Actin-related proteins in Anabaena spp. and Escherichia coli

Alma L. Guerrero-Barrera,¹ Claudia M. García-Cuellar,¹,4 José D. Villalba,¹
Magdalena Segura-Nieto,³ Carlos Gómez-Lojero,² Magda E. Reyes,¹
José M. Hernández,¹ Rosa M. García¹ and Mireya de la Garza¹

1,2 Departamento de
Biología Celular¹, and
Departamento de
Bioquímica², Centro de
Investigación y de
Estudios Avanzados del
IPN, Apartado Postal 14-740, México, DF 07000, Mexico
3 Departamento de
Ingeniería Genética,
Centro de Investigación y de
Estudios Avanzados del
IPN, Km 9.6,
Libramiento Norte,
Carretera Irapuato-León,
Irapuato, Gto, Mexico
4 División de Investigación
Básica, Instituto Nacional
de Cancerología, San
Fernando No. 22, Tlalpan
DF 14000, Mexico

Actin has been described in all eukaryotic cells as the major microfilament
cytoskeletal protein. Although prokaryotic cells do not have a cytoskeleton,
proteins related to the latter have been found in different prokaryotic species.
We have found prokaryotic actin-related proteins in the enterobacterium
Escherichia coli and in the cyanobacteria Anabaena cylindrica and Anabaena
variabilis. They were identified by the following criteria: (1) by cross-reaction
with a fluorescent conjugated anti-actin (rat-brain) mAb by Western blot
analysis (in total cellular extracts); (2) specific binding of acetone powder
and soluble cellular extracts to DNase I; and (3) specific binding of cells and total
cellular extracts to phalloidin. In E. coli, specific binding of phalloidin labelled
with rhodamine to cells was detected by spectrofluorometry. In total cellular
extracts, three bands of 60, 43 and 35 kDa were weakly recognized by the mAb
by Western blot analysis; this recognition increased when phalloidin was
added to the extracts. Furthermore, three polypeptides of 60 kDa were
isolated by binding to DNase I, showing pl values of 6.7, 6.65 and 6.6, less acidic
than all reported actin pl values. In A. cylindrica and A. variabilis,
specific binding of phalloidin labelled with rhodamine to cells was also detected
by spectrofluorometry. In total and soluble cellular extracts, the mAb recognized
two bands of 45 and 40 kDa by Western blot analysis, but only the first was
purified by binding to DNase I, and it showed three isoforms of pl values 6.8,
6.5 and 6.4. These results suggest the presence, in prokaryotes, of proteins with
similar biochemical characteristics to eukaryotic actin.

Keywords: actin and related proteins, cytoskeleton, Anabaena spp., Escherichia coli,
Entamoeba histolytica

INTRODUCTION

Actin is one of the most conserved proteins throughout
Rasheda & Sodja, 1983; Hightower & Meagher, 1986;
Meagher & MacLean, 1990). This protein, in association
with actin-binding proteins, has an important role in cell
motility and muscular contraction, contributing to cellular
shape (Drubin, 1990) and to vital functions like
phagocytosis, cytoplasmic flux (Heat, 1990), chromosomal
segregation, organelle transportation (Norris et al.,
1991) and signal transduction, amongst others (Janmey,
1994).

Recently, actin-related proteins have been described in
eukaryotic cells. They are proposed to be primitive actin
ancestors (Herman, 1993) which show low sequence
homology with actin (less than 50%). In some yeasts and
in the nuclei of vertebrate cells, these proteins apparently
participate in cytoskeleton rearrangement in mitosis
(Less-Miller et al., 1992; Schwob & Martin, 1992). In
addition, a possible evolutionary relationship between the
heat-shock proteins and actin has been proposed, since a
structural homology between the 40 kDa ATPase segment
of Hsc 70 (heat-shock cognate protein) and actin has been
found (Bork et al., 1992). A common ancestral
molecule for both proteins has been inferred (Kabsch
et al., 1990; Flaherty et al., 1991; Sánchez et al., 1994).

Although in prokaryotic cells a cytoskeleton has not been
described so far, different researchers have reported the

Abbreviations: CD, cytochalasin D; PARP, prokaryotic actin-related
proteins; TRITC, tetramethylrhodamine isothiocyanate.

