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The role of actin binding proteins in epithelial morphogenesis: models based upon *Listeria* movement

R.M. Golsteyn *, D. Louvard, E. Friederich

Morphogenèse et Signalisation Cellulaires, Centre National de la Recherche Scientifique, UMR 144, Institut Curie, 26 rue d'Ulm, Paris 75248 Cedex 05, France

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Abstract

We summarize recent findings on the organization of the protein actin in eucaryotic cells. In particular we focus on how actin can be used to generate a vectorial force that is required for cell movement. These forces arise from protein molecules that recruit actin to the plasma membrane in such a manner that actin filaments extend outward from the cell body. This type of actin dependent force generation has been described in a nucleation-release model, which is one of several models currently being tested to explain actin dependent cell movement. Data in support of this model has arisen unexpectedly from studies of an intracellular bacteria, *Listeria monocytogenes*. This bacteria uses actin to propel itself during infection of eucaryotic cells. By studying *Listeria* movement, the roles of several eucaryotic actin interacting proteins have been identified. One of these is zyxin, a human protein that shares important structural and possibly functional properties with ActA, an actin dependent force generating protein of *Listeria*. We intend to test the function of these and other actin interacting proteins in a simplified system that should facilitate precise measurement of their properties of force generation in vitro. © 1997 Elsevier Science B.V.

Keywords: ActA; Actin; Cell motility; *Listeria*; Polymerization; Zyxin

1. Introduction

By a simple property of polymerization and depolymerization, actin can be orchestrated to produce movement in almost all cell types. Biochemical studies have identified many molecules, including proteins, that interact with actin and its partners to generate vectorial forces in vitro. Transferring this knowledge to studies made in living cells has revealed not only that we are still far from understanding how actin is controlled, but it is likely that important actin regulatory proteins remain to be found. A major obstacle to understanding how actin operates in vivo is that its dynamic properties are inaccessible to study by classical biochemical analysis. Recently, however, the study of an intracellular bacterial parasite that uses eucaryotic actin to move itself, has given new insights to fundamental properties of actin in normal eucaryotic cells. We describe these bacteria and how they may be useful in testing actin dependent force generation. By using this system, we have identified a protein that may have an important role in forming regulatory complexes with

* Corresponding author. Tel.: (33) 1 42346375; fax: (33) 1 42346377; e-mail: golsteyn@curie.fr

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actin binding proteins, and possibly in generating force. The identification and characterization of actin interacting proteins is an important step in designing and testing various models that describe how actin is used to move eucaryotic cells.

2. Actin and actin binding proteins

Actin is found in a large variety of states and structures in cells. But as the repertoire of pure actin is limited to monomer (G-actin) and chain-like polymer (F-actin) states, it is clear that the diversity of actin structures and forces observed in cells arises from the interaction with other molecules, notably proteins. Most actin interacting proteins fall into a number of different groups that describe their effects upon actin [1,2]. For example, bundling proteins can form large actin cables from single actin fibres (alpha-actinin) and crosslinking proteins (filamin) can join actin filaments to form meshes which may be important for cell shape. In addition, there are proteins (capping protein) that block actin polymerization by binding to the end of a filament that is favoured for polymerization [3]. Together with monomer binding proteins such as profilin and thymosin $\beta 4$, actin filament formation can be regulated at the site of polymerization and by subunit availability. Nucleating proteins, such as gelsolin increase the rate of polymerization by favouring the production of actin nuclei, a rate-limiting step in actin polymerization. Other examples of actin interacting proteins exist, and the in vivo activity of many of the 50 or so members remains to be described [4]. An in vivo role is difficult to ascertain because some actin interacting proteins have multiple functions in vitro, such as gelsolin. Furthermore, many actin interacting proteins are regulated by combinations of tissue specific expression, response to chemical second messengers such as calcium and polyphosphoinositides, and phosphorylation state. When one considers the possible interactions of a large number of actin binding proteins, that are either singly or collectively regulated, it becomes easy to imagine the complexity of actin regulation in living cells. This complexity is manifest by the variety of actin structures observed in the cell, the rapidity with which actin structures can be modified, and the ability to have different actin structures in different places of the same cell at the same time.

