Isoforms of α-Actinin from Cardiac, Smooth, and Skeletal Muscle Form Polar Arrays of Actin Filaments

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Abstract. We have used a positively charged lipid monolayer to form two-dimensional bundles of F-actin cross-linked by α-actinin to investigate the relative orientation of the actin filaments within them. This method prevents growth of the bundles perpendicular to the monolayer plane, thereby facilitating interpretation of the electron micrographs. Using α-actinin isoforms isolated from the three types of vertebrate muscle, i.e., cardiac, skeletal, and smooth, we have observed almost exclusively cross-linking between polar arrays of filaments, i.e., actin filaments with their plus ends oriented in the same direction. One type of bundle can be classified as an Archimedian spiral consisting of a single actin filament that spirals inward as the filament grows and the bundle is formed. These spirals have a consistent hand and grow to a limiting internal diameter of 0.4–0.7 μm, where the filaments appear to break and spiral formation ceases. These results, using isoforms usually characterized as cross-linkers of bipolar actin filament bundles, suggest that α-actinin is capable of cross-linking actin filaments in any orientation. Formation of specifically bipolar or polar filament arrays cross-linked by α-actinin may require additional factors that either determine the filament orientation or restrict the cross-linking capabilities of α-actinin.

Key words: electron microscopy • actinin ultrastructure • actinin metabolism • actins ultrastructure • molecular structure

Introduction

The F-actin cross-linking protein α-actinin is an antiparallel homodimer. Each peptide has an NH_2-terminal actin-binding domain followed by four spectrin-like triple helical motifs arranged in tandem, and a COOH-terminal domain with a pair of E-F hand motifs (Blanchard et al., 1989). α-Actinin is localized to many different sites within cells where actin filaments are arranged in oriented arrays. It is found at anchoring sites of antiparallel actin filaments, such as the Z-disk of striated muscle (Masaki et al., 1967; Lazarides and Granger, 1978) and the cytoplasmic dense bodies of smooth muscle (Schollmeyer et al., 1976; Geiger et al., 1981), to anchoring points of polar arrays of actin filaments, such as the membrane-associated adhesion plaques of smooth muscle (Schollmeyer et al., 1976; Geiger et al., 1981; Small, 1985) and the focal adhesions of nonmuscle cells (Burridge et al., 1990) as well as to regions of poorly oriented actin filaments in the leading edge of motile cells (Langanger et al., 1986). These varied types of filament orientation in actin bundles formed in vivo with α-actinin imply a lack of specificity of the cross-linking to the filament orientation. However, it remains unclear whether the formation of bipolar or unipolar arrays is determined by an isoform-specific intrinsic cross-linking orientation of α-actinin or is dictated by the presence of other cytoskeleton proteins.

Examination of F-actin orientation in bundles formed in vitro in bulk solution using the smooth muscle isoform of α-actinin indicated a preference for bipolar cross-linking (Meyer and Aebi, 1990). Smooth muscle α-actinin is somewhat unique among muscle isoforms in its localization to both cytoplasmic dense bodies and to membrane-associated adhesion plaques (Schollmeyer et al., 1976; Geiger et al., 1981; Small, 1985). α-Actinin isoforms from skeletal muscles are confined to the nonterminal Z-disks (Endo and Masaki, 1984; Atsuta et al., 1993). Conversely, antibodies to smooth muscle α-actinin stain the sarcolemma of skeletal muscle cells and myotubes, locations where polar arrays of actin filaments form (Atsuta et al., 1993). These observations suggest a somewhat greater specificity for actin filament orientation for skeletal muscle isoforms or the effect of targeting proteins that direct the different isoforms to specific sites.

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The numerous other proteins that interact with α-actinin complicate the understanding of α-actinin–actin interactions. These include the β-integrins (Otey et al., 1990), vinculin (Wachsstock et al., 1987), intercellular adhesion molecule (ICAM) (Carpen et al., 1992; Heiska et al., 1996), titin (Ohtsuka et al., 1997; Sorimachi et al., 1997), zyxin (Crawford et al., 1992), and nebullin (Nave et al., 1990). These proteins, which bind to different domains on α-actinin, may affect its interaction with actin in ways not yet understood.

Previously, we had investigated the possibility of using lipid monolayers as a vehicle for producing two-dimensional (2-D)[1] bundles of actin cross-linked with actin-binding proteins for the purpose of investigating these kinds of effects (Taylor and Taylor, 1994). Specimens that are consistently single-layered facilitate structural studies by eliminating the superposition effects that complicate interpretation of images from three-dimensional (3-D) bundles. Our earlier study using α-actinin from smooth muscle revealed a propensity to form polar bundles, in contradiction to the earlier studies examining α-actinin–actin bundles formed in solution (Meyer and Aebi, 1990). In an effort to better define the specificity of filament orientation, we have repeated the earlier study, this time using skeletal, cardiac, as well as smooth muscle isoforms of α-actinin. The results demonstrate that polar actin bundle formation is as much an intrinsic property of α-actinin as bipolar bundle formation. The results suggest that consistently polar or bipolar α-actinin–actin bundles require extrinsic factors to promote one polarity over the other.

Materials and Methods

Protein Purification

Actin was prepared from rabbit muscle acetone powder (Pardee and Spudich, 1982) with the modification that the chromatography step was done on a Superose-12 column. G-actin was prepared from F-actin by overnight dialysis at 2°C against buffer A, which consisted of 2 mM Tris-Cl, 0.2 mM Na_2ATP, 0.02% β-mercaptoethanol, 0.2 mM $CaCl_2$, 0.01% NaN_3, pH 8.0 (at 25°C). The G-actin preparation was clarified by high-speed centrifugation immediately before use.

α-Actinin from rabbit skeletal and cardiac muscle was obtained from a myofibril preparation by first dissolving the protein in 1.0 M NaI, 25 mM Tris, 2 mM $MgCl_2$, pH 8, in the presence of 0.02% β-mercaptoethanol, followed by clarification and elution from hydroxyapatite with a gradient of 5–350 mM sodium phosphate in 0.6 M NaCl, 2 mM $MgCl_2$, pH 7.5 (Schachat, F.H., unpublished data). α-Actinin from chicken gizzard was prepared according to Feramisco and Burridge (1980).

Preparation of 2-D paracrystalline arrays of F-actin–α-actinin was done as described previously (Taylor and Taylor, 1994). This method involves polymerizing G-actin in the presence of α-actinin under the positively charged lipid monolayer composed of didodecyldimethylammonium bromide and dilaurylphosphatidylcholine in a molar ratio of ~1:1. Protein concentrations are typically 0.3 μM in a polymerization medium buffered with phosphate. Polymerization is carried out at 4°C. Arrays are stable for months, although they may change order, morphology, and extent over long periods.

Actin spirals were formed the same way as cross-linked spirals, using a concentration of G-actin in the wells of 0.3 μM. No α-actinin was present in these experiments. In one experiment, actin spirals were formed in large numbers when polymerization was done at room temperature and the samples recovered after 12 h.

[1]*Abbreviations used in this paper*: 2-D, two-dimensional; 3-D, three-dimensional.

EM

Specimens for EM were made by transferring the bundles to 200–300-mesh copper grids coated with a reticulated carbon support film (Kubalek et al., 1991). Reticulated carbon films were prepared according to Fukami and Adachi (1965). Specimens were stained using 1% aqueous uranyl acetate, air-dried, and before EM examination, stabilized by vacuum deposition of a thin layer of carbon.

Image Processing

The filtered image was obtained by interpolating the original micrograph along the unit cell edges with an integral number of samples per unit cell. After Fourier transformation, a mask with an axial height of one pixel but with a width that spanned the entire transform was then applied to the computed Fourier transform. This procedure results in an image filtered along only the b-axis (the filament axis) with no filtering along the a-axis (the interfilament axis). We refer to this process as column averaging. After filtering, the image was orthogonalized back to its original dimensions.