Printed in Great Britain
presence of several structures and proteins associated with this organelle (Eda, 1977; Nakamura & Watanabe, 1978; Nakamura et al., 1978; Göbel et al., 1981; Sioud et al., 1987; Hiraga, 1993; Luktenhaus, 1993; Korolev et al., 1994). Functions proposed for prokaryotic actin-like proteins have included motility in the absence of locomotor appendages and maintenance of cell shape without a cell wall in Mycoplasma pneumoniae (Neimark, 1977). Actin-like proteins have been sought in different species of both eubacterial and archaeobacterial phyla (Minkoff & Damadian, 1976; Neimark, 1977; Beck et al., 1978; Nakamura et al., 1978; Göbel et al., 1981; Sioud et al., 1987; Barnett & Cunningham, 1992; Bork et al., 1992; Foster, 1993; Sánchez et al., 1994). Minkoff & Damadian (1976) proposed that the actin-like proteins account for cell swelling and contraction cycles accompanying potassium uptake.

Our group has been interested in learning if an ancestral molecular related to actin is present in enterobacteria (García-Cuáller et al., 1990) and cyanobacteria (Guerrero-Barrera et al., 1994), and elucidating its possible function in these types of cells. We used the method of Pardee & Spudich (1982) for actin purification in order to obtain the prokaryotic actin-related proteins (PARP). Furthermore, other strategies used to characterize actin were utilized, such as binding to phallolidin and to DNase I. The results, and the cross-reaction with a mAb able to recognize actin from different sources, such as skeletal muscle, Entamoeba histolytica (Manning-Cela et al., 1994) or bean (Pérez et al., 1994), and now the enterobacterium Escherichia coli and the cyanobacteria Anabaena cylindrica and Anabaena variabilis, suggest the presence of PARP in these two kinds of non-related and evolutionarily separated eubacteria.

**METHODS**

**Micro-organisms.** *Escherichia coli* K12 W3110 was donated by Dr Carmen Gómez (Universidad Nacional Autónoma de México) and the strains of *Anabaena cylindrica* and *Anabaena variabilis* were from the collection of the Biotechnology Department, CINVESTAV-México. *Entamoeba histolytica* HM-1:IMSS was from our collection and was cultured in TYI-S-33 medium (Diamond et al., 1978); only trophozoites were used, harvested after 48 h culture.

**Bacterial cell extracts.** All bacteria were grown with shaking at 150 r.p.m. The *E. coli* cells were grown to OD_600_ 0.6 in Luria–Bertani (LB) medium. *A. cylindrica* and *A. variabilis* were grown to stationary phase at 25 °C under 6000 lx for 15 d in BG-11 cyanobacterium medium (Castenholz, 1988). Cells were harvested by centrifugation at 36000 g (Sorvall microspin 12), washed twice with PBS (0.145 M NaCl, 0.15 M sodium phosphate; pH 7.4), centrifuged and resuspended in a protease inhibitor cocktail (for *E. coli*: 625 mM Tris/HCl, 5 mM dithiothreitol, 2 mM N-ethylmaleimide, 10 mM 6-hydroxymercaptobenzene and 2 μM leupeptin; for cyanobacteria: 0.0625 M Tris/HCl, 1 mM benzamidine and 5 mM ε-aminocapric acid; all the inhibitors were from Sigma). Cells were broken by 10 freezing and thawing cycles and centrifuged at 36000 g (soluble cellular extracts) or, in the case of extracts used for Western blotting, they were broken in the presence of a mixture (1:1) of the protease inhibitor cocktail and 2X sample buffer (Laemmli, 1970) by boiling for 1 min (total extracts) and centrifuging at 36000 g. The protein concentration was determined by the Bradford (1976) method.

**SDS-PAGE.** SDS-PAGE (10%, w/v, gel) was prepared according to Laemmli (1970). The proteins were separated by electrophoresis in a Mighty-Small II SE250 cell (Hoefler) at 100 V for 1.5 h. The gels were stained with Coomassie blue R250 (Sigma) and destained with water/methanol/acetic acid (5:4:1, by vol.).

**Two-dimensional gel electrophoresis.** The PARPs were first separated using isoelectric focusing according to O’Farrell (1975) with the SE250 Tube Gel Adapter for the Mighty-Small II SE250 cell (Hoefler). The pH gradient was obtained using amphotlies from Pharmacia, at 2.5% (v/v) final concentration (50% pH 5–8 and 50% pH 3–10). The second dimension was in 12.5% (w/v) SDS-PAGE by the Laemmli (1970) technique. Finally the gels were stained and destained as described above.

**Acetone powder preparation and polymerization of PARP.** The Pardee & Spudich (1982) procedure was followed to purify muscular actin and to obtain the PARP of *E. coli* K12 W3110.

