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REVIEW ARTICLE

Eduardo Villalobo · Pilar Pérez-Romero
Rocío Sánchez-Silva · Antonio Torres

Unusual characteristics of ciliate actins

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Abstract Actin is a cytoskeletal protein that is ubiquitous in eukaryotes, hence the corresponding genes and proteins have been isolated from numerous organisms as different as animals, plants, fungi and protozoa. Several atomic models are available for the monomeric as well as the filamentous form, and more than 70 proteins that bind actin and control filament dynamics have been isolated from diverse eukaryotes. Moreover, the function and dynamics of the actin cytoskeleton in several eukaryotic systems have been depicted in depth. Unlike other protozoa, such as amoeba, actin is not an abundant protein in ciliates, whose cytoskeleton is mainly composed of microtubular arrays. Ciliate actin has been studied in several species, and it was established early on that this ciliate protein is very different from that of other eukaryotes. Similarly, the actin-binding proteins studied in ciliates display great differences with those of other eukaryotes. Consequently, ciliate actin has been considered as "unconventional," and this review focuses on molecular data leading to this conclusion.

Keywords Actin · Actin-binding protein · Ciliate · Cytoskeleton

Introduction

Actin is one of the most highly conserved and best studied eukaryotic proteins. It may be present as monomeric G-actin in the absence of salt, or as microfilaments (F-actin) in the presence of physiological concentrations of salt. Monomeric actin binds 1 mol of ATP and one divalent cation per mol of protein. It is a Mg^{2+}-stimulated ATPase, but at low salt concentrations, in which it does not polymerise, this ATPase activity is low. Under polymerisation conditions, monomers are incorporated into filaments in the ATP state and the nucleotide is hydrolysed to ADP, although filament assembly is not dependent on this hydrolysis.

Actin protein is a major cellular component of both the cytoskeleton and the muscle sarcomere. In muscle cells, actin is involved in myofibrillar construction. In non-muscle cells, the actin cytoskeleton plays an essential role in multiple cellular processes, including cell elongation and shape determination, cytoplasmic streaming, cell motility, division-plane localisation, chromosome segregation, secretion, endocytosis, and organelle transport [45]. All of these processes rely on the capacity of the actin cytoskeleton to respond to cellular signals and reorganise spatially and temporally into a variety of specific structures.

The 42-kDa G-actin monomer typically consists of 375 amino acid residues. Primary structures of actins from different eukaryotic species exhibit exceptionally high sequence identities. There are at least six different isoforms of actin in mammals; two cytoplasmic actins, β and γ, and four muscle actins. The latter include two striated muscle (α-skeletal and α-cardiac) and two smooth muscle (α-aortic and γ-enteric) actins. In most eukaryotes, actin is encoded by a multigene family [7], although organisms such as yeasts [19], *Giardia* [13], and several flagellated protista [2] contain only a single actin gene. The multigene families are likely to have arisen from gene duplications followed by divergence of the duplicated copies during evolution. The different actin isoforms produced by the members of the actin gene families are typically highly conserved at the amino acid sequence level, apparently due to their critical functional roles in cells.

Crystallographic data [5, 29,48] indicate that actin monomer is a globular protein consisting of two domains connected by a hinge region. These two domains, originally termed large and small (although now they are

E. Villalobo · P. Pérez-Romero
R. Sánchez-Silva · A. Torres (✉)
Departamento de Microbiología,
Facultad de Biología. Universidad de Sevilla.
Apdo 1095. 41080 Seville, Spain
E-mail: trueda@cica.es
Tel.: +34-954557115
Fax: +34-954557830