Results

Morphology of 2-D α-Actinin–F-Actin Bundles

Our experimental approach utilizes a positively charged lipid monolayer to form 2-D paracrystalline complexes of F-actin and α-actinin (Taylor and Taylor, 1994), thereby eliminating superposition effects that complicate interpretation of 3-D bundles formed in situ or in solution. This makes it easier to observe subtle differences, if any, between bundles formed by different isoforms. Typical α-actinin–F-actin arrays formed consist of 4–10 filaments heavily cross-linked by α-actinin molecules (Fig. 1).

Arrays formed by this method always reveal the same morphology with regards to the α-actinin cross-linkers (Fig. 1 and Fig. 2 a). This holds for all three muscle isoforms of α-actinin tested as well as with the nonmuscle isoform from *Dictyostelium*. α-Actinin molecules bind periodically along the actin filament, most commonly with one to two α-actinins per crossover repeat. Rarely as many as three α-actinins can be seen clustered together. The α-actinin molecules predominately maintain a single orientation within the bundle. The cross-links do not usually form paired, chevron-like structures with a pseudomirror-line parallel to the filament axis. Some bundles have cross-links that are angled in opposite directions between the same filament pair, thereby producing triangular struts between the filaments. This kind of structure was usually observed in the best ordered bundles (Fig. 1 a and Fig. 2 a), presumably because the arrangement produces a more regular filament separation and a bundle that resists deformation better than those with a single orientation of α-actinin cross-linkers.

The filaments themselves are quite regularly positioned within the unit cell, despite the rather disordered arrangement of the α-actinin cross-linkers, thereby producing a sampled computed diffraction pattern (Fig. 2 b). This differential ordering between filaments and cross-linkers is manifest in the filtered image. Filtering the image in one dimension along the actin filament axis reveals the individual actin subunits while at the same time smearing out the density due to α-actinin. Some density due to α-actinin remains in the filtered image, thereby identifying the location on the actin filament where α-actinin binds. It is clear from comparing the filtered image and the raw image that

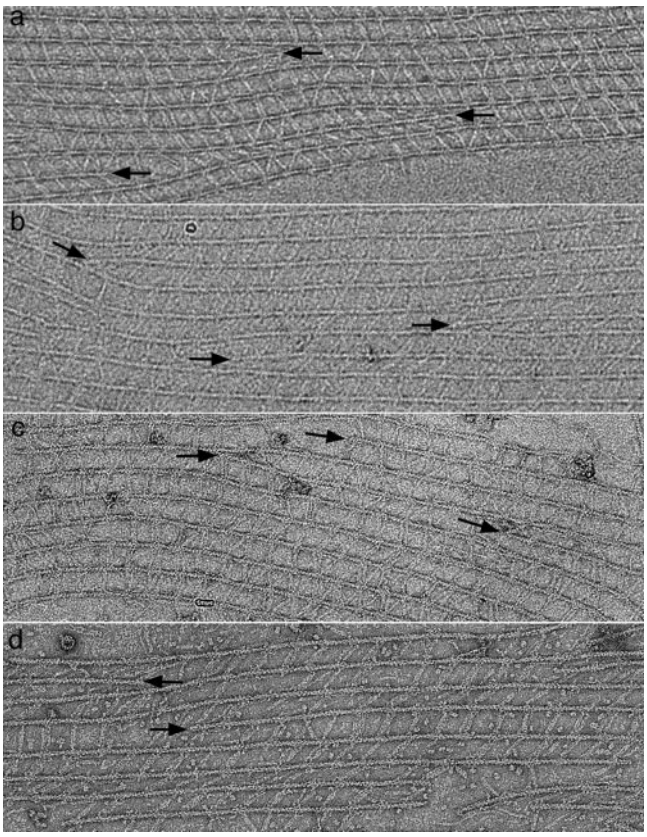

Figure 1. Arrays of actin filaments cross-linked with α-actinin from (a) skeletal muscle, (b) smooth muscle, (c) cardiac muscle, and (d) *Dictyostelium discoidium*, a nonmuscle isoform. In all three cases, the morphology of the bundles is similar, including the insertion of filaments into the bundle. Arrows denote filament insertions. Note that insertion does not disrupt the cross-linking after the filament spacing has adjusted to the optimal amount. There is also no alteration in the preferred attachment angle of the cross-linkers once the filament spacing has adjusted, suggesting that the filaments are incorporated in the same orientation as the previously cross-linked filaments. The bundles shown in b and c are portions of spirals.

the binding of α-actinin to actin occurs where the actin filament profile is at its widest (Fig. 2 c). This location is also consistent with that deduced from 3-D reconstructions of actin filaments decorated with the 27-kD actin-binding domain of α-actinin (McGough et al., 1994). Also note that this location places α-actinins on the left and right sides of an actin filament at different heights relative to the lipid monolayer. This difference in height is important for understanding how polar bundles may be favored when grown on a surface (see below).

Differences between bundles formed with the different isoforms may be related to the stability of the bundles. Although all muscle isoforms produced bundles with the same general appearance of α-actinin cross-links, the best ordered bundles were formed using skeletal muscle α-actinin. The arrays formed using skeletal muscle α-actinin are very stable and increase in size with time. We have observed some preparations ~6 mo of age that contained filament arrays many micrometers in extent (Taylor and Taylor, 1999). The arrangement of α-actinin and actin in these large bundles is morphologically indistinguishable from that in the smaller bundles shown here. The large size and more ordered appearance of bundles formed using skeletal muscle α-actinin occur because this isoform does not form 2-D crystals under the conditions used to form the 2-D bundles. Smooth and cardiac muscle α-actinin will form 2-D crystals under these conditions. The competing processes of bundle formation and crystallization seem to favor crystallization.

Orientation of Actin Filaments within the Bundles

Evidence for the cross-linking orientation comes from four observations. One of these is the orientation of the α-actinin cross-linkers. We expect a cross-linker forming a bipolar array of actin filaments to be arranged with a narrow angular distribution with respect to the filament axis. The expected structure would have a chevron-like appearance with respect to individual filaments. Proceeding from one actin filament to the next should reveal a systematic alternation in the angle that the α-actinin makes with respect to successive filaments, thereby creating a herringbone pattern. As noted previously, the majority of the bundles have cross-links with a single orientation across the bundle, with only a small proportion angled oppositely (Fig. 1 a and Fig. 2 a). However, when two orientations are observed, they occur between the same pair of actin filaments.

Second, the ability of filaments to insert into the cross-linked array depends on cross-linking specificity. If α-actinin were a constitutive cross-linker of antiparallel actin filaments, insertion of an actin filament must disrupt the pattern of cross-links to one of the two adjacent filaments. If α-actinin were a constitutive cross-linker of identically oriented actin filaments, actin filaments could only be incorporated into the bundle if they were of the same orientation. If the cross-linker had no orientation specificity, we at least expect some change in the orientation of the cross-linker. Experimentally, when actin filaments are incorporated into the bundle, after the spacing between filaments adjusts, cross-links of identical appearance reform, indicating that the additional filament was incorporated into the bundle in the same orientation as those preceding it (Fig. 1). At the very least, this observation would support the conclusion that α-actinin lacks specificity with respect to the orientation of the actin filaments that it cross-links. However, the observation seems to support the conclusion that these are polar bundles and that filaments are incorporated into the array in the same orientation as those preceding it.