3. Actin as a force that moves cells

Cells use actin to move. There are at least two ways this is accomplished. In the first of these, which is well documented, actin is used as a solid support or as a rail to which force generating molecules are attached. The classical example is the actino–myosin complex found in muscle tissue. The force generated by a myosin molecule has been measured by laser traps and has given us some of the first insights to the molecular forces that may exist in a cell [5]. Variations on this theme, but less well documented, are the interactions between actin and non-muscle myosins that hydrolyse adenosine triphosphate (ATP) as an energy source to drive intracellular transport in cells [6,7].

In this review, we concentrate on another actin dependent force found in cells. This mechanism is conceptually different from that of molecular motors in which actin itself is inert in the force generation system. Here, the source of force is the growing actin polymer; the addition of monomers that pushes against a surface and results in the movement of that surface. In cells, the addition of actin monomers to the tips of actin filaments is postulated to generate a force sufficient to move cells in the direction of the growing actin filament (reviewed in [8]).

4. Cell movement and actin

Cell movement is a co-ordinated process that involves steps of membrane extension, attachment, translocation, and retraction of rear components of the cell [9,10]. Membrane extension occurs at the leading lamellae, a thin, actin rich region that extends away from the cell body. In Fig. 1 we present a diagram of a lamellipodium of a cell moving in the direction of actin filament polymerization. In the model presented here, the lamellipodium contains short and long actin filaments with the 'barbed' end of the filament orientated to the plasma membrane [10]. The term barbed refers to the orientation of S1 myosin heads on filaments, the end which is favoured

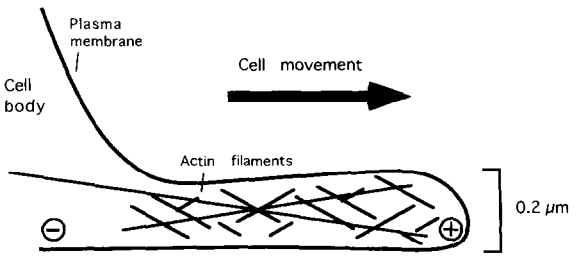

Fig. 1. Actin organization in the lamellipodia of moving cells. This diagram highlights the basic features of actin organization at the membrane of a moving mammalian cell. The cell would be moving from left to right as is marked by the heavy arrow. In the thin lamellipodium, both relatively long and relatively short actin filaments are orientated with their barbed ends towards the plasma membrane (marked by +) and their pointed ends towards the cell body (marked by −). The kinetically favoured site of actin polymerization is the barbed end, which in conjunction with actin organizing proteins, would generate a force to move the plasma membrane towards the right. The scale bar is shown on the right.

kinetically for polymerization relative to the non-favoured or 'pointed' end [11]. The addition of actin monomers to the barbed ends contributes to a force that pushes the plasma membrane in the direction of the growing actin filament. The molecules that regulate this process, and how they contribute to force generation are not well understood.

There are several different models that describe the production of F-actin at the plasma membrane. In the nucleation-release model, short actin filaments are produced at new sites of actin polymerization at the membrane [8,12]. In a treadmilling model, longer F-actin filaments are made and they contribute to a polarized framework that may in addition support other actin interacting, force producing proteins [13,14]. These models are similar in that actin polymerization occurs at the barbed end of filaments that are orientated towards the plasma membrane. They differ in that the nucleation-release model suggests that new, short filaments are generated at the plasma membrane in addition to polymerization at pre-existing filaments, whereas the treadmilling model emphasizes that polymerization occurs at pre-existing filaments resulting in long filaments.

In the lamellipodia of moving cells such as keratocytes, actin filaments are organized such that the barbed end is orientated towards the cell membrane [14]. This is believed to be the site of actin polymerization as suggested by direct observation of actin in cells [12,15]. Depending upon the method of analysis used, both long and short actin filaments can be found in this cell type, and likely in all moving cells. There are isolated examples, such as in leucocytes, in which the number of actin filaments increases during membrane extension, in support of the nucleation-release model [16]. Conversely, in platelets, capping and uncapping of pre-existing actin filaments, under the control of polyphosphoinositides, is believed to be a major regulator of actin polymerization [17,18].