**Western blotting.** The total cell extract proteins were separated by SDS-PAGE as described above and transferred to Immobilon-P membranes (Millipore) in Transphor electrophoresis unit TE 22 (Hoefler) at 253 mA for 1.5 h. The transfer buffer was 0.25 M monobasic and dibasic sodium phosphate, pH 6.5. An anti-actin (rat-brain) mAb from ascitic fluid was used as the first antibody at pH 7.4 and a goat anti-mouse antibody conjugated with peroxidase (1:4000) was the second one. The whole procedure was carried out at room temperature.

**PARP purification by binding to DNase I.** The method of Etho et al. (1990) with some modifications was followed, using DNase I and Sepharose 4B activated with CNBr (10 mg DNase I to 1 g Sepharose 4B; Pharmacia). The PARP extract obtained by the Pardee & Spudich (1982) method or the soluble cellular extracts obtained by freezing and thawing cycles were solubilized (1:1) with buffer A (10 mM HEPES, pH 7.5, 2 mM CaCl₂, 2 mM MgCl₂, 5 mM ATP, 0.5 mM β-mercaptoethanol). The solutions were coupled overnight in the column at 4 °C (Barnett & Cunningham, 1992). The charged column was gently washed with buffer A supplemented with 0.1 M KCl. The PARP were eluted with buffer A without CaCl₂ and supplemented with 5 mM EGTA and 1 M KCl. The next elution step was with buffer A without calcium and supplemented with 40% (v/v) formamide. The elution fractions were mixed and dialysed three times against buffer A with calcium. The protein concentration was measured according to Bradford (1976), and the PARP were concentrated with PVP-360 (Sigma) and lyophilized.

**Binding to phalloidin**

**Microscopy.** *E. coli* cells from an OD_600_ 0.6 culture were permeabilized with 2 vols methanol for 30 min, then fixed with 3.7% (v/v) formaldehyde/PBS (pH 7.4) for 20 min and washed with PBS (pH 7.4). They were then incubated with phalloidin labelled with tetramethylrhodamine isothiocyanate (TRITC, Sigma; 500 ng ml⁻¹) for 6 h and washed four times with PBS and once with distilled water. The cells were resuspended in 1% (w/v) gelatin, mounted in glycerol and observed by phase contrast and epifluorescent microscopy (Universal Zeiss) (García-Cuáller et al., 1990). Photographs were taken with Tri-X Pan 400 Kodak film.

Ameoba cells were collected, washed with PBS, and deposited on glass coverslips with fresh culture medium at 37 °C. After the attachment of the cells to the coverslips, they were rinsed with PBS and fixed with 3.7% (v/v) formaldehyde/PBS (pH 7.4) for 20 min. After several rinses in PBS, cells were permeabilized for 6 min with cold acetone. After air drying, coverslips were
incubated for 20 min with TRITC-phalloidin (100 ng ml\(^{-1}\); Sigma), previously resuspended in PBS. The coverslips were rinsed with several changes of PBS and mounted on glass slides with glycerol/PBS (9:1, v/v) (De la Garza et al., 1989).

**Spectrofluorometry.** This was performed using the same method described above for microscopy, but at the last step the cellular pellet was resuspended in 200 \(\mu\)l PBS, and finally the readings were taken in the spectrofluorometer (Sim Aminco DMX-1000, SLM Instruments) at an excitation wavelength of 550 nm and an emission wavelength of 574 nm. *Ent. histolytica* trophozoites in solution were stained as above and were used as a eukaryotic control. For all the strains (prokaryotic and eukaryotic), the quantity of cells used contained 900 ng protein ml\(^{-1}\). In order to test the specificity of phalloidin binding for microscopy and spectrofluorometry, phalloidin without TRITC (100 ng ml\(^{-1}\)) and cytochalasin D (CD, 250 \(\mu\)g ml\(^{-1}\)) were used, before the addition of TRITC-phalloidin (5 \(\mu\)g ml\(^{-1}\)), and TRITC alone (1.65 ng ml\(^{-1}\)) was used to test for non-specific staining of proteins (this quantity of fluorochrome is the same as that binding to 5 \(\mu\)g phalloidin in TRITC-phalloidin). The autofluorescence of permeabilized cells was also checked.