Third, many arrays give rise to sampled computed diffraction patterns (Fig. 1 b), indicating a high degree of order in the actin filaments or the α-actinin cross-links. The distribution of sampled spots in the diffraction pattern can be used to determine orientation as well as relative rotation and axial shift between successive filaments, provided the resolution extends to the 5.9- and 5.1-nm layer lines (Sukow and DeRosier, 1998). This resolution is usually sufficient to unambiguously determine filament polarity in isolated actin filaments by image processing. There are three cases relevant to the question of polarity: (1) If the bundle was bipolar, the unit cell would contain two oppo-

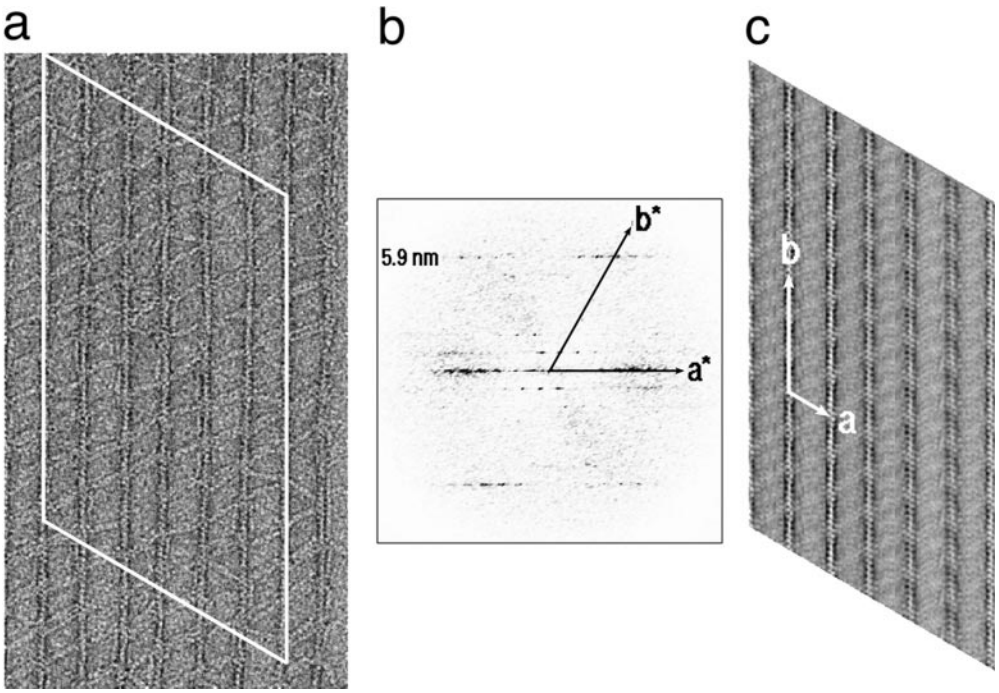

Figure 2. Array of actin filaments cross-linked by rabbit erector spinae muscle α-actinin. Typically, one or two α-actinin cross-links form within each crossover period between adjacent filaments. Paired links can be either parallel to each other or differently angled to produce triangular struts between actin filaments. The unit cell has dimensions of a = 45.4 nm, b = 112.1 nm, γ = 120.4°, and contains only one actin filament. The interfilament spacing is 39.1 nm. (b) Computed diffraction pattern from the region outlined in a. Sampling on all layer lines indicates that the actin filaments in the array are oriented in the same direction. The helical structure of the actin filaments is 41 subunits in 19 turns of the 5.9-nm genetic helix. (c) Filtered image of the region outlined in a.

sitely oriented actin filaments and the spacing of spots on the equator would be twice the spacing on the outer layer lines. The diffraction would be sampled on all layer lines if the translations and rotations between successive filaments were periodic. (2) If the actin filaments were oriented randomly, the outer layer lines would be unsampled and the inner parts of the equator would be sampled. (3) If the bundle was polar and the filaments regularly arranged in the bundle, the diffraction would be sampled on all layer lines and the spacing of spots on the equator would be the same as those on the outer layer lines. Moreover, if successive filaments were related by fixed translation along the filament axis, the row lines on the inner and outer layer lines will be angled and intersecting. It can be seen from the optical diffraction pattern that this third case describes the 2-D bundles. The repeating unit cell contains a single actin filament, a result predicted for polar cross-linking.

Spirals of F-Actin

The fourth and strongest piece of evidence comes from single actin filaments coiled into spiral figures by α-actinin cross-linking. This kind of structure can only occur if α-actinin can cross-link a polar arrangement of actin filaments (Fig. 3). Spirals have been obtained for all muscle isoforms tested, demonstrating that there is no qualitative difference in their ability to form polar bundles. Within the spirals, the appearance of α-actinin cross-links is indistinguishable from those in the more ordered bundles with straight filaments.

Actin filaments that coil into spirals apparently do so from the outside inwards. Because coiling occurs on a plane surface, the hand depends on the direction of view. When viewed from the direction of the solution onto the monolayer, coiling from the outside inwards is in a clockwise direction, or with a left-handed sense. We have observed 33 α-actinin–actin spirals of different shapes and sizes, all of which have had the same hand. In some spirals, particularly the ones with an oval shape, well-ordered regions of straight filaments have been observed in regions of the spiral structure. The largest number of turns observed in a spiral was 19. The longest filament length measured in a spiral was 31.5 μm.

Our experiments with muscle isoforms so far have not produced ordered arrays that could be identified as bipolar. Potentially, cross-links between oppositely oriented actin filaments (bipolar cross-links) might be formed between actin filaments of adjacent spirals. All spirals have the same hand, so where they touch, the actin filaments are oppositely oriented. Spirals are found in proximity to each other and cross-links form between them where they touch. These structures have not been found positioned within the holes in the support film in such a way that the actin filaments in each spiral could be traced uninterrupted to the point of inter-spiral contact. However, based on the arguments given above for inserted filaments, even if an actin filament or small bundle were interposed between spirals, a bipolar cross-link must nevertheless form at some point between the two spirals.

Quite often, when spirals form, the actin filament will appear to break toward the center of the spiral (Fig. 3). In many cases, it appears that the polymerization continues from the break point through initiation of a new filament. This second filament usually grows a short distance before it too stops and another filament begins to grow. The smallest inner diameter formed before a filament break was 0.44 μm (Fig. 4).

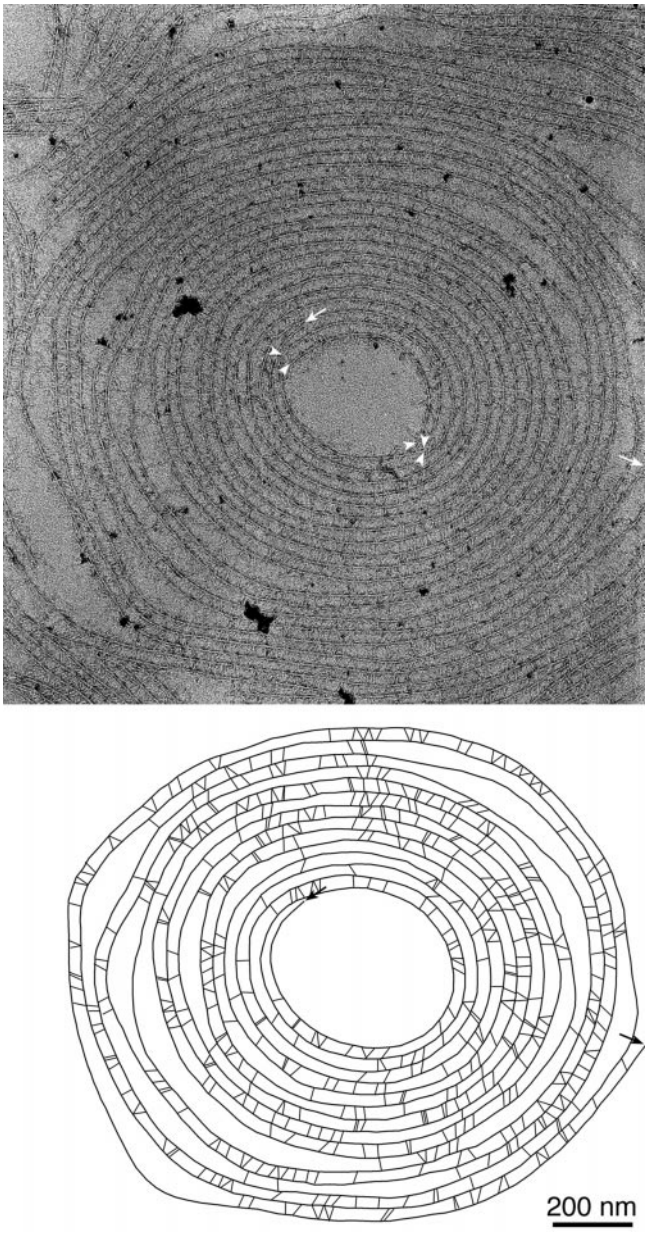

Figure 3. α-Actinin–F-actin spiral figures. (Upper panel) Spiral figure of one actin filament cross-linked with skeletal muscle α-actinin. The two segments of actin filament cross-linked by α-actinin are always oriented in the same direction; the resulting bundle is always polar. (Lower panel) Drawing indicating the path of the filaments and the position of the most clearly identified cross-links. When viewed in a direction from the solution phase onto the monolayer, the spirals coil in a left-handed (clockwise) sense, as the filament grows inward from the outside. The arrows indicate the start and end of the continuous filament run. After the long segment stops growing, at the center of the bundle, additional filaments grow for short periods and they too stop. The beginning and ending points of these filament fragments are indicated by arrowheads in the upper panel.