Models that describe the forward movement of a plasma membrane predict several phenomena which are currently not well characterized in living cells. For example, the polymerization of actin at the plasma membrane requires the presence of actin monomers at the site of polymerization. This suggests that either transmembrane or membrane associated proteins may be present to attract actin monomers to maintain maximum actin polymerization. Similarly, nucleating proteins may be present at the plasma membrane. These proteins could promote F-actin formation by eliminating the rate-limiting step of nuclei formation. A membrane associated protein with nucleating properties (ponticulin) has been described in the amoebae *Dictyostelium*, but it is not known if related proteins are present in higher eucaryotic cells [19,20]. Finally, filaments, once formed would have to be released from the membrane to permit the production of new filaments.

Whether actin filaments are formed in cells by nucleation or by uncapping of pre-existing filaments, in either case, the forward motion of a surface resting against a polymerizing actin filament requires both a force and a space for the insertion of the actin monomer. This space may be provided by thermal fluctuation at the molecular level. A growing actin filament may then rectify a surface such as a plasma membrane rather than push it directly. The physical properties for this process have been named a Brownian ratchet [21]. The polymerization of actin in cells may be of sufficient strength to move a membrane forward. For example, in vitro, it has been shown that under polymerizing conditions actin can generate enough force to distort lipid membranes [22]. An important difference in cells would be the temporal and spatial control of this force by actin interacting proteins. Clearly there is much to be explained be-

fore any actin polymerization dependent cell movement can be understood, but observations in intact cells suggest that this mechanism does exist and suggests possible characteristics for proteins that may regulate this process.

5. *Listeria*: A procaryote used to study actin in eucaryotic cells

The nature of actin dependent force generation does not readily lend itself to characterization by biochemical approaches. Standard techniques of cell fractionation and protein purification are not compatible with isolating proteins that are either in low concentration in a cell or exercise their effects only in complex, dynamic systems such as moving cells. Therefore to identify new actin interacting proteins we examined a recently characterized bacteria whose method of movement shares several important properties with eucaryotic cell movement.

Listeria monocytogenes is a bacteria pathogen that is capable of invading eucaryotic cells and spreading from cell to cell in a tissue without passing to the extracellular medium [23]. Analysis of infected eucaryotic cells by electron microscopy revealed that after invasion of a cell, the *Listeria* become coated with actin that elongates behind the bacteria [24]. By causing the polymerization of actin, the bacteria move in cells at a rate of 1 μm per second, which approaches that of the maximal theoretical rate of actin polymerization [25,26]. Conversely, if actin polymerization is inhibited by the addition of cytochalasin, the bacteria stop moving [24]. This unusual form of infection and its relationship with host cell actin was immediately recognized as a potential experimental system to study actin organization in eucaryotic cells [24,27].

Molecular dissection of the genes responsible for *Listeria* movement in cells has identified the actA gene product, a protein of approximately 90 kDa, as being required for its mobility [28,29]. ActA is expressed at the surface of the bacteria and is therefore in contact with the host cell cytoplasm. Its expression results in the formation of short actin filaments which, as described above for mammalian cells, exhibit a polarity with the barbed end being closest to the bacteria (Fig. 2) [24,30–32]. This collection of actin filaments, or 'comet' which describes its image when viewed by fluorescent microscopy, propels the bacteria in the host cell until it contacts an adjacent cell, thereby continuing its infection and effectively evading host humoral immune response.

There are similarities between the organization of actin in comets of *Listeria* and of actin at the leading edge of moving eucaryotic cells [30,33]. The site of actin polymerization is at the barbed end which is immediately adjacent to the bacteria. This is reminiscent of the orientation of actin filaments relative to the plasma membrane of eucaryotic cells. The addition of actin monomers at this site, by an mechanism as yet unexplained, generates a force sufficient to

