, M.S. (1994) J. Mol. Biol. 235, 1351–1356.
[9] Fyrberg, C. and Fyrberg, E. (1993) Biochem. Genet. 31, 329–341.
[10] Fyrberg, C., Ryan, L., Kenton, M. and Fyrberg, E. (1994) J. Mol. Biol. 241, 498–503.
[11] Harata, M., Karvan, A. and Wintersberger, U. (1994) Proc. Natl. Acad. Sci. USA 91, 8258–8262.
[12] Muhua, L., Karpova, T.S. and Cooper, J.A. (1994) Cell 78, 669–679.
[13] Nellen, W., Datta, S., Reymond, C., Sivertsen, A., Mann, S., Crowley, T. and Firtel, R.A. (1987) in: Molecular Biology in *Dictyostelium*: Tools and Applications. Methods in Cell Biology (Spudich, J.A. ed.) pp. 67–100, Academic Press, Orlando, FL.
[14] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.
[15] Berks, M. and Kay, R.R. (1990) Development 110, 977–984.
[16] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorf, J.W. (1990) Methods Enzymol. 185, 60–89.
[17] Romans, P. and Firtel, R.A. (1985) J. Mol. Biol. 186, 321–335.
[18] Vandekerckhove, J. and Weber, K. (1980) Nature 284, 475–477.
[19] Romans, P., Firtel, R.A. and Saxe III, C.L. (1985) J. Mol. Biol. 186, 337–355.
[20] Witke, W., Schleicher, M., Lottspeich, F. and Noegel, A. (1986) J. Cell Biol. 103, 969–975.
[21] Luna, E.J., Wuestehube, L.J., Chia, C.P., Shariff, A., Hitt, A.L. and Ingalls, H.M. (1990) Dev. Genet. 11, 354–361.
[22] Wada, K., Wada, Y., Ishibashi, F., Gojobori, T. and Ikemura, T. (1992) Nucl. Acids Res. 20 (Suppl.), 2111–2118.
[23] Kabsch, W., Mannherz, H.G., Suck, D., Pai, E.F. and Holmes, K.C. (1990) Nature 347, 37–44.
[24] Holmes, K., Popp, D., Gebhard, W. and Kabsch, W. (1990) Nature 347, 44–48.
[25] Wertman, K.F., Drubin, D.G. and Botstein, D. (1992) Genetics 132, 337–350.
[26] Wertman, K.F. and Drubin, D.G. (1992) Science 258, 759–760.
[27] McLaughlin, P.J., Gooch, J.T., Mannherz, H.G. and Weeds, A.G. (1993) Nature 364, 685–692.
[28] Milligan, A., Whittaker, M. and Safer, D. (1990) Nature 348, 217–221.
[29] Vandekerckhove, J.S., Kaiser, D.A. and Pollard, T.D. (1989) J. Cell Biol. 109, 619–626.
[30] Rozycki, M.D., Myslik, J.C., Schutt, C.E. and Lindberg, U. (1994) Curr. Biol. 6, 87–95.
[31] Machesky, L.M., Atkinson, S.J., Ampe, C., Vandekerckhove, J. and Pollard, T.D.(1994) J. Cell Biol. 127, 107–115
[32] Paschal, B.M., Holzbaur, E.L.F., Pfister, K.K., Clark, S., Meyer, D.I. and Vallee, R.B. (1993) J. Biol. Chem. 268, 15318–15323.
[33] Witke, W., Schleicher, M. and Noegel, A.A. (1992) Cell 68, 53–62.
[34] Gerisch, G., Noegel, A.A. and Schleicher, M. (1991) Annu. Rev. Physiol. 53, 607–628.
[35] Sutoh, K., Ando, M., Sutoh, K. and Toyoshima, Y.Y. (1991) Proc. Natl. Acad. Sci. USA 88, 7711–7714.
[36] Sheterline, P. and Sparrow, J.C. (1994) Protein Profile 1, 1–121.
[37] Way, M., Pope, B., Gooch, J., Hawkins, M. and Weeds, A.G. (1990) EMBO J. 9, 4103–4109.
[38] Waterston, R., et al. (1992) Nature Genet. 1, 114–123.
[39] Way, M., Pope, B. and Weeds, A.G. (1992) J.Cell Biol. 116, 1135–1143.
[40] Clark, S.W. and Meyer, D.I. (1993) Current Biology 3, 54–55.
[41] Troll, H., Malchow, D, Muller-Taubenberger, A., Humbell, B., Lottspeich, F., Ecke, M., Gerisch, G., Schmid, A. and Benz, R. (1992) J. Biol. Chem. 267, 21072–21079.
[42] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) Nucl. Acids Res. 22, 4673–4680.
[43] Bisson, R., Schiavo, G. and Papini, E. (1985) Biochemistry 24, 7845–7852.
[44] Stuchell, R.N., Weinstein, B.I. and Beattie, D.S. (1975) J. Biol. Chem. 250, 570–576.
[45] Varma, M., Aebi, U., Fleming, J. and Leavitt, J. (1987) Expt. Cell Res. 173, 17–22.
[46] Watts, D.J. and Ashworth, J.M. (1970) Biochem. J. 119, 171–174.