To obtain some sense of whether the inner diameter represents an actual limit, we measured the internal diameter of all 33 spirals found to date. The range of limiting inner diameters for the α-actinin–F-actin spirals is fairly

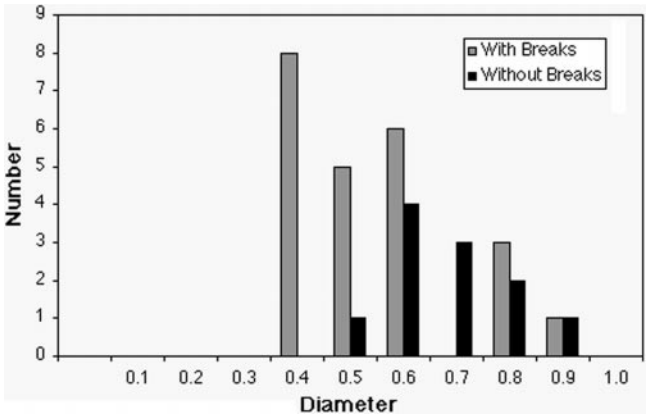

Figure 4. Histogram showing the range of internal diameters of actin spirals cross-linked by α-actinin. Many spirals are not perfectly circular. Some have oval or ellipsoidal shapes. In these cases, the width of the minor axis was taken to be the limiting diameter. In cases where the shape was more irregular, we attempted to draw a circle tangential to the actin filament at the point of the break.

large and encompasses both spirals with filament breaks and spirals with an unbroken end. The smallest diameter observed with no break was 0.56 μm, but only a single unbroken spiral was found that small. Most unbroken spirals had an inner diameter of ∼0.65 μm. Comparing the histograms for all spirals (Fig. 4), it seems clear that a limit is reached between 0.4 and 0.5 μm inner diameter, below which the spirals cannot continue grow.

To test whether spiral formation required α-actinin binding, we attempted to form spirals using actin alone (Fig. 5). Spirals formed readily using G-actin alone, indicating that α-actinin is not required to form this structure. Moreover, based on the limited number of these spirals formed to date, the hand of the coiling is consistent and the same as that with the cross-linked spirals. This indicates that α-actinin merely adds to the growing F-actin spiral, perhaps stabilizing it. Although we have not yet observed as many F-actin spirals as cross-linked spirals, there nevertheless seems to be a limiting inner diameter of ∼0.4 μm, below which the spiraling filament cannot continue to grow.

Discussion

Cross-linking Orientation in 3-D α-Actinin–F-Actin Bundles

α-Actinin has been localized to a wide range of actin-containing structures, some of which contain a polar arrangement of actin filaments, whereas others have a bipolar arrangement. However, in vivo, these α-actinin–containing structures are accompanied by other proteins that may be affecting the interaction between α-actinin and actin. It is therefore important to examine the relative orientation of actin filaments cross-linked by α-actinin in vitro, where the influence of other factors can be controlled. The results obtained in this study suggest an unexpected tendency of muscle isoforms of α-actinin to form polar actin bundles

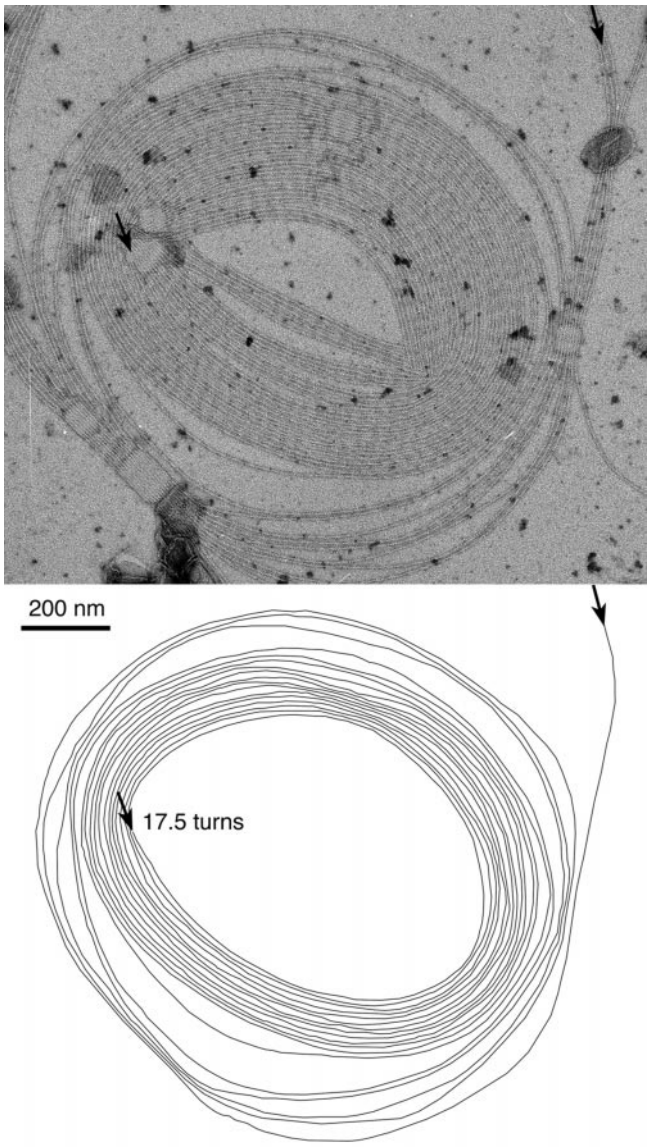

Figure 5. (Upper panel) Electron micrograph of a spiral figure formed from G-actin polymerization alone. Arrows indicate the start and end of the continuous filament. (Lower panel) Drawing of the continuous filament. In the center, the short lengths of the filaments have condensed into a paracrystalline raft near the arrow.

on a surface, whereas the cellular localization to Z-disks and homologous structures would suggest a predominately bipolar cross-linking orientation.

Our results for 2-D actin bundles differ somewhat from the only previous investigation into the polarity of α-actinin–F-actin cross-links determined from 3-D bundles formed in bulk solution (Meyer and Aebi, 1990). That work investigated cross-linked actin filaments using α-actinin isoforms from chicken gizzard smooth muscle as well as nonmuscle isoforms from *Acanthamoeba* and *Dictyostelium*. These isoforms differ in actin affinity, muscle isoforms having greater affinity than nonmuscle isoforms, as well as molecular length, *Acanthamoeba* being ~10 nm longer than the others. There are also several similarities and differences between this study and that reported earlier. Both studies used polymerization of actin in the presence of α-actinin, which optimizes the accessibility of the filaments to the cross-linker. However, in this work, the concentration of actin is 40× lower (0.3 vs. 12 μM), the low actin concentration chosen to minimize the concentration of actin filaments in the bulk phase. The ratio of α-actinin to actin is also high in this work, which favors bundle formation and helps prevent the actin from forming paracrystals on its own, which it will do in this system (Taylor and Taylor, 1992).