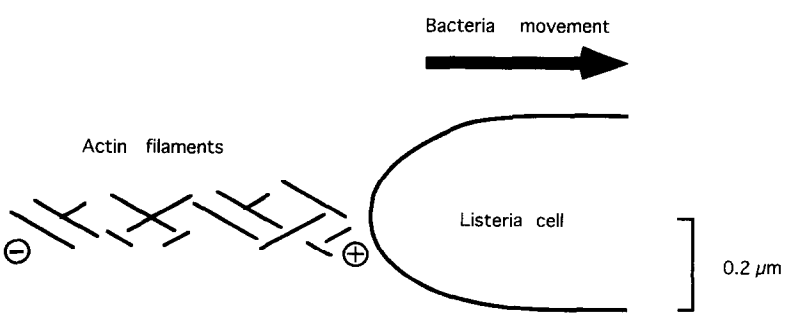

Fig. 2. Actin organization in the comet of a moving *Listeria monocytogenes*. This diagram highlights the basic features of actin organization in the comet that is used to propel *Listeria* upon infection of eucaryotic cells. The bacteria would be moving from left to right as marked by the heavy arrow. The comet is composed of short actin filaments that are orientated with their barbed ends towards the bacteria (marked by +) and their pointed ends away from the bacteria (marked by −). The kinetically favoured site of actin polymerization is the barbed end, which in conjunction with actin organizing proteins, would generate a force to move the bacteria towards the right. The scale bar is shown on the right.

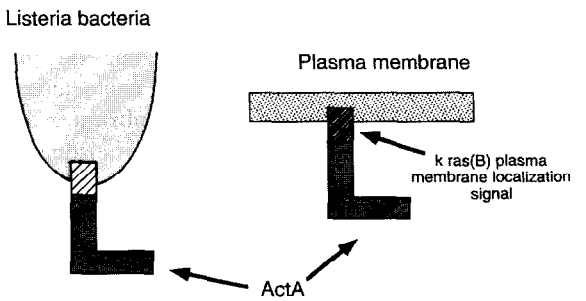

Fig. 3. Targeting ActA protein to the inner plasma membrane of eucaryotic cells. The orientation of ActA protein on the surface of *Listeria* (left) is compared to the orientation of a variant of ActA expressed at the inner plasma membrane of a mammalian cell (right). In both cases the C-terminal portion of the protein is adjacent to a membrane allowing the N-terminal and central portions to extend into the cytoplasm. In the mammalian cell this is the result of a CAAX box motif (k ras B plasma membrane localization signal) which is inserted into the ActA coding sequence. The CAAX box motif replaces a membrane localization signal that is specific for bacterial or mitochondrial membranes [36]. The CAAX box motif can be used to target any actin interacting protein (and other proteins) to the inner plasma membrane and observe their effects upon the actin cytoskeleton [33].

propel the bacteria through a viscous medium (cytoplasm). Furthermore, *Listeria* movement does not appear to use myosin motors, since it is not inhibited by chemical agents that disrupt mechano-motors although this does not exclude the possibility of the presence of uncharacterized motor proteins [34].

The interaction between ActA of *Listeria* and host actin may be a simplified model for understanding how eucaryotic cells use actin to generate a vectorial force. Molecular analysis of *Listeria* movement offers several experimental advantages over similar studies in eucaryotic cells. For example, the bacteria appear to move in cells without responding to 'stop' and 'start' biochemical signals that control eucaryotic cell movements. As a bacteria, *Listeria* is amenable to genetic manipulation which facilitates modification of proteins involved actin dependent movement. It is postulated, therefore, that *Listeria* movement may represent an example of a basic actin organizing system, and that this same system is present in eucaryotic cells, but without additional components that confound biochemical and biophysical analysis.

ActA protein is essential for *Listeria* movement in eucaryotic cells. *Listeria* that are mutant for ActA are capable of infecting eucaryotic cells but cannot spread to other cells and do not form actin tails [28,29]. When ActA is expressed, or attached to bacteria that are not related to *Listeria monocytogenes*, these bacteria acquire actin dependent comets and movement [31,35]. Further evidence that suggests that ActA interacts with actin arises from experiments in which ActA protein is ectopically expressed in eucaryotic cells. Transient transfection of mammalian cells with cDNA constructs encoding ActA results in a redistribution of actin and the disruption of normal actin containing structures

