Disruption of the Actin Cytoskeleton after Microinjection of Proteolytic Fragments of α-Actinin

Fredrick M. Pavalko and Keith Burridge

Department of Cell Biology and Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Abstract. α-Actinin can be proteolytically cleaved into major fragments of 27 and 53 kD using the enzyme thermolysin. The 27-kD fragment contains an actin-binding site and we have recently shown that the 53-kD fragment binds to the cytoplasmic domain of β_1 integrin in vitro (Otey, C. A., F. M. Pavalko, and K. Burridge. 1990. *J. Cell Biol.* 111:721–729). We have explored the behavior of the isolated 27- and 53-kD fragments of α-actinin after their microinjection into living cells. Consistent with its containing a binding site for actin, the 27-kD fragment was detected along stress fibers within 10–20 min after injection into rat embryo fibroblasts (REF-52). The 53-kD fragment of α-actinin, however, concentrated in focal adhesions of REF-52 cells 10–20 min after injection. The association of this fragment with focal adhesions in vivo is consistent with its interaction in vitro with the cytoplasmic domain of the β_1 subunit of integrin, which was also localized at these sites. When cells were injected with >5 μM final concentration of either α-actinin fragment and cultured for 30–60 min, most stress fibers were disassembled. At this time, however, many of the focal adhesions, particularly those around the cell periphery, remained after most stress fibers had gone. By 2 h after injection only a few small focal adhesions persisted, yet the cells remained spread. Identical results were obtained with other cell types including primary chick fibroblasts, BSC-1, MDCK, and gerbil fibroma cells. Stress fibers and focal adhesions reformed if cells were allowed to recover for 18 h after injection. These data suggest that introduction of the monomeric 27-kD fragment of α-actinin into cells may disrupt the actin cytoskeleton by interfering with the function of endogenous, intact α-actinin molecules along stress fibers. The 53-kD fragment may interfere with endogenous α-actinin function at focal adhesions or by displacing some other component that binds to the rod domain of α-actinin and that is needed to maintain stress fiber organization.

α-ACTININ is an actin binding protein that cross-links actin filaments in vitro (Maruyama and Ebashi, 1965; Burridge and Feramisco, 1981). Its native structure is a homodimer with a subunit molecular weight of 103 kD (Singh et al., 1977; Suzuki et al., 1979; Baron et al., 1987) and is visualized in the electron microscope as a dumbbell-shaped molecule, 30–40 nm long and 3–4 nm wide with an enlarged head domain at each end (Podlubnaya et al., 1975; Bretscher et al., 1979; Pollard, 1981). Distinct α-actinin isoforms have been identified in muscle (Endo and Masaki, 1982) and nonmuscle cells (Burridge and Feramisco, 1981; Duhaiman and Bamburg, 1984; Bennett et al., 1984; Landon et al., 1985); however, the only known functional difference between the isoforms is that binding of muscle α-actinin to actin is calcium insensitive, whereas the binding of nonmuscle α-actinins to actin is inhibited by calcium (Mimura and Asano, 1979; Burridge and Feramisco, 1981; Duhaiman and Bamburg, 1984; Bennet et al., 1984; Landon et al., 1985).

Based on the complete amino acid sequence of α-actinin from various sources (Baron et al., 1987; Noegel et al., 1987) it has become clear that the α-actinin monomer is organized into three domains: a globular NH_2-terminal domain which contains the binding site for actin (Mimura and Asano, 1986; Imamura et al., 1988; Baron et al., 1987), an extended rodlike domain which is responsible for dimer formation and consists of four internal repeating units of 122 amino acids each (Mimura and Asano, 1987; Imamura et al., 1988), and a COOH-terminal domain containing two EF-hand calcium-binding regions (Baron et al., 1987; Noegel et al., 1987). Comparison of α-actinin sequences with those of spectrins and dystrophin has revealed extensive homologies, leading to the suggestion that these three proteins are members of a single family of cytoskeletal proteins (Hammond, 1987; Koenig et al., 1988; Davison and Critchley, 1988; Davison et al., 1989).