Using smooth muscle and *Dictyostelium* α-actinin, which are isoforms of similar molecular length, Meyer and Aebi (1990) observed predominately bipolar cross-linking, although a relatively small proportion of polar cross-links were also observed. Their observation may have less to do with a bias toward bipolar cross-linking than with a natural tendency of actin filaments to form mixed polarity bundles in which the proportion of polar and bipolar α-actinin cross-links simply reflect the filament orientation. Francis and DeRosier (1990) investigated an unusual disorder in bipolar bundles (paracrystals) of actin filaments formed in the presence of Mg^{2+}. This disorder was also said to explain observations made on polylysine-induced actin paracrystals (Fowler and Aebi, 1982). The Francis and DeRosier bundles consist of nearly equal amounts of filaments in each orientation, but with triangular groupings of nearest neighbor filaments that consist of two that are parallel to each other and one that is antiparallel to the other two (Fig. 6). In such bundles, there will be, on average, a 67% chance that a cross-link will occur between filaments of opposite orientation, and a 33% chance that a cross-link will occur between filaments of the same orientation (Fig. 6, a–c). To test this, we generated 20 bundles of 19 filaments according to Francis and DeRosier (1990) with the result that 65 ± 2.5% of cross-linking opportunities were bipolar and 35 ± 2.5% polar. This prediction is rather similar to that observed (82 vs. 18% for chicken gizzard and 70 vs. 30% for *Dictyostelium*), which was based on a sampling of only 10 or 11 filaments in each case.

Somewhat surprisingly, for cross-linking to next nearest neighbor filaments, the bipolar–polar cross-linking probabilities are just the opposite, even in the same bundle; there is generally a higher probability of cross-linking parallel actin filaments than antiparallel actin filaments (Fig. 6, d–f). The proportion of polar vs. bipolar cross-links found by Meyer and Aebi (1990) was 80 vs. 20% for *Acanthamoeba* α-actinin, which is, fortuitously, the proportion shown in the diagram. In our 20 bundles, the change in cross-linking orientation was less dramatic. Of the 20 bundles, 18 favored polar next nearest neighbor cross-linking, with an average probability of 63 ± 10% for polar and 37 ± 10% bipolar cross-linking, and two cases favored bipolar cross-linking. However, the change in trend is very clear. Next nearest neighbor filaments are further apart than nearest neighbor filaments, and require a longer cross-linker, which is the case with *Acanthamoeba* α-actinin. Bundles of actin filaments cross-linked by *Acanthamoeba* α-actinin consist of closely spaced filaments, so that a next nearest neighbor cross-link is geometrically possible (Wachsstock et al., 1993). This implies that a bundle formed initially with predominately bipolar cross-links between nearest neighbor filaments could change to one

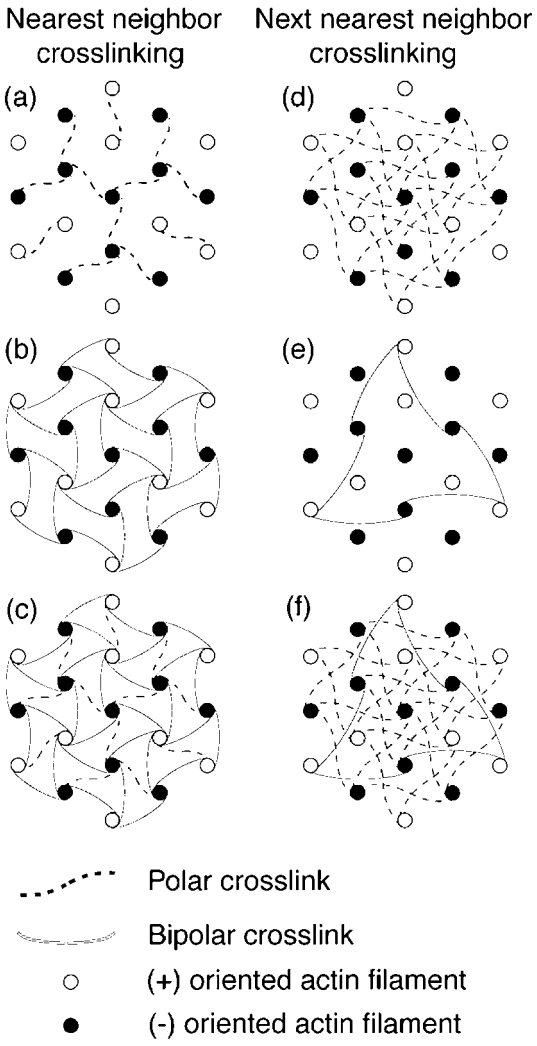

Figure 6. Filament arrangements in bipolar 3-D bundles with random filament orientations. This particular bundle was adapted from Fig. 4 a of Francis and DeRosier (1990). There are 19 filaments in the bundle (9 in one orientation and 10 in the other). The actin filaments occur in random orientations with respect to + or − directions, with the added constraint that any triplet contains two filaments in one orientation and one in the other. Open and filled circles represent the two filament orientations. Bipolar cross-links (C-shaped) have been drawn so that they begin and end on one side of the center line adjoining the two filaments. A local twofold symmetry axis centered on the cross-link is possible for this positioning. Polar cross-links (sigmoid shape) cross the center line to bind the other side of the neighboring actin filament. In projection, a polar cross-link would appear to have twofold symmetry about an axis parallel to the filaments and through the cross-link. However, an actual twofold axis would require that the cross-link be perpendicular to the filament axis. (a–c) Crosslinking pattern between nearest neighbor filaments. (a) Polar nearest neighbor cross-links. (b) Bipolar nearest neighbor cross-links. (c) Complete pattern of 42 nearest neighbor cross-links, of which 12 are polar (28%) and 30 are bipolar (72%). Note that cross-links can be drawn without crossing each other. (d–f) The same bipolar actin bundle shown in a but with cross-links drawn to next nearest neighbor filaments. (d) Next nearest neighbor polar cross-linking pattern. (e) Next nearest neighbor bipolar cross-linking pattern. (f) Overall next nearest neighbor cross-linking pattern. In this case there are only 30 cross-links, 6 of which are bipolar (20%) and 24 of which are polar (80%), which is the opposite trend for nearest neighbor cross-linkers. Note also that in this arrangement, cross-links drawn in this planar view cross each other, unlike for the nearest neighbor cross-links. This does not necessarily mean that they interfere with each other, since the actual pattern has 3-D depth to it.

with predominately polar cross-links between next nearest filaments by rearrangement of the cross-links during bundle formation as the actin filaments are drawn closer together. With a long cross-linker, such as *Acanthamoeba* α-actinin, it may become more favorable to detach from the nearest neighbor actin filament and rebind to a filament further away. Rearrangement of cross-links as needed to achieve this is a necessary requirement in the formation of bundles from gels (Wachsstock et al., 1994).

Actin bundles such as those described by Francis and DeRosier (1990) cannot be formed by addition of randomly oriented filaments, which would produce some triangular groupings of filaments with a single orientation. To produce triangular groupings with a 2:1 ratio with respect to filament orientation requires some energetic factor, which they suggested was entropy. A disordered, bipolar bundle would have higher entropy than an ordered polar bundle. Moreover, they argued that 2:1 actin bundles had a higher proportion of bipolar cross-links and would thus be more stable. However, positively charged agents like Mg^{2+} and polylysine are nonspecific actin binders, which produce paracrystals by neutralizing surface charges on actin. We suggest that the Francis and DeRosier actin bundles explain the proportion of polar and bipolar cross-links produced by α-actinin in 3-D bundles. It would therefore seem that α-actinin is behaving like a nonspecific actin cross-linker, at least as regards filament orientation, even though it binds actin at a specific site.

Image analysis of 2-D arrays of skeletal muscle α-actinin reveals a molecular twofold rotation axis perpendicular to the long axis of the molecule (Tang, J., D.W. Taylor and K.A. Taylor, unpublished observation). Such a twofold axis is readily expressed locally in a bipolar bundle, but is not so easily expressed in a polar bundle unless the α-actinin is oriented perpendicular to the filament axis, or when the cross-links are angled, a twofold screw axis could be present. Nevertheless, the expression of a twofold axis in the same molecule in a polar and a bipolar bundle requires that the actin-binding domain be capable of rotating up to ~90° about the long axis of the molecule. Similar capabilities have been observed in other proteins, for example myosin, which can rotate 180° to bind actin filaments of either orientation, even when incorporated into an array of thick filaments (Reedy et al., 1989). An alternative possibility is the presence of two different modes of interaction between actin and α-actinin, in support of which there is at present no data.