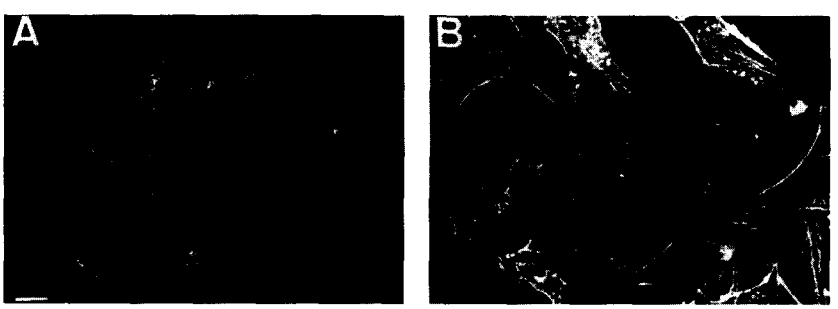

Fig. 4. ActA protein at the inner plasma membrane of mammalian cells reorganizes the actin cytoskeleton. Mammalian culture cells were transfected with cDNAs that encode ActA in fusion with a CAAX motif to direct the protein to the inner plasma membrane of the cell. Panel A. A transfected cell shows strong expression of ActA protein when fixed and stained with anti-ActA antibody. The expression of ActA results in a dramatic change in cell shape. Panel B. The same group of cells have been fixed and stained with rhodamine-labelled phalloidin to mark F-actin. The neighbouring cells which do not express ActA (therefore are not seen in Panel A) show normal actin containing structures and normal shape. The ActA transfected cell shows a striking disruption of F-actin structures. In addition, ActA transfected cells contain more F-actin than normal cells, suggesting that ActA can nucleate actin filaments. The bar represents 15 μm. These results have been previously described in [33].

[33,36]. In a series of transfection experiments, ActA cDNA constructs were prepared to encode ActA protein with an addition CAAX box motif at its C-terminus (Fig. 3). The CAAX box is a peptide sequence from the protein k-ras; it confers the property of inner plasma membrane association to proteins which contain the sequence [37,38]. As shown in Fig. 3, by placing a CAAX motif at the C-terminus of ActA, one can target ActA to the inner plasma membrane of cells in an orientation that is similar to that of ActA on the outer surface of the bacteria. In Fig. 4 we show a cultured fibroblast cell that expresses an ActA–CAAX variant [33]. The transfected eucaryotic cell shows a striking disruption of the actin cytoskeleton and loses its normal shape. Furthermore, by placing ActA at the plasma membrane one can measure an increase in total F-actin content of the cell suggesting that ActA can induce F-actin polymerization.

The response of the cell to ActA reveals that there are eucaryotic ligands that interact directly or indirectly with ActA. One of these is profilin, a protein of 15 kDa that binds to G-actin to form dimer complexes. It is believed to participate in signal transduction pathways because it is capable of binding to signalling molecules such as polyphosphoinositides [39,40]. The function of profilin in vitro and in vivo is difficult to describe because its effects are sensitive to the presence and the concentration of other actin interacting proteins [41]. Briefly, profilin is thought to enhance actin polymerization by promoting the production of the polymerization competent form of G-actin, G-ATP actin, in doing so it may effect the transfer of G-actin from thymosin β4 to F-actin [42]. Conversely, in vitro, profilin can inhibit actin polymerization by binding to actin monomers and blocking nucleation. Profilin is associated with *Listeria* dependent movement by several different experimental approaches. Firstly, it is found at the bacteria–comet interface, which is the site of actin polymerization [43]. Depletion of profilin from cell free extracts retards bacteria movement, and this movement is replenished upon the addition of purified profilin [43], however, profilin independent bacteria movement in cell free extracts has also been reported [44].

VASP (vasodilator stimulated protein) is a ligand of profilin and binds directly to ActA in vitro [45]. VASP is a 46–50 kDa, proline rich protein, found in actin rich sites in cells such as focal adhesions and the cell cortex [4,46]. It was originally characterized as a cAMP/cGMP protein kinase substrate, and therefore likely to be important in signal transduction pathways [47]. In *Listeria* infected cells, VASP is found at the intersection of the bacteria and its actin comet, suggesting that it has a role in co-ordinating actin filaments with ActA [48]. VASP is a member of a family of proline rich proteins whose members include Mena and Evl gene products. Like VASP, these proteins are localized in actin rich structures and in the *Listeria* actin comet interface [49]. Originally these proteins have been characterized by their role in signal transduction pathways, however, their precise role in *Listeria* motility is not known [50].