known to have almost identical sizes), are further divided into subdomains. The small domain is composed of subdomain 1 (amino acid residues 1–32, 70–144, 338–375, rabbit α-actin numbering) and subdomain 2 (amino acid residues 33–69), whereas the large domain comprises subdomain 3 (amino acid residues 145–180 and 270–337) and subdomain 4 (amino acid residues 181–269). The large subdomain has a structural core made up of residues from both subdomains 3 and 4, whereas the small-domain core is made up exclusively of residues from subdomain 1. Subdomains 1 and 3 are thought to have evolved by duplication of an ancestral gene coding for a polypeptide of about 150 amino acids that appears to be an ancient nucleotide-binding pocket also found in heat shock proteins, sugar kinases and several polypeptides known to regulate the prokaryote cell cycle [3]. Subdomains 2 and 4 were probably inserted subsequently into subdomains 1 and 3, respectively.

The nucleotide/cation complex is bound at the bottom of the cleft between subdomains 1 and 3, with the adenosine base resting in a hydrophobic pocket formed between subdomains 3 and 4. There are 15 residues involved in nucleotide-binding sites, of which 13 belong to subdomains 1 and 3, and two are within subdomain 4. The region of contact with DNase I consists of hydrogen bonds, electrostatic and hydrophobic interactions involving subdomain 2 (amino acid residues 39–46; 60–64) and subdomain 4 (amino acid residues 202–204 and 207). Residues 40–50 within subdomain 2 are highly disordered and form the DNase I loop. Actin-actin interactions in the filament involve about 45 residues. Subdomains 2 and 4 of the actin monomer have actin-actin interfaces interacting with subdomains 1 and 3 of other actin monomers. Several amino acid residues in subdomain 2, including a region of the sequence that is nearly identical to that of the DNase I contact, have been shown to be in contact with other actin monomers. The actin helix is stabilised through a loop of 11 amino acids (262–272) that includes a four-residue hydrophobic plug. This loop inserts into a hydrophobic pocket formed by subdomains 2 and 3 of two adjacent monomers on the opposing strand.

In the cell, many actin-binding proteins (ABPs) regulate the nucleation and assembly-disassembly of actin into filament. Most families of ABPs have been widely conserved over phylogeny, in both primary structure and biochemical properties, and they can be found in organisms as diverse as humans and yeast. This suggests that these proteins already existed in a common eukaryotic ancestor and that the basic mechanisms regulating the dynamics of the actin cytoskeleton are conserved among diverse organisms and cell types. More than 70 ABPs that can control filament dynamics have been identified [35,37]. Some affect monomers by controlling sequestration or nucleotide exchange; others control filament formation and stability by regulating capping, nucleating, cross-linking, bundling, and severing. Finally, cellular signalling molecules and small GTPases control actin remodelling by regulating the activities of ABPs and numerous direct and indirect effectors [36,44].

Ciliate actin

This cytoskeletal protein has been studied in several species, although results are scarce, sometimes contradictory, and generally restricted to *Tetrahymena* and *Paramecium*. The following sections are dedicated to reviewing the most relevant knowledge on ciliate actin.

The protein

The first attempts to demonstrate the presence of actin in ciliates were carried out by indirect techniques, such as heavy meromyosin (HMM) decoration or immunodetection. Thus, actin was localised in the cortex of *Discophrya* [23], *Paramecium* [49] and *Tetrahymena* [31], and in the oral apparatus of *Pseudomicrothorax* [25]. Lately, *Paramecium* actin was found in food vacuoles, around contractile vacuoles [9], and in the epiplasm [8]. However, it was not detected deeper in the cortex, as previously suggested. In *Tetrahymena*, actin was also found in the food and contractile vacuole, in the division furrow [38], and in the basal body-cage complex [28]. The link between ciliate actin and basal bodies is not an exception, since actin has also been reported to be associated with axonemes in algae [43] and birds [47], and with centrosomes in vertebrates [6]. Evidence of the association of ciliate actin with basal bodies has been obtained by us using an antibody raised against centractin, a centrosome-associated actin. In *Paramecium*, this anti-centractin antibody labelled basal bodies not only in the cortical rows but also in the oral apparatus (our unpublished data).