Factors Favoring 2-D α-Actinin–F-Actin Bundles

If the orientation and separation of the actin filaments govern the polarity of bundles obtained in solution, what then is the role of the monolayer in directing the cross-linking of a predominately polar arrangement of filaments? The monolayer is fluid at the temperatures used in

the study, and therefore does not restrict the motions of the molecules bound to it except to rotation and translation within the monolayer plane during the time they are bound. However, by providing a planar surface for assembly, it can affect the final structure in ways not possible in a bulk solution. First, it may sterically block the accessibility of α-actinin to actin monomers, and second, it may stabilize the bond between actin and α-actinin if one or both are attracted to the lipid. To understand these effects it is necessary to first explore the spatial relationships between filament and cross-linker in bipolar and polar filament arrays.

The helical symmetry of the actin filament presents α-actinin with actin monomers in different orientations depending on whether the filaments are parallel or antiparallel. As long as the cross-linker binds actin stereospecifically, the cross-linked actin monomers will lie in different positions with respect to the interfilament axis for bipolar and polar cross-linking. A bipolar cross-link between α-actinin and F-actin would be positioned on one side of the interfilament axis (Fig. 6 b), thereby expressing a local twofold symmetry axis perpendicular to the filament axis. Between any two cross-linked filaments, all the α-actinin cross-links will lie on one side of this axis. This also means that the cross-linkers to the next adjacent filament would form on the opposite side of the interfilament axis. In a polar bundle, the opposite occurs. Cross-links between two parallel actin filaments would pass from the side of one filament, through the interfilament axis to the other side (Fig. 6 a).

In a 2-D bundle growing on a surface, accessibility of an actin monomer to a cross-linker is affected by its proximity to the monolayer surface. Obviously, actin monomers that are closer to the bulk solution are more accessible to cross-linkers than those near the surface, which in some cases may be sterically blocked. In the bipolar 2-D bundle, cross-linkers between successive filament pairs alternate from the bulk solution side to the monolayer side across the filament array (Fig. 7, a and b). The accessibility of actin-binding sites to the cross-linker would be very different for alternate filament pairs. In addition, the surface of α-actinin facing the monolayer would also change, which might affect its interaction with lipid. On the other hand, cross-links between actin filaments close to the monolayer surface may be stabilized by charge attraction to the monolayer. This might be a key factor in promoting cross-linking if the concentrations of α-actinin and actin are low.

For polar 2-D arrays between any filament pair, one of the two binding sites for cross-linker would be in proximity to the solution and the other in proximity to the monolayer. The resulting average accessibility of actin-binding sites to α-actinin and potential stabilization of the cross-link by the monolayer would be the same between any filament pair (Fig. 7, c and d). Growth of the bundle could thus proceed within the plane of the monolayer without alternation between two spatial arrangements. α-Actinin would also present the same molecular surface to the monolayer everywhere across the bundle, thereby facilitating interaction with the lipid.

Both actin and α-actinin are acidic proteins (Pollard et al., 1986; Sheterline et al., 1996) that bind to the positively charged lipid monolayer. However, charge attraction is

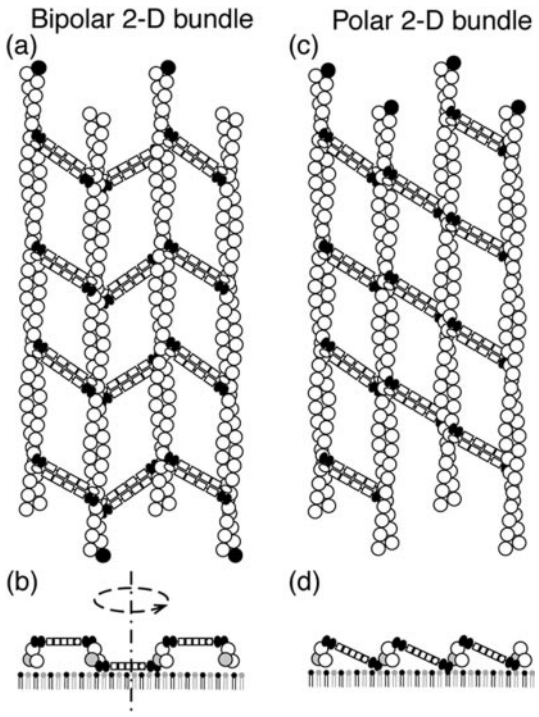

Figure 7. Diagram showing the relative positions of filaments and cross-links in 2-D bundles formed on a lipid surface. (a and b) Bipolar 2-D bundle in (a) longitudinal and (b) transverse view down the filament axis. Note that free cross-linkers must approach from the side opposite from the monolayer, which may make actin monomers appropriately oriented for cross-linking between alternate filament pairs relatively inaccessible. In the accessible orientation, the cross-link is distant from the lipid surface and probably cannot be stabilized by lipid binding. In the less accessible position close to the monolayer, the cross-linker can be stabilized by lipid binding. (c and d) Polar 2-D bundle in (c) longitudinal and (d) transverse view. Note that in this orientation, each filament pair has equal accessibility to cross-linker and that the cross-linker also is accessible over at least part of its surface to the lipid layer.

critically dependent on the distance between the protein and the lipid. Some stabilization would always be possible in the case of polar cross-linking, because at least part of the cross-linker could come in proximity with the monolayer. For a bipolar cross-link, only for alternate filament pairs would α-actinin be favorably positioned to bind both actin and the monolayer. Actin-binding sites on the intervening filament pairs would be accessible to α-actinin from the solution. However, a greater distance separates these cross-links from the monolayer, thereby weakening any interaction that might stabilize the bond with actin.

Bipolar cross-linking is not completely inhibited by the monolayer. We have formed mixed polarity bundles with this monolayer system using other F-actin cross-linkers (Taylor, D.W., and K.A. Taylor, unpublished observation), demonstrating that the monolayer does not force formation of exclusively polar cross-links, but we have not obtained any extensive bipolar bundles with regularly alternating filament orientations. Thus, the monolayer favors polar cross-linking over bipolar cross-linking,

thereby acting as a factor defining the type of bundle produced.

In vivo, lipids are predominately acidic or neutral, rather than basic as used here. However, α-actinin has affinity for some natural lipids (Meyer et al., 1982; Burn et al., 1985; Niggli and Gimona, 1993) as well as the cytoplasmic domain of β-integrin (Otey et al., 1990), factors which may stabilize the cross-linked actin filaments and thus facilitate growth of a polar filament array. In particular, specific interactions between α-actinin and the membrane would be dependent on both distance from the membrane and presentation of the correct molecular surface to the membrane, which could not be consistently done for bipolar arrays. Arrays of actin filaments originating from cell membranes are polar, and it seems at least possible that the interaction of the membrane with the cross-linker could contribute to controlling this polarity.

Myofibrils from striated muscle are a convenient source for obtaining polar actin bundles with a limited amount of bipolar bundle possible at the Z-line. However, they also contain other proteins that may affect the interaction with proteins applied exogenously. Tropomyosin is one such protein and is known to affect the binding of α-actinin to actin in a temperature-dependent manner (Goll et al., 1972). Treatments that remove tropomyosin from skeletal muscle I-segments will facilitate binding of exogenous α-actinin to the polar array of actin filaments in the I-band at low temperatures (Goll et al., 1972; Stromer and Goll, 1972; Sanger et al., 1984). Electron micrographs of these decorated I-bands are surprisingly similar to the images reported here. α-Actinin is also an important component of the comet tails produced by *Listeria monocytogenes* in infected cells (Dodd et al., 1994), which have a polar arrangement of actin filaments (Tilney et al., 1992).

The ability of α-actinin to cross-link actin filaments in vitro is clearly demonstrated (Goll et al., 1972; Wachsstock et al., 1994), but its role in complicated multicomponent systems such as Z-disks and adhesion plaques is less clear. Averaged 3-D image reconstructions of vertebrate Z-disks (Morris et al., 1990; Luther, 1991) contain cross-linking density connecting antiparallel actin filaments of the appropriate length for α-actinin. However, the identification of this density with α-actinin is circumstantial. The cross-links may contain α-actinin but other proteins may be colocalized as well. Limited proteolytic treatment of Z-disks results in removal of α-actinin without modification of its molecular structure (Goll et al., 1991; Astier et al., 1993), thereby indicating involvement of other molecules in formation of the bipolar array of actin filaments. The most likely candidate is titin, which is known to interact with α-actinin (Ohtsuka et al., 1997; Sorimachi et al., 1997) and which is degraded by similar treatments with proteolytic enzymes (Astier et al., 1993).