6. ActA like proteins in eucaryotic cells

The interaction of profilin and VASP like proteins with ActA of *Listeria* suggested that ActA like molecules may be present normal (noninfected) cells. In addition, it has been shown that eucaryotic proteins interact with ActA in the absence of any bacterial protein. For example, by transient transfection assays that place ActA at the plasma membrane, ActA is competent to recruit actin and to disrupt previously existing actin containing structures [33]. As there are proteins in eucaryotic cells that react with ActA it would be logical to assume that there may be proteins in cells that are structurally related to ActA, and may have a role in generating actin dependent movement. Further evidence for this comes from experiments in which peptides that share sequence identity to proline rich regions of the ActA molecule disrupt normal actin containing structures upon microinjection [51,52].

Based upon this evidence we suggest that ActA like molecules are present at the inner plasma membrane of eucaryotic cells as ActA is present at the surface of *Listeria*. This model is described in Fig. 5. This protein would be structurally and functionally similar to ActA such that it would be capable of generating short actin filaments that polymerize at the plasma membrane. Through the interaction with other host cellular proteins including, but not limited to, profilin and VASP-like proteins, actin is recruited

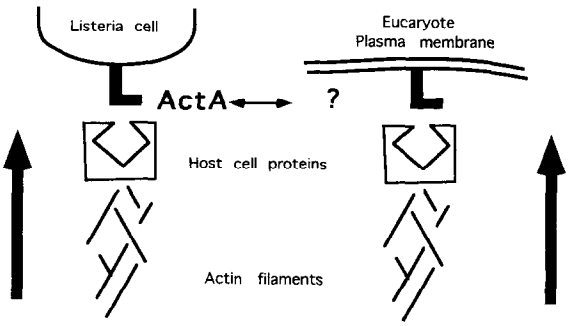

Fig. 5. Comparison of actin organization and movement between *Listeria* and a lamellipodia of a mammalian cell. There are similarities between the way that actin is organized in the comets of moving *Listeria* and the way actin is organized at the lamellipodia of mammalian cells. Actin filaments tend to be short (although it is possible that longer filaments also are present) and they are orientated with the barbed end toward the direction of movement. Host cell proteins such as VASP and profilin are found at the sites of actin polymerization in both *Listeria* and in the cortex of the lamellipodia. *Listeria* use ActA to recruit and organize actin by an unknown mechanism to generate force. Given the similarities between the two systems, it is likely that eucaryotic cells contain a protein that is structurally and functionally similar to ActA to move the plasma membrane. The arrows indicate the direction of movement.

to the barbed end of filaments that push the cell membrane forward. From this basic system which may be similar to *Listeria*, eucaryotic cells would likely have additional proteins which would enable them to be receptive to biochemical signals that control cell movement.

An important step in this model is whether or not eucaryotic cells contain molecules that are structurally and functionally related to ActA. Here we summarize our recent results in which we have identified a human protein, zyxin, that shares striking structural features with ActA (Golsteyn, Beckerle, Louvard and Friederich, submitted). The identification of this protein will facilitate studies of the biophysical properties of actin in cell movement.

7. Zyxin may be an ActA like protein in human cells

We used an immunological approach to identify human proteins that share similar epitopes and actin organization properties to ActA. Fig. 6 shows staining of a mammalian fibroblast cell by an affinity purified antibody directed against ActA of *Listeria*. The antibody strongly stains focal adhesions and the actin cortex of the lamellipodia. The antigen recognized by the antibody is human zyxin, a protein originally identified as an antigen that localizes to actin rich structures of cells as shown in Fig. 6 [53].