Actin has been purified in *Climacostomum* [18], *Paramecium* [49] and *Tetrahymena* [27]. In these ciliates, the purified actin corresponds to a protein of about 43 kDa, if some confusing reports on *Tetrahymena* actin are not considered [39,41]. Conventional methods were used to purify actin in *Paramecium* and *Tetrahymena*, whereas DNase I-affinity chromatography was used in *Climacostomum*. Using this last method, a 43-kDa protein was also isolated in *Paramecium* [8], but the authors questioned whether this protein was actually actin. In fact, although its peptide mapping did not coincide with that of rabbit muscle actin, an antibody raised against this 43-kDa protein [8] decorated the epiplasm, the same structure decorated by a monoclonal antibody against rat skeletal actin [34].

Far from shedding light on ciliate actin, these results pose new questions as to its biochemical nature. Nevertheless, to date a detailed biochemical characterisation of actin is available only in *Tetrahymena* [27]. In some respects, *Tetrahymena* actin shows biochemical properties similar to that of rabbit muscle actin, namely: (1) it polymerises in vitro into microfilaments in an

ion-dependent-manner; (2) it forms arrowhead structures with HMM; and (3) it activates the Mg^{+2}-ATPase of myosin S-1. However, *Tetrahymena* actin shows unusual properties in two respects: first, it does not bind to phalloidin; second, it does not inhibit DNase I activity. This latter feature of *Tetrahymena* actin raises the possibility that the purification of *Paramecium* and *Climacostomum* actin by DNase-I affinity chromatography is artefactual. This is supported by the fact that the DNase-I-purified actin of *Climacostomun* forms microfilaments in vitro but independently of salt concentration. All these unusual properties allow us to assume that, overall, ciliate actin can be considered unconventional. This would explain in part why many antibodies raised against conventional actin fail to recognise any protein in the 43 kDa range in total protein extracts of many ciliate species. For instance, in total protein extracts, the antibody of Lin [34] did not detect any polypeptide in *Paramecium* and *Tetrahymena*, although the antibody recognised a polypeptide of about 43 kDa in *Climacostomum* [18] and, surprisingly, a set of polypeptides ranging from about 110 to 97 kDa in *Euplotes* (our unpublished results).

The genes

Actin genes have been sequenced in many species, despite the fact that the protein has been isolated in only a few ciliate species. As in yeasts, the number of macronuclear actin genes in ciliates is low, ranging from one in *Histriculus* [42] and *Tetrahymena* [10,26] to three in *Oxytricha* [12,30] and *Stylonychia* [24]. The size of the actin macronuclear molecules in hypotrichs, in which the size of the whole gene unit can be obtained, ranges from about 1,200 bp to about 1,600 bp, excluding telomere repetitions. The micronuclear counterparts of the macronuclear actin genes have been sequenced only in *Oxytricha* and *Urostyla*. Interestingly, in the micronuclear DNA of *Oxytricha trifallax*, ten macronuclear-destined sequences (MDSs) corresponding to actin I are dispersed and disordered in the chromosome, a phenomenon called "scrambling" [14]. Scrambled MDSs must be rearranged during macronuclear development to form a functional macronuclear molecule.

Macronuclear actin genes are relatively A + T-rich (mean of about 56%), although this richness depends on the region considered and the species analysed. In general, the A + T content in non-coding regions (5′- and 3′-untranslated region and introns) is higher than in the coding regions. This A + T content is especially high (75–91%) in the two short introns present in *Paramecium* [11], *Vorticella* and *Opisthonecta* actins [46], the only available ciliate actin genes known to be interrupted by introns. Note that, in *Paramecium*, one intron is located within subdomain 1 and the other one within subdomain 3, which supports the idea that both domains arose by duplication of an ancient polypeptide and/or by exon shuffling.