Taken in context with earlier results that studied cross-linking orientation in bulk solution (Meyer and Aebi, 1990) our results demonstrate a lack of specificity to the orientation of actin filaments cross-linked by α-actinin. The ability to cross-link actin filaments in any orientation would make α-actinin by itself a poor candidate to direct the formation of an ordered array of actin filaments of a particular polarity. Thus, it seems likely that the polarity of actin-containing structures is not specified by α-actinin, but by components that limit its access to actin-binding sites, such as tropomyosin, factors that favor one type of polarity over another, such as the cell membrane, or by components that specify, by themselves, the orientation of the actin filaments within the bundles. In this regard, the ability of cellular titin, myosin, and α-actinin to form stress fiber–like structures in the absence of actin (Eilertsen et al., 1997) may indicate that a bipolar scaffold is necessary to form a bipolar actin bundle in solution.

Spiral Formation

The spiral structures represent one of the more interesting α-actinin–actin arrays produced in this study. Their utility for determining filament orientation in the cross-linked array is clear, but the question remains as to how they form. Once a single revolution is produced, continued spiraling is guaranteed by actin polymerization in the 2-D plane. The problem is to produce the first complete revolution. Several observations are important for any mechanism that could explain spiraling.

Spirals form under polymerization conditions in samples containing both α-actinin and actin as well as G-actin alone. This suggests that spiral formation is an inherent property of actin polymerization on a positively charged surface. Spiraling, in cases where filaments can be tracked unambiguously, occurs in only a single direction, because all spirals observed so far, whether cross-linked or not, have a single hand when viewed from one side of the monolayer. The unique handedness suggests that cross-linking does not lock in random curvature that might be induced by collisions or crowding between growing filaments, because most likely, curvature induced by crowding would produce both left- and right-handed spirals if indeed a complete turn could be produced on a crowded monolayer.

Actin filaments consist of a helical arrangement of actin monomers, but in the spiral, the helical structure is perturbed because the filaments are systematically curved in one direction. It seems likely that the monolayer is providing the force to alter the filament curvature. The attraction of the actin filament to the monolayer is due to opposite net charge. However, this attractive force may be asymmetric if the charge distribution on actin is not uniform across the surface of the filament. That is to say, the charge distribution on actin has chirality. Such a charge distribution might alter the pitch of one of the helical tracks to differing degrees on left and right sides of the filament. The needed changes to produce the curvature in the first turn (average diameter 1.8 μm) are actually quite small, and are ~0.03 nm per actin monomer.

Both the F-actin spirals and the spirals cross-linked by α-actinin had a limiting internal diameter, below which the filament either broke or ceased to grow. Spirals form under polymerization conditions, so it seems likely that breaks in the filament are actually interruptions in the polymerization process. Two factors may contribute to this observation. In one case, the growing end may collide with the side of the filament, which would block the addition of monomers. Alternatively, the actin filament may not be capable of incorporating additional monomers if the radius of curvature is too small at the growing end. Never-

theless, the blocked end seems to be capable of seeding a new polymerization site, because new filaments begin growing close to the blocked end.

Recently, it has been shown that actin filaments bent below a limiting diameter of 0.36 μm will break (Arai et al., 1999). This value is very similar to the limiting diameter observed in this work, although the two approaches differ considerably. Arai et al. (1999) explain the breaking radius as a consequence of the ~0.15 nm/monomer differential between the inner and outer circumference of the actin filament. By comparison, the amount of stretch that an actin filament undergoes during an isometric muscle contraction is 0.007 nm/monomer (Huxley et al., 1994; Wakabayashi et al., 1994).

The limiting amount of bending that occurs in our result and in that of Arai et al. (1999) is a factor of two or more less than the mean bending flexibility per actin subunit reported for actin (Orlova and Egelman, 1993). The common denominator in the knots and spirals is systematic bending in a plane, whereas other measures of actin flexibility measure accumulated bending in a volume. Our results may indicate that actin has anisotropic bending flexibility that is more readily expressed on a planar surface. In a plane, the twist of the actin filament would sample bending modes systematically, with the most flexible mode accommodating most of the needed bending. For example, if the bending flexibility of one mode were ~3° and all other modes 0 (an over-simplification), the average over seven actin monomers (to the midpoint between crossovers) would be ~0.4°, which is approximately the amount of bend/monomer at the point of the break in the spirals.

We have shown here a novel method for producing actin filament bundles that are constrained to growth in a plane, thereby facilitating relatively straightforward image interpretation. The results suggest that bundle formation on a surface attractive to α-actinin is biased in favor of polar cross-linking, which is consistent with the observed polar orientation of filament arrays formed on membranes. Although bipolar bundles in 2-D have not been produced with simple α-actinin–actin mixtures, these may be possible by adding additional protein components found in Z-disks and homologous structures. The techniques used here may facilitate structural studies of these more complex actin-containing structures.

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References

Arai, Y., R. Yasuda, K. Akashi, Y. Harada, H. Miyata, K. Kinosita, and H. Itoh. 1999. Tying a molecular knot with optical tweezers. *Nature.* 399:446–448.

Astier, C., J.-P. Labbé, C. Roustan, and Y. Benyamin. 1993. Effects of different enzymic treatments on the release of titin fragments from rabbit skeletal myofibrils. *Biochem. J.* 290:731–734.

Atsuta, F., K. Sato, K. Maruyama, and Y. Shimada. 1993. Distribution of connectin (titin), nebulin and α-actinin at myotendinous junctions of chicken pectoralis muscles: an immunofluorescence and immunoelectron microscopic study. *J. Muscle Res. Cell Motil.* 14:511–517.

Blanchard, A., V. Ohanian, and D. Critchley. 1989. The structure and function of α-actinin. *J. Muscle Res. Cell Motil.* 10:280–289.

Burn, P., A. Rotman, R.K. Meyer, and M.M. Burger. 1985. Diacylglycerol in large α-actinin/actin complexes and the cytoskeleton of activated platelets. *Nature.* 314:469–472.

Burridge, K., G. Nuckolls, C. Otey, F. Pavalko, K. Simon, and C. Turner. 1990. Actin-membrane interaction in focal adhesions. *Cell Differ. Dev.* 32:337–342.

Carpen, O., P. Pallai, D.E. Staunton, and T.A. Springer. 1992. Association of intercellular adhesion molecule-1 (ICAM-1) with actin-containing cytoskeleton and alpha-actinin. *J. Cell Biol.* 118:1223–1234.

Crawford, A.W., J.W. Michelsen, and M.C. Beckerle. 1992. An interaction between zyxin and α-actinin. *J. Cell Biol.* 116:1381–1393.

Dodd, F.G., J.M. Sanger, and J.W. Sanger. 1994. Intact alpha-actinin molecules are needed for both the assembly of actin into the tails and the locomotion of *Listeria monocytogenes* inside infected cells. *Cell Motil. Cytoskelet.* 28:97–107.

Eilertsen, K.J., S.T. Kazmierski, and T.C. Keller III. 1997. Interaction of α-actinin with cellular titin. *Eur. J. Cell Biol.* 74:361–364.

Endo, T., and T. Masaki. 1984. Differential expression and distribution of chicken skeletal- and smooth-muscle-type α-actinins during myogenesis in culture. *J. Cell Biol.* 99:2322–2332.

Feramisco, J.R., and K. Burridge. 1980. A rapid purification of α-actinin, filamin and a 130,000-dalton protein from smooth muscle. *J. Biol. Chem.* 255: 1194–1199.

Fowler, W., and U. Aebi. 1982. A consistent picture of the actin filament related to the orientation of the actin molecule. *J. Cell Biol.* 93:452–458.

Francis, N.R., and D.J. DeRosier. 1990. A polymorphism peculiar to bipolar actin bundles. *Biophys. J.* 58:771–776.