**RESULTS AND DISCUSSION**

**PARP in whole cells**

To search for the presence of PARP in different bacteria, the cells were incubated overnight in the presence of TRITC-phalloidin, a drug which very specifically binds to actin by accelerating the G- to F-actin polymerization. It is known that this interaction results in a dramatic increase in the stability of actin filaments, not only towards chaotropic ions, DNase I and cytochalasins (Danker et al., 1975; Low et al., 1975), but also towards heat denaturation and proteolytic degradation (Lengsfeld et al., 1974; Wieland, 1977; Vandekerckhove et al., 1985). A specific fluorescence was seen in *E. coli* (Fig. 1) and it remained even when cells were subjected to the action of lysozyme, exhaustive washes or sonication. This fluorescence was also observed in *Salmonella typhimurium* (data not shown).

Control cells stained with TRITC alone gave some background but the staining intensity was significantly lower than with TRITC-phalloidin. To quantify the contribution of TRITC alone in the fluorescence detected in the cells treated with TRITC-phalloidin, the interaction was measured by spectrofluorometry as shown in Fig. 2(a–c), where the relative contributions of internal background and specific fluorescence from the phalloidin interaction are shown in spectrums 5 and 1, respectively. By microscopy, both *A. cylindrica* and *A. variabilis* have a natural fluorescence at the wavelength range for the rhodamine filter (540–570 nm). Spectrofluorometry at 550 nm (rhodamine wavelength emission) was used in order to test the specific binding of phalloidin to these cells. Binding specificity was detected in the same form that occurred in *E. coli* and in the eukaryotic control *Ent. histolytica*. Thus, both apparently showed similar specificity towards phalloidin, since the competitors (phalloidin without TRITC, or CD) interfered with the binding and the cells tested with the fluorochrome alone lost it with the washes (Fig. 2).

Phalloidin has preference for the actin-F form in eukaryotes (Wulf et al., 1979). However, we cannot say that only filamentous PARP were binding to phalloidin, because of the low resolution of the light microscope. When the cells were preincubated with phalloidin without rhodamine, the fluorescence considerably diminished, almost to basal level (Fig. 2, spectrum 2), and when the cells were pretreated with CD (a drug that depolymerizes actin), they did not fluoresce (in the microscope) and the level of fluorescence detected by spectrofluorometry declined (Fig. 2, spectrum 3). Furthermore, long chains of swollen cells of *E. coli* were seen in the presence of CD, indicating alterations in cell shape (data not shown). Cytochalasins are fungal metabolites with inhibitory effects on a variety of processes in eukaryotic cells, such as the uptake of small sugars (glucose) or nucleosides (thymidine), and cell motility due to actin (Tannenbaum, 1986). Gosh et al. (1978) tested cytochalasin B (CB) in *Mycoplasma gallisepticum*, where it inhibited cell division through its action on some specific target, but it did not affect the transport of sugars or nucleosides. Betina et al. (1972) found that CB had no antibacterial effect, but cytochalasin A inhibited the growth of *Bacillus subtilis* and *E. coli*, increasing motility in the latter, and other authors have also studied bacterial sensitivity to CB or CD, obtaining similar results (Sioud et al., 1987).

**PARP in cellular extracts: Western blotting**

No evidence for the existence of PARP has been previously found in cyanobacteria. In spite of these prokaryotes having a bigger cellular size than enterobacteria...
bacteria, and showing phototaxis and gliding like some eukaryotes (Van Liere et al., 1979), there are reports that refer to these processes in cyanobacteria as being independent of cytoskeleton-related proteins (Burchard, 1982).

Here we could clearly recognize two polypeptides of 45 and 40 kDa by Western blotting of total cellular extracts of both cyanobacterial species; this shows that in this cellular system there exist epitopes related to actin (Fig. 3, lane 3). In contrast, with the same antibody for *E. coli* extracts, there was a weak recognition of four polypeptides of 60, 43, 35 and 30 kDa (Fig. 3, lane 4). This recognition was increased with phalloidin addition (compare lane 6 with lane 5, where there was no addition of phalloidin). The evolutionary distance between different bacterial groups and eukaryotic cells used in this study probably marks the difference in the recognition with the mAb. In another prokaryote, the halophilic archaeobacterium *Halobacterium halobium*, a major 80 kDa polypeptide cross-reacted with a polyclonal anti-skeletal-muscle chicken actin antibody (Sioud et al., 1987). These observations encourage further study of these proteins.