A promoter sequence motif (TATA-box) can be found in most but not all the available actin sequences, but whether this motif is directly implied in transcription is not known. In *Histriculus cavicola* [42], there are two potential TATA boxes (40 and 65 bp upstream of the initiation codon) in its unique actin gene, and the initiation of transcription occurs in the fourth nucleotide (A) of the first motif (position –40). Therefore, it is assumed that the second motif (position –65) might be the actual promoter. This second motif (position –65) is 22 bp upstream of the transcription initiation, resembling that described in *Euplotes crassus* actin (a potential TATA-box is situated 21 bp upstream of the transcription initiation, which is also an A [22]). Note that the 64 nucleotides upstream of the initiation codon are rather similar in *H. cavicola*, *O. fallax*, and *O. trifallax* actin sequences, and that a perfect alignment can be produced around the TATA box [42].

Related to the polyadenylation consensus sequence (AATAAA), it is uncommon to find perfect matches in ciliate actin genes, but this is not surprising, as yeast and plants would not require this motif to add the poly(A) tract [50]. In fact, in *E. crassus*, where no perfect polyadenylation signal can be recognised in actin, two different polyadenylation sites have been described. These correspond to two different developmental stages, although whether polyadenylation addition to a particular site is related to a particular stage of development remains to be determined [22].

The expression level of actin has been examined in a few cases. In *Tetrahymena*, for instance, actin seems to be actively expressed, but with little fluctuation throughout the cell cycle [54]. In *Sterkiella histriomuscorum*, our previous results indicated that actin is also expressed actively, although to a lesser extent than α-tubulin. Moreover, actin is down-regulated during starvation (reaching a minimum in the cyst) and up-regulated during excystment, probably until the level of the vegetative cell is reached.

The primary amino acid sequence

At this level, the primary amino acid sequences of ciliate actins shows a relatively low level of identity with those of actins deposited in databases. In some cases, ciliate actins are no more similar to each other than to those of other organisms. For example, *Paramecium* actin is approximately 71% and 61.3% identical to *Arabidopsis thaliana* and *Oxytricha trifallax* actins, respectively. In the same manner, *Paramecium* actin is more similar to actin of an early diverging protozoan (58.3% identity with *Giardia lamblia* actin) than to the actin of a member of the ciliates (56.5% identity with *Euplotes crassus* actin). This situation is accentuated in *Oxytricha nova*, which has three highly divergent actin genes (actin II and actin III are only 50% identical). These striking differences among ciliate actin sequences show the high evolutionary rate of actin within the phylum Ciliophora.

As a consequence of this unusually rapid rate of evolution, ciliates appear to be polyphyletic, emerging quite low in the actin phylogenetic tree [13]. However, a wealth of morphological evidences and phylogenetic trees of rRNA and tubulins [1] unquestionably demonstrates that ciliates constitute a solid monophyletic group. Maybe actin, in contrast to tubulin, is poorly used in ciliates. If this is true, actin would have less functional constraints and, therefore, it could evolve at a high mutation rate. This assumption could explain, in part, why ciliate tubulins are so similar in their amino acid sequences, whereas ciliate actins are not. It could also explain the unusual characteristics of ciliate actin, which might have evolved new functions.

The tertiary structure

Since the X-ray structures of monomeric actin have become available [5, 29,48], it has been possible to determine precisely which amino acids are significant for any given actin property, such as binding to divalent cation, to nucleotide, or to DNase I. Superimposition of ciliate actin sequences on the three-dimensional structure of rabbit actin has allowed us to analyse the degree of conservation of several ligand-binding sites in the actin of ciliates. Furthermore, we have analysed in more detail the structure of *H. cavicola* actin by in silico modelling.