Fukami, A., and K.J. Adachi. 1965. A new method of preparation of a self-perforated micro plastic grid and its application (I). *J. Electron Microsc.* 14:112–118.

Geiger, B., A.H. Dutton, K.T. Tokuyasu, and S.J. Singer. 1981. Immunoelectron microscope studies of membrane-microfilament interactions: distributions of α-actinin, tropomyosin, and vinculin in intestinal epithelial brush border and chicken gizzard smooth muscle cells. *J. Cell Biol.* 91:614–628.

Goll, D.E., A. Suzuki, J. Temple, and G.R. Holmes. 1972. Studies on purified α-actinin. I. Effect of temperature and tropomyosin on the α-actinin/F-actin interaction. *J. Mol. Biol.* 67:469–488.

Goll, D.E., W.R. Dayton, I. Singh, and R.M. Robson. 1991. Studies of the α-actinin/actin interaction in the Z-disk by using calpain. *J. Biol. Chem.* 266: 8501–8510.

Heiska, L., C. Kantor, T. Parr, D.R. Critchley, P. Vilja, C.G. Gahmberg, and O. Carpen. 1996. Binding of the cytoplasmic domain of intercellular adhesion molecule-2 (ICAM-2) to alpha-actinin. *J. Biol. Chem.* 271:26214–26219.

Huxley, H.E., A. Stewart, H. Sosa, and T. Irving. 1994. X-ray diffraction measurements of the extensibility of actin and myosin filaments in contracting muscle. *Biophys. J.* 67:2411–2421.

Kubalek, E.W., R.D. Kornberg, and S.A. Darst. 1991. Improved transfer of two-dimensional crystals from the air/water interface to specimen support grids for high-resolution analysis by electron microscopy. *Ultramicroscopy.* 35:295–304.

Langanger, G., M. Moeremans, G. Daneels, A. Sobieszek, M. De Brabander, and J. De Mey. 1986. The molecular organization of myosin in stress fibers of cultured cells. *J. Cell Biol.* 102:200–209.

Lazarides, E., and B.L. Granger. 1978. Fluorescent localization of membrane sites in glycerinated chicken skeletal muscle fibers and the relationship of these sites to the protein composition of the Z disc. *Proc. Natl. Acad. Sci. USA.* 75:3683–3687.

Luther, P.K. 1991. Three-dimensional reconstruction of a simple Z-band in fish muscle. *J. Cell Biol.* 113:1043–1055.

Masaki, T., M. Endo, and S. Ebashi. 1967. Localization of the 6S component of α-actinin in the Z-band. *J. Biochem.* 62:630–632.

McGough, A., M. Way, and D. DeRosier. 1994. Determination of the α-actinin–binding site on actin filaments by cryoelectron microscopy and image analysis. *J. Cell Biol.* 126:433–443.

Meyer, R.K., and U. Aebi. 1990. Bundling of actin filaments by α-actinin depends on its molecular length. *J. Cell Biol.* 110:2013–2024.

Meyer, R.K., H. Schindler, and M.M. Burger. 1982. α-Actinin interacts specifically with model membranes containing glycerides and fatty acids. *Proc. Natl. Acad. Sci. USA.* 79:4280–4284.

Morris, E.P., G. Nneji, and J.M. Squire. 1990. The three-dimensional structure of the nemaline rod Z-band. *J. Cell Biol.* 111:2961–2978.

Nave, R., D.O. Fürst, and K. Weber. 1990. Interaction of α-actinin and nebulin in vitro. Support for the existence of a fourth filament system in skeletal muscle. *FEBS Lett.* 269:163–166.

Niggli, V., and M. Gimona. 1993. Evidence for a ternary interaction between α-actinin, (meta)vinculin and acidic-phospholipid bilayers. *Eur. J. Biochem.* 213:1009–1015.

Ohtsuka, H., H. Yajima, K. Maruyama, and S. Kimura. 1997. The N-terminal Z repeat 5 of connectin/titin binds to the C-terminal region of α-actinin. *Biochem. Biophys. Res. Commun.* 235:1–3.

Orlova, A., and E.H. Egelman. 1993. A conformational change in the actin subunit can change the flexibility of the actin filament. *J. Mol. Biol.* 232:334–341.

Otey, C.A., F.M. Pavalko, and K. Burridge. 1990. An interaction between α-actinin and the β1 integrin subunit in vitro. *J. Cell Biol.* 111:721–729.

Pardee, J.D., and J.A. Spudich. 1982. Purification of muscle actin. *Meth. Enzymol.* 85:164–181.

Pollard, T.D., P.C.-H. Tseng, D.L. Rimm, D.P. Bichell, R.C. Williams, Jr., J. Sinard, and M. Sato. 1986. Characterization of alpha-actinin from *Acanthamoeba. Cell Motil. Cytoskelet.* 6:649–661.

Reedy, M.C., C. Beall, and E. Fyrberg. 1989. Formation of reverse rigor chevrons by myosin heads. *Nature.* 339:481–483.

Sanger, J.W., B. Mittal, and J.M. Sanger. 1984. Analysis of myofibrillar structure and assembly using fluorescently labeled contractile proteins. *J. Cell Biol.* 98:825–833.

Schollmeyer, J.E., L.T. Furcht, D.E. Goll, R.M. Robson, and M.H. Stromer. 1976. Localization of contractile proteins in smooth muscle cells and in normal and transformed fibroblasts. *In* Cell Motility. Vol. A. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 361–388.

Sheterline, P., J. Clayton, and J.C. Sparrow. 1996. Actins. Academic Press, London. 116 pp.

Small, J.V. 1985. Geometry of actin-membrane attachments in the smooth muscle cell: the localization of vinculin and α-actinin. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:45–49.

Sorimachi, H., A. Freiburg, B. Kolmerer, S. Ishiura, G. Stier, C.C. Gregorio, D. Labeit, W.A. Linke, K. Suzuki, and S. Labeit. 1997. Tissue-specific expression and α-actinin binding properties of the Z-disc titin: implications for the nature of vertebrate Z-discs. *J. Mol. Biol.* 270:688–695.

Stromer, M.H., and D.E. Goll. 1972. Studies on purified α-actinin II. Electron microscopic studies on the competitive binding of α-actinin and tropomyosin to Z-line extracted myofibrils. *J. Mol. Biol.* 67:489–494.

Sukow, C., and D. DeRosier. 1998. How to analyze electron micrographs of rafts of actin filaments crosslinked by actin-binding proteins. *J. Mol. Biol.* 284:1039–1050.

Taylor, K.A., and D.W. Taylor. 1992. Formation of 2-D paracrystals of F-actin on phospholipid layers mixed with quaternary ammonium surfactants. *J. Struct. Biol.* 108:140–147.

Taylor, K.A., and D.W. Taylor. 1994. Formation of 2-D complexes of F-actin and crosslinking proteins on lipid monolayers: demonstration of polar α-actinin-F-actin crosslinking. *Biophys. J.* 67:1976–1983.

Taylor, K.A., and D.W. Taylor. 1999. Structural studies of cytoskeletal protein arrays formed on lipid monolayers. *J. Struct. Biol.* 128:75–81.

Tilney, L.G., D.J. DeRosier, and M.S. Tilney. 1992. How *Listeria* exploits host cell actin to form its own cytoskeleton. *J. Cell Biol.* 118:71–81.

Wachsstock, D.H., J.A. Wilkins, and S. Lin. 1987. Specific interaction of vinculin with α-actinin. *Biochem. Biophys. Res. Commun.* 146:554–560.

Wachsstock, D.H., W.H. Schwarz, and T.D. Pollard. 1993. Affinity of α-actinin for actin determines the structure and mechanical properties of actin filament gels. *Biophys. J.* 65:205–214.

Wachsstock, D.H., W.H. Schwaartz, and T.D. Pollard. 1994. Cross-linker dynamics determine the mechanical properties of actin gels. *Biophys. J.* 66: 801–809.

Wakabayashi, K., Y. Sugimoto, H. Tanaka, Y. Ueno, Y. Takezawa, and Y. Amemiya. 1994. X-ray diffraction evidence for the extensibility of actin and myosin filaments during muscle contraction. *Biophys. J.* 67:2422–2435.