**PARP in cellular extracts: acetone powder preparation and polymerization of PARP**

Nakamura et al. (1978) used the Spudich & Watt (1971) technique for muscular actin purification. This method is based on the characteristic of actin polymerization/depolymerization and the great concentration of this protein in muscle. They made some modifications for enterobacterial cells and reported the purification of an *E. coli* actin-like protein. However, we failed to repeat their observations, because during the preparation of acetone...

---

**Fig. 2.** Emission spectrofluorometry of the binding of phalloidin to whole cells (excitation wavelength 550 nm; emission wavelength 574 nm). (a) *A. cylindrica*; (b) *E. coli*; (c) *Ent. histolytica*. 1, Cells incubated with TRITC-phalloidin (5 µg ml⁻¹); 2, cells pre-incubated with phalloidin alone (100 µg ml⁻¹) followed by TRITC-phalloidin (5 µg ml⁻¹); 3, cells pre-incubated with CD (250 µg ml⁻¹) followed by TRITC-phalloidin (5 µg ml⁻¹); 4, washed cells; 5, permeabilized cells incubated with TRITC (1.65 µg ml⁻¹). The table shows the corresponding maximum emission value (relative units) for every spectrum.

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>191</td>
<td>296</td>
<td>206</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>80</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>92</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>27</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>59</td>
<td>29</td>
</tr>
</tbody>
</table>

**Fig. 3.** Western blotting with the anti-actin (rat-brain) mAb. Lanes: 1, total cellular extract of *E. coli* (150 µg) probed only with secondary antibody conjugated with peroxidase; 2, muscle actin (10 µg); 3, total cellular extract of *A. cylindrica* (50 µg); 4, total cellular extract of *E. coli* (150 µg); 5, total cellular extract of *E. coli* (50 µg); 6, total cellular extract of *E. coli* (50 µg) with 5 ng phalloidin added to the extract.
powder in accordance with their method, the PARP was lost. We found that using the Spudich & Watt (1971) procedure to obtain PARP was even less effective in yielding polymeric material than the Pardee & Spudich (1982) method. Thus, instead of the method of Nakamura et al. (1978) we used the original Pardee & Spudich (1982) procedure. Although with this method we could achieve three cycles of polymerization, the protein pellet contained many polypeptides instead of the clean PARP. Western blot analysis of the proteins from the pellet revealed three main polypeptides of 60 kDa, 43 kDa and 35 kDa, of which the 35 kDa was the most abundant and increased after every polymerization cycle (data not shown).

These observations are in agreement with the results of Barnett & Cunningham (1992), who also reported an inability to purify a Streptococcus pyogenes actin-like protein with the Pardee & Spudich (1982) method.

PARP in cellular extracts: binding to DNase I

PARP from the Pardee & Spudich (1982) method. One of the characteristics of actin, except in Ent. birstlytica and Tetrahymena thermophila (Hennessy et al., 1993), is the high-affinity binding to DNase I in the presence of CaCl2 (Lazardis & Lindberg, 1974). This property is also present in mitochondrial actin (Stozharov, 1985; Etho, 1990). Our major interest was to know if the PARP from E. coli copolymerized with several proteins after the Pardee & Spudich (1982) procedure, had this capacity. From the proteins obtained by the Pardee & Spudich (1982) polymerization experiments, only one 60 kDa polypeptide was isolated by binding to DNase I (Fig. 4). Barnett & Cunningham (1992) reported the isolation of two actin-like proteins from S. pyogenes with similar molecular masses of 60 kDa and 43 kDa by this procedure. It is interesting to note that these authors also used the Pardee & Spudich (1982) procedure as the first step.

PARP from soluble cellular extracts. The Pardee & Spudich (1982) method is designed for a tissue that contains actin and myosin as major proteins. It is not convenient for either non-muscular cells because of their low actin content or bacterial cells because of the disadvantages of managing large volumes of culture (20 l), protein degradation, loss of PARP with the successive steps of polymerization and the copolymerization with other proteins. We decided to test if the PARP from soluble cellular extracts of the bacterial strains studied would also bind DNase I. A 60 kDa polypeptide could be isolated from E. coli, and one of 45 kDa from both cyanobacteria (Fig. 4). In Anabaena, the 45 kDa polypeptide was better recognized by the antibody (Fig. 5). The PARP were labile and tended to degrade with manipulation. In the case of E. coli, the 60 kDa protein apparently generates both the 43 kDa and 35 kDa polypeptides. On the other hand, in both species of Anabaena the main degradation product had a molecular mass of 40 kDa. More studies are required to investigate the degradation of these PARP.