First, we aligned the actin amino acid sequences of several ciliates with that of rabbit α-actin and calculated the percentage of identity by subdomain (Table 1). The highest degree of conservation is found in subdomain 1, especially in the region of amino acids 70–144, and in the region of amino acids 145–180 of subdomain 3. These zones constitute the inner hydrophobic core of actin and they form the hydrophobic pocket involved in nucleotide- and cation-binding. Accordingly, the nucleotide-binding sites are well-conserved motives in ciliate actins (Table 2). Subdomains 2 and 4, and the region of amino acids 270–337 of subdomain 3 are less conserved (Table 2), with the greatest divergence mainly located on the surface of the molecule (Fig. 1); therefore, the DNase loop (amino acids 40–50) of subdomain 2 and the peripheral regions of amino acids 194–203 and 223–242 inside of subdomain 4 are not well-conserved (Table 2, Fig. 1). The DNase-I-binding sites of ciliate actins share 35.3–70.6% identity with the corresponding sequence of rabbit α-actin (Table 2). Since *T. pyriformis* actin, with 58.8% identity of the DNase-I-binding sites, lacks DNase-I-binding activity, it is likely that hypotrich, oligotrich and peritrich actins, with a DNase-binding motif less conserved than in *Tetrahymena*, also fail to bind DNase I. The actin-actin interactions in the microfilament include a region that is almost identical to that associated with DNase I–actin contact, the DNase I loop (divergent in ciliate actins) being of primary importance for the stabilisation of the actin filament. Moreover, in rabbit α-actin, a loop (amino acids 262–272) that includes a four- residue hydrophobic plug inserted into a hydrophobic pocket formed by two adjacent monomers on the opposing strand is also crucial for stabilisation of the actin helix. In a conventional actin, such as that of yeast, a mutation ($L_{266}D$) in the region of amino acids 262–272 produces disruptions in hydrophobic interactions, which results in the inhibition of actin polymerisation at low temperature [4]. The 262–272 loop is not well-conserved in ciliate actins (Table 2). In this region, hypotrich and *Halteria* actins share only 27.3–45.5% identity with rabbit α-actin, and hydrophobic residues within the plug are replaced by hydrophilic ones. These modifications in ciliate actin

Table 1 Percentages of identity between rabbit muscle actin (whole molecule and subdomains) and actins from different ciliates

	Whole molecule	Subdomain 1			Subdomain 2	Subdomain 3		Subdomain 4
	Amino acids 1–375	1a Amino acids 1–32	1b Amino acids 70–144	1c Amino acids 338–375	Amino acids 33–69	3a Amino acids 145–180	3b Amino acids 270–337	Amino acids 181–269
Hypotrichs								
Histriculus cavicola	65.5	72.4	85.3	67.5	48.6	69.4	44.1	65.2
Oxytricha nova I	65.8	64.5	85.3	67.5	45.9	69.4	52.9	64.0
Oxytricha nova II	67.2	71.9	75.7	85.0	58.3	80.0	56.1	58.0
Oxytricha fallax	66.2	73.3	69.3	67.5	45.9	69.4	50.0	65.2
Oxytricha trifallax	66.8	73.3	85.3	67.5	48.6	69.4	50.0	66.3
Euplotes crassus	61.9	59.4	65.3	67.5	54.1	72.2	60.3	58.4
Paramecium tetraurelia	73.6	78.9	83.1	83.7	70.2	83.3	73.1	61.3
Tetrahymena pyriformis	74.9	53.1	82.0	89.4	75.6	86.4	69.5	66.3
Peritrichs								
Vorticella microstoma	69.8	68.4	78.6	76.3	62.2	83.7	69.1	58.4
Opisthonecta matiensis	64.2	61.4	77.3	73.3	62.2	67.0	58.8	55.4
Olygotrichs								
Halteria sp.	66.2	68.7	81.3	65.7	48.6	81.3	48.5	67.4

Table 2 Percentages of identities between nucleotide-binding sites, DNase-I-binding sites, DNase loop (amino acids 40–50), and amino acid 194–203, 223–242 and 262–272 loops of rabbit muscle actin and the corresponding regions in ciliate actin sequences