Zyxin is a protein of 573 amino acids that is expressed in many embryonic and adult tissues [54,55]. Its function is not known, but it is localized at focal adhesions and at the actin cortex, it is likely to be a component of the actin cytoskeleton. Further evidence of a role in the actin cytoskeleton comes from biochemical studies that show that zyxin interacts with proteins including alpha-actinin, CRP (cysteine rich protein) and VASP-like proteins [49,56–58]. It is striking that zyxin interacts with VASP like proteins that have been also described as ActA-binding proteins.

Protein sequence alignments of bacterial ActA and human zyxin are particularly informative for comparing the function of ActA with zyxin. First, as independent ActA antibodies react with human zyxin it is likely that the two proteins have a similar

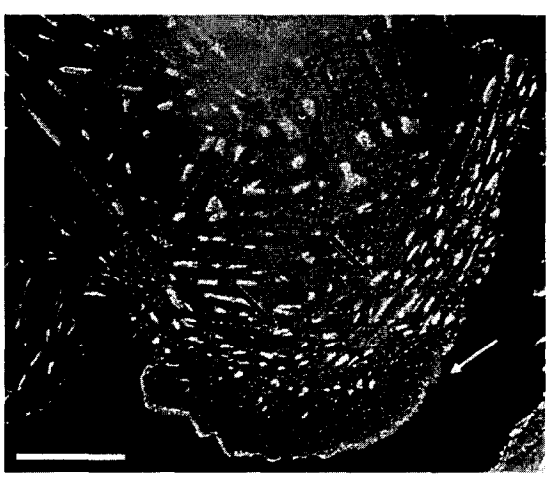

Fig. 6. Actin rich structures in mammalian cells are detected with an anti-ActA antibody. Mammalian culture cells were fixed and treated with an anti-ActA antibody. The antibody was detected with a fluorescently labelled secondary antibody and the cells were observed by microscopy. Numerous actin rich structures were strongly labelled, including focal adhesions (black arrows) and the actin cortex of the lamellipodia (white arrow). The antigen was shown to be zyxin, a protein that shares structural features with ActA of *Listeria*. The bar shown in white represents 2 μm.

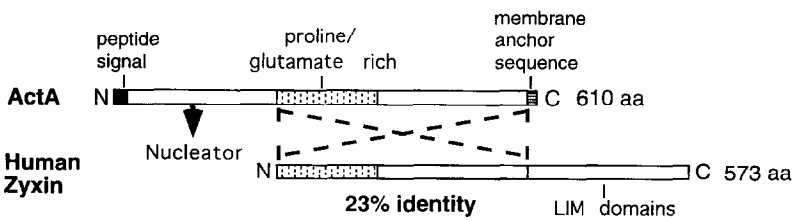

Fig. 7. Bacterial ActA and human zyxin proteins share a major structural domain. The structural domains of ActA protein (610 amino acids) and zyxin protein (573 amino acids) are represented by line diagrams. The two proteins show significant structural similarities in a large central portion of the protein that is enriched in proline and glutamate amino acids. The shared sequence identity between the two molecules is 23%. Regions required for ActA localization at the bacterial surface are labelled as the peptide signal sequence and the membrane anchor sequence, however, these peptide sequences are not required for actin organization activity. It is noteworthy is that the N-terminal domain of ActA, which is required for actin recruitment (labelled nucleator), is not present in zyxin and that LIM domains, found in the C-terminus of zyxin, are not found in ActA.

structure. Overall the proteins share only 23% sequence identity, which is found within a proline and glutamate rich region that composes the central portion of ActA and the N-terminal portion of zyxin (Fig. 7). The high number of prolines in this region is reminiscent of domains required for protein–protein interactions, and may explain the ability of zyxin to interact with other cellular proteins [55,59].