Fig. 4. Isoelectric points (SDS-PAGE; 10%) for PARP of E. coli and A. variabilis. (a) E. coli (gel stained with Coomassie blue). Lanes: 1, molecular mass markers; 2, PARP unidimensional electrophoresis. (i) pI 6.6, (ii) 6.65 and (iii) 6.7. (b) A. variabilis. Molecular mass markers are shown in lane 1. (i) pI 6.4, (ii) 6.5 and (iii) 6.8.

PARP in cellular extracts: PARP molecular masses and isoelectric points

Eukaryotic class I and II actins show pI values between 5.4 and 5.8 (Herman, 1993). For the most conserved actin isoforms (α, β and γ), it is proposed that pI variation is a direct consequence of amino acid substitution in its most variable region: the N-terminus (Vandekerckhove & Weber, 1981).

E. coli PARP showed remarkable differences from actins. They had a higher apparent molecular mass (60 kDa) than most eukaryotic actins (42–48 kDa), albeit similar to the actin-like protein from S. pyogenes (Barnett & Cunningham, 1992), and the pI values of the three isoforms from E. coli (6.60, 6.65 and 6.70; Fig. 4) were less acidic than most conserved actins. The anti-actin (rat-brain) mAb weakly recognized these PARP. In contrast, in both A. cylindrica and A. variabilis the mAb recognized two bands with molecular masses similar to actin (45 and 40 kDa). Only the 45 kDa form was bound to DNase I, but showed three less-acidic isoforms of pI values 6.8, 6.5 and 6.4. The different affinity of the mAb for the PARP from E. coli and both cyanobacteria reflects differences in their primary structure as well as the differences in size.
We are continuing the study of these PARP to try to learn more about their physico-chemical properties.

Studies of actin-like proteins in mitochondria have revealed the presence of two isoforms of 43 kDa with a pI value around 5.6; they are related to the β and γ isoforms from eukaryotic actins (Stozharov, 1985). Although this organelle probably has a prokaryotic origin, more studies are necessary to characterize these actin-like proteins and compare them with PARP. Recent findings show that vertebrate cells contain actin-like proteins (class III) (Herman, 1993), such as actin-RPV (actin-related protein of vertebrates). This is a major component of the brain dynactin and centractin complexes which is associated with centriosomes. In fungal cells such as Saccharomyces cerevisiae or Schizosaccharomyces pombe, actin class III proteins are involved in cytoskeletal rearrangements during mitosis (Less-Miller et al., 1992; Schwob & Martin, 1992). Herman (1993) has proposed that these proteins are ancestral acts in eukaryotes, and it is interesting to note that they have a molecular mass above 45 kDa and pI values of 6.6–6.8, which are similar to the PARP reported here, in addition to being able to hydrolyse ATP and sequester divalent cations.

The origin of bacterial actin-like proteins is unknown. Hybridization of a Drosophila actin gene to S. pyogenes DNA in Southern blots, under low stringency conditions, was negative in autoradiograms (Barnett & Cunningham, 1992). This result is in agreement with our hybridization experiments for L. coli DNA using two genomic actin probes (from Taenia solium and Acanthamoeba castellanii) and a cDNA actin probe from Ent. histolytica; in all cases they showed low hybridization by dot blotting at low stringency for the washes (García-Cuellar et al., 1990). However, we have not been able to detect a specific hybridization by Southern blotting (data not shown). These results suggest that if there are actin-like proteins in S. pyogenes or in the strains under study here, their nucleotide sequence does not have homology with the Drosophila actin gene or with the probes tested, but they could have tridimensional structures and functions similar to those of eukaryotic actin, as is proposed by Flaherty et al. (1991). It is possible that PARP represent a group of interactive actin-like proteins that may function in cell cycle and cell division (Norris et al., 1991), motility, cellular swelling and cell shape (Minkoff & Damadian, 1976; Nakamura et al., 1978; Neimark, 1977).

ACKNOWLEDGEMENTS

We thank Mr Esteban Molina and Mr Pedro Escalera for their technical assistance. We also thank the Electron Microscopy Unit of the Cell Biology Department of CINVESTAV-IPN. This work was supported by CONACYT grant 1462-N9207.

REFERENCES


Prokaryotic actin-related proteins


Van Liere, L., Mu, L. R., Gibson, C. E. & Herdman, M. (1979). Growth and physiology of Oscillatoria agardhii Gomont cultivated in...


Received 13 April 1995; revised 8 November 1995; accepted 13 December 1995.