	Nucleotide binding	DNase I binding	DNase I loop	194–203 loop	223–242 loop	262–272 loop
Histriculus cavicola	73.3	35.3	9.1	60	26.6	36.4
Oxytricha nova I	86.7	35.3	9.1	40	26.6	36.4
Oxytricha nova II	73.3	52.9	45.5	40	26.6	27.3
Oxytricha fallax	86.7	35.3	9.1	40	26.6	36.4
Oxytricha trifallax	93.3	35.3	9.1	40	26.6	36.4
Euplotes crassus	86.7	58.9	45.5	30	26.6	45.5
Paramecium tetraurelia	86.7	70.6	72.7	40	20.0	63.6
Tetrahymena pyriformis	80.0	58.9	63.6	30	26.6	54.4
Vorticella microstoma	86.7	41.2	45.5	30	20.0	45.5
Opisthonecta matiensis	86.7	47.0	45.5	30	13.3	54.5
Halteria sp.	86.7	35.3	9.1	60	20.0	27.3

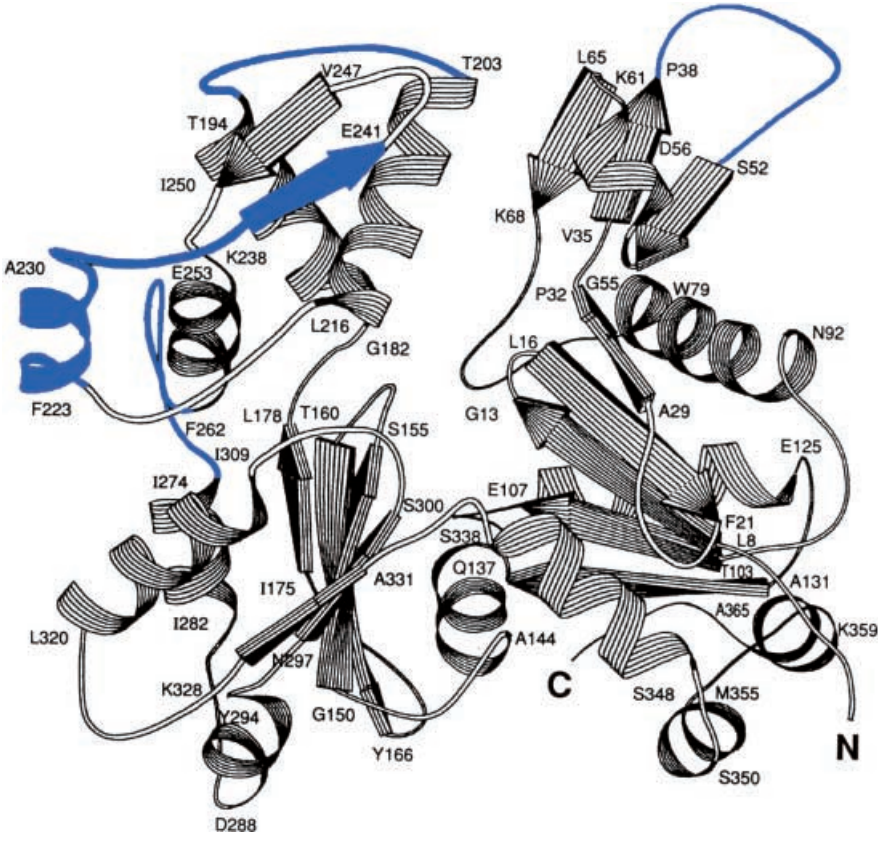

Fig. 1 The three-dimensional structure of actin–DNaseI complex according to Kabsch et al. [30]. The first and last amino acids residues in helices and sheet strands are specified. Regions of subdomains 2 and 4 that are highly divergent in ciliate actins are shown in *blue* (amino acids 40–50, 194–203, 223–242 and 262–272)

sequences may indicate unusual polymerisation properties or an inability to polymerise.