Further comparisons of the amino acid sequences of ActA and zyxin emphasize that the N-terminal region of ActA is not found in zyxin (Fig. 7). This result requires particular attention because the N-terminal region of ActA is essential for F-actin recruitment in transfection assays [33,36] and for *Listeria* movement as detected by infecting cell free extracts with *Listeria* harbouring ActA mutants [60]. Friederich and co-workers [33] found that the proline/glutamate rich region of ActA disrupted pre-existing actin containing structures such as stress fibres and focal adhesions when directed to the inner plasma membrane of a mammalian cell line. This effect, however, was limited when compared to full-length ActA, which contains an N-terminal domain which is not found in human zyxin. In this case, cells that express ActA at the plasma membrane are able to recruit actin filaments, thus increasing the amount of total F-actin present in the cell. Interestingly, the N-terminal domain of ActA, is also able to recruit actin filaments although it is less effective than full-length ActA, suggesting an enhancement effect of the proline/glutamate rich region of ActA. In a complementary assay, ActA, and various truncated forms were directed to the mitochondria of eucaryotic cells rather than the plasma membrane [36]. It was shown that the proline/glutamate rich region of the ActA molecule was important for F-actin recruitment, although not essential. Both of these experimental systems indicated that although the N-terminus of ActA is essential for actin recruitment, the efficiency of this recruitment is reduced in the absence of the proline and glutamate rich region. This result points to the possibility that zyxin may have another protein partner, whose activity may resemble that of the N-terminal domain of ActA.

Sequence comparisons also highlight that zyxin has protein domains that are not found in ActA. These domains (LIM domains), originally described in *Caenorhabditis elegans* Lin-11 protein, are found in three copies in zyxin [55,61]. The role of LIM domains is not known, although they have been shown to be required for protein–protein interaction with in a variety of proteins, some which are not directly related to the actin cytoskeleton [62]. The presence of LIM domains in zyxin and the absence of a known nucleator domain indicates, by sequence analysis, that zyxin and ActA are not identical structurally. Nonetheless, they share a large sequence that is rich in proline and glutamate residues whose function remains poorly defined. Experiments are required to test if the structural similarities between ActA and zyxin described here represent functional similarities.

Biochemical data suggests that the proteins that are required to propel *Listeria* in cells and the proteins found in actin rich complexes are similar up to the point of the proline and glutamate rich region

of ActA. One could predict then that a novel protein, similar in function to the N-terminus of ActA may also exist in eucaryotic cells. Experiments to test the function of the N-terminus of ActA reveal that it is essential for *Listeria* movement [50,60], and that it has actin nucleating activity [33]. It is also possible however, that a presumptive zyxin partner may have actin barbed end uncapping activity. Since actin polymerization at the leading edge of cells is regulated in part by proteins that cap the barbed end of actin, uncapping proteins would be required to restart polymerization on pre-existing actin filaments [3,63]. In studies of *Listeria* movement in cell free experimental systems, an uncapping activity present at the surface of *Listeria* has been predicted by estimating G-actin concentration in the presence of sequestering proteins [44]. Calculations predict that capping proteins must be present in cell extracts to account for the G-actin concentration, hence, actin polymerization at the *Listeria* surface (or at a plasma membrane) requires uncapping proteins. Direct biochemical testing of the N-terminus of ActA will be required to identify its function, and in turn may provide clues to help identify similar eucaryotic proteins in cells.

8. Perspectives: Moving forward with *Listeria*

The arrangement and organization of actin in moving cells remains obscure despite the impressive knowledge base of actin interacting proteins and their activity in vitro. Recently, the identification of actin dependent *Listeria* movement and characterization of its surface protein ActA has pointed to new directions for experimental approaches. This bacterial system generates movement that avoids the complex regulation found in wholly eucaryotic systems. It also provides the possibility of direct physical measurements of actin dependent force. A number of previously described actin binding proteins such as profilin and the VASP family members have been implicated in *Listeria* movement, suggesting that these proteins may have an important role in normal eucaryotic cell movement. By searching for ActA like molecules, human zyxin has been identified, which supports the model that ActA like molecules may be important in generating actin dependent movement.

The biochemical and biophysical mechanisms for actin dependent movement remains poorly resolved, in part due to the difficulties in working with cells. By identifying and purifying key cellular components, however, we are closer to being able to reproduce totally synthetic force generating systems in vitro. These simplified systems would contain actin, actin interacting proteins, an adenosine triphosphate dependent energy system and inert, polarized particles (C. Sykes and E. Friederich, personal communication). Ideally, by the addition specific proteins, one would be able to control and subsequently measure actin filament formation and force generation. Such studies should greatly further our understanding of cell movement in vivo.

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