In striated and smooth muscles, α-actinin is localized at Z-lines (Masaki et al., 1967; Goll et al., 1969) and dense bodies (Geiger et al., 1981), respectively, where it seems to be involved in anchoring actin thin filaments. In cultured nonmuscle cells α-actinin is localized along actin stress fibers with a periodic distribution reminiscent of muscle sarcomeres (Lazarides and Burridge, 1975). Here it may also be involved in anchoring actin filaments as well as bundling

F. M. Pavalko's current address is Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN 45202.

them into stress fibers. α-Actinin is also present at sites where actin filaments attach to the cytoplasmic face of the plasma membrane which are known as focal adhesions (Wehland et al., 1979). Several other proteins including vinculin (Geiger, 1979; Burridge and Feramisco, 1980), talin (Burridge and Connell, 1983), and integrin (Damsky et al., 1985; Chen et al., 1985; Kelly et al., 1987) are also present in focal adhesions and together with α-actinin these proteins have been proposed to link actin to integrin in the membrane via a series of protein interactions (reviewed in Burridge et al., 1988).

The role of α-actinin in mediating the attachment of actin to the membrane in nonmuscle cells is not entirely clear. Some work has suggested that actin can attach to the membrane via mechanisms that do not involve α-actinin. For example, it was possible to extract α-actinin from isolated plasma membranes obtained from cells grown in suspension without dissociating most of the actin (Burridge and McCullough, 1980). Immunoelectron microscopy of cells with stress fibers and focal adhesions indicated that much of the α-actinin in the focal adhesions of cultured cells was located farther from the membrane than was vinculin (Chen and Singer, 1982). Also, the intensity of staining for α-actinin in focal adhesions varies among cell types and even among adhesions in the same cell (our unpublished observations) suggesting that the role of α-actinin at these sites may also vary. Based on these findings, together with the discovery that α-actinin can bind directly to vinculin (Wachsstock et al., 1987), α-actinin in focal adhesions has generally been thought either to be involved in bundling actin filaments or to be one of several links in a chain of protein interactions between actin and the membrane.

Recently, however, we found that α-actinin purified from chicken gizzard smooth muscle was able to interact directly with the cytoplasmic domain of the β_1 subunit of integrin in vitro (Otey et al., 1990). The binding site on α-actinin for integrin was localized to a 53-kD proteolytic fragment of α-actinin that dimerizes in solution and corresponds to the rod domain (Otey et al., 1990). This study suggested a more direct role for α-actinin in linking actin to the membrane.

One prediction of this earlier work is that the 53-kD fragment of α-actinin would target to focal adhesions that contain integrin after its microinjection into live cells. We tested this prediction and found that microinjected 53-kD fragment of α-actinin concentrated in focal adhesions shortly after injection into cells, whereas the microinjected 27-kD fragment localized along stress fibers. When cells were cultured for longer periods of time following injection of either fragment of α-actinin, actin stress fibers and focal adhesions were disassembled. Possible mechanisms whereby α-actinin fragments may disrupt the actin cytoskeleton are discussed.

Materials and Methods

Cell Culture and Microinjection

Rat embryo fibroblasts (REF-52), primary chick embryo fibroblasts, African green monkey kidney cells (BSC-1), MDCK cells, and gerbil fibroma cells (IMR-33) were cultured in DMEM-H supplemented with 10% fetal calf serum (FCS), 50 U/ml penicillin, and 50/ml streptomycin in a humidified incubator with 7% CO_2 at 37°C. Cells for microinjection were passaged by brief trypsinization and subcultured to 50% confluency on glass coverslips for 24–48 h. Coverslips were transferred to media containing 10% FCS and 15 mM Hepes, pH 7.3 for microinjection and a small square was etched in the center of the coverslip to aid in locating injected cells on the fluorescence microscope. Injections were performed at room temperature. After returning to the incubator for a postinjection incubation period of 10 min to 18 h, the coverslips were fixed in 3.7% formaldehyde for 5 min.

Needles for microinjection were prepared from glass capillaries (Kwikfil; World Precision Instruments, Inc., New Haven, CT) pulled on a micropipette puller (Brown-Flaming; Sutter Instrument Co., San Francisco, CA). For microinjection, cells were viewed on a Diavert microscope using a 32× phase objective and microinjections were performed using a micromanipulator (Leitz, Inc., Rockleigh, NJ).

Protein Purification

α-Actinin and vinculin were purified from frozen chicken gizzards as described previously (Feramisco and Burridge, 1980), with an additional, final, purification by chromatography on an FPLC Mono Q column (Pharmacia Fine Chemicals; Piscataway, NJ). Actin was prepared from rabbit skeletal muscle acetone powder by the method of Spudich and Watt (1971) and further purified by gel filtration on a Sephadex G-150 column.

Thermolysin Cleavage of α-Actinin and Isolation of Proteolytic Fragments

8 ml of purified α-actinin (2 mg/ml) was treated with the enzyme thermolysin (Sigma Chemical Co., St. Louis, MO) at a 1:25 enzyme/protein ratio for 2 h at 37°C with frequent mixing. The fragments were separated by FPLC using a Mono Q column equilibrated with buffer B (20 mM Tris-acetate, 20 mM NaCl, 0.1 mM EDTA, 0.1% β-mercaptoethanol, pH 7.6) and eluted with a 0–400 mM NaCl gradient in buffer B. The 53-kD fragment of α-actinin was further purified on a Mono S column equilibrated in MES buffer (20 mM MES, 0.1 mM EDTA, 0.1% β-mercaptoethanol, pH 6.2). Under these conditions the 53-kD fragment flowed through the column and several trace contaminating proteins were retained by the column.

Fluorescent Labeling of α-Actinin and the 27- and 53-kD Fragment

Purified intact α-actinin and the 53-kD fragment were labeled with either fluorescein isothiocyanate (FITC), tetramethylrhodamine-isothiocyanate (TRITC; Research Organics, Inc., Cleveland, OH), or iodoacetaminotetramethyl rhodamine (IATR; Molecular Probes, Inc., Junction City, OR). The 27-kD fragment was labeled with either FITC or TRITC. For the IATR labeling, ~5 ml of purified protein at 1.5–2 mg/ml was dialyzed against 200 mM sodium borate buffer, pH 7.5. 22 μg of IATR (dissolved in DMSO) was added to the protein solution and incubated at 4°C for 6 h. The reaction was stopped by addition of DTT to a final concentration of 0.1%. For FITC and TRITC labeling, protein at approximately the same concentration as for IATR labeling was dialyzed against 200 mM sodium carbonate/bicarbonate (C/B) buffer at pH 9.5. The dialysis bag was then transferred to 600 ml of the C/B buffer and 18 mg of FITC or TRITC was added with stirring. The reaction was continued for 6 h at 4°C and then terminated by dialyzing against buffer B at pH 7.6. Free dye was removed from labeled proteins by extensive dialysis against microinjection buffer (75 mM KCl, 0.1% β-mercaptoethanol, 10 mM potassium phosphate, pH 7.5). Intact α-actinin and both of the labeled fragments were concentrated by ultrafiltration to a final concentration of ~200 μM. Dye/protein molar ratios for the labeled proteins were between 0.1 and 0.5 as determined by absorbance at 575/280 nm. Estimates of the α-actinin concentration in fibroblasts were made by RIA using rabbit polyclonal anti-α-actinin antisera with purified α-actinin as a standard.

Fluorescence Microscopy

Cells (REF-52, BSC-1, IMR-33, MDCK, and CEF) were grown on 12-mm round glass coverslips and prepared for fluorescence microscopy and photographed as previously described (Pavalko et al., 1989). Antibodies specific for the cytoplasmic domain of the β_1 subunit of integrin were generously provided by Dr. Richard Hynes (Massachusetts Institute of Technology, Cambridge, MA). Polyclonal antibodies against α-actinin, talin and vinculin were raised against purified chicken gizzard proteins.

SDS-PAGE, Vinculin Iodination, and Blot Overlays