Second, we aligned the actin amino acid sequence of *H. cavicola* with that of rabbit α-muscle actin and then carried out in silico modelling, taking into account the tertiary structure of the complex actin–DNase I available from the Protein Data Bank (accession number 1ATN). These in silico structures have also served to calculate a molecular dynamics model and can be used to search for conformational differences among them. The in silico structures of *H. cavicola* and rabbit α-muscle actin are similar, although small differences can be seen in subdomains 2 and 4. These differences can also be seen by comparing the average structures created with molecular dynamic simulations (Fig. 2), as well as by comparing the percentage of identity of these subdomains at the amino acid level (see the precedent paragraph). Moreover, during the simulation, subdomain 2 moved toward subdomain 4, the displacement being more pronounced in *H. cavicola* actin than in rabbit α-muscle actin. The tighter interaction between subdomains 2 and 4 in *H. cavicola* actin caused an allosteric hindrance that could inhibit DNase I binding.

The actin-binding proteins

As can be deduced from the preceding sections, knowledge on ciliate actin is still very scarce and ABPs are not an exception but rather an extreme case. In fact, data are

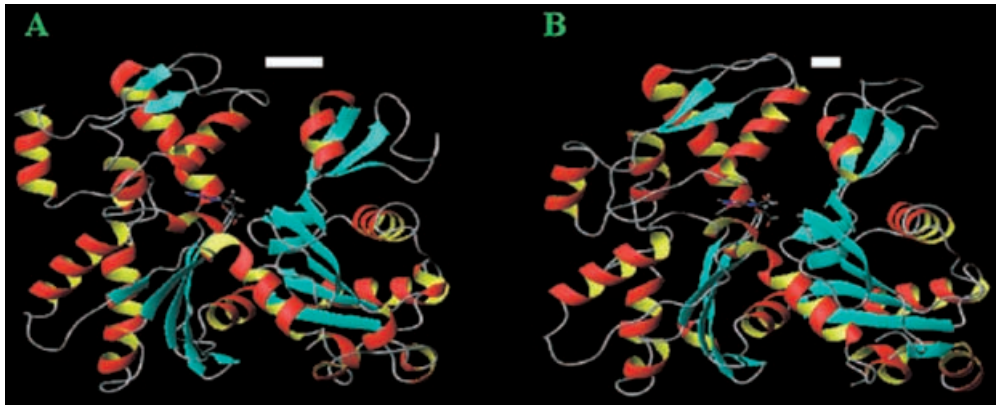

Fig. 2 Computer-simulated three-dimensional structure of rabbit α-actin (**A**) and *Histriculus cavicola* actin (**B**). These representations correspond to the average structures of molecular dynamic simulations. *White bars* Distance between subdomains 2 and 4, which is shorter in *H. cavicola* actin than in rabbit α-actin

restricted to *Tetrahymena*. It is generally believed that all eukaryotic cells contain actin and its filament-binding motor protein myosin. This is certainly true, but in ciliates the demonstration of myosin has been difficult, maybe because of the high divergence of ciliate myosin. A report on the presence of *Tetrahymena* myosin appeared in 1995 [21], i.e. more than a decade after the first reports on *Tetrahymena* actin. The authors described the presence of two polypeptides of 180 and 15 kDa in the basal-body cage complex of *Tetrahymena* that could correspond, based on biochemical evidence, to the heavy and light chains of myosin. Furthermore, authors in the same laboratory described the cloning of a myosin heavy chain gene [20] which is indeed expressed in growing cells. The predicted amino acid sequence of this gene shows that all the signature motives for the head domain of known myosins are conserved. Nonetheless, a phylogenetic analysis shows that *Tetrahymena* myosin heavy chain belongs to a new myosin family. Moreover, disruption of this gene affects endocytosis and macronuclear elongation [53]. Profilin, an actin-sequestering protein that modulates actin polymerisation, has also been described in *Tetrahymena*. A conventional profilin of 12.8 kDa was isolated in *T. pyriformis* by poly (L-proline) affinity column [16]. The same procedure was used to isolate profilin in *T. thermophila*, and the purified protein was used to obtain its corresponding cDNA [52]. This cDNA sequence predicts a polypeptide of 16.7 kDa with little homology with the previously reported *T. pyriformis* profilin gene [15]. This last *Tetrahymena* profilin gene is also divergent when compared to mammalian profilins but its N- and C-terminal regions are relatively conserved. By immunofluorescence, profilin was detected in the division furrow of *Tetrahymena* [17]. Profilin seemed not to be the only ABP that co-localised with actin in the division furrow, since three other *Tetrahymena* co-localising proteins have been described: fimbrim, elongation factor 1α (EF-1α) and calmodulin. *Tetrahymena* fimbrin was isolated as a 61-kDa polypeptide that was shown to be the partial degradation product of a 71-kDa polypeptide. A cDNA corresponding to this fimbrin was sequenced and predicted to be a protein of about 65.1 kDa with two actin-binding domains, but lacking the EF-hand-binding domain. This last feature and the low homology with other known fimbrins suggests that this protein is a new member of the fimbrin/plastin family. *Tetrahymena* fimbrin co-localises with actin in dividing cells, but in interphase cells also at the oral apparatus and vacuole pores [51]. EF-1α is involved in protein synthesis in eukaryotes, although some reports show that it can perform other functions, usually related to cytoskeleton regulation. In fact, *Tetrahymena* EF-1α co-precipitates with F-actin and has F-actin bundling activity, as shown by electron microscopy [33]. Furthermore, it has been shown that Ca^{+2}/calmodulin directly interacts with EF-1α inhibiting its F-actin bundling activity [32]. Both EF-1α and calmodulin have been detected in the oral apparatus and apical region of contractile vacuoles in interphase *Tetrahymena* cells [40], as has also been observed with fimbrin.

Summarising, the results described here show that the ciliate ABPs identified thus far are somehow different from those of other eukaryotes. This is similar to the case of ciliate actin when compared to conventional actins, thus reaffirming the unconventional character of the ciliate actin cytoskeleton.

Future directions

It is clear that the function of ciliate actin is still unknown. We can assume that ciliate actin plays the same or a similar role that conventional actin does. This extrapolation, however, could be inexact, since the primary amino acid sequence and biochemical properties of ciliate actins differ from those of conventional actins. Indications on ciliate actin function come from the localisation of this protein in the cell. These data suggest that actin may be involved in some steps of phagocytosis, the contractility of the cortex and the division furrow. Nevertheless, implication of ciliate actin in these or other processes needs more experimental evidence, which can be obtained using current molecular approaches, such as transformation. This technique has been successfully applied to *Tetrahymena*, *Paramecium*, *Stylonychia* and *Euplotes*. Transformation would allow, for example, observation of actin dynamics in vivo through fusion with green fluorescent protein. It would

also be possible to use "gene silencing" to reduce actin expression, if this were not lethal for cells. This phenotype can be obtained whenever the role of actin becomes less essential for organelle movements, cell shape or chromosome rearrangements in ciliates than in other eukaryotes. Preliminary experiments developed in collaboration with A. Fleury (Université Paris-XI, Orsay, France) have allowed us to obtain a non-lethal phenotype in actin-transformed *Paramecium*. However, as actin expression has not been analysed yet, we do not know whether the gene has actually been silenced.

The role of ciliate actin as both regulator of cellular signalling pathways and co-ordinator of cellular behaviour should be exploited. Attention to ABPs, kinases, phosphatases, calmodulin, or small GTPases and to morphogenetic processes, such as conjugation, encystment/excystment, regeneration and polymorphic transformation, will no doubt lead to very promising lines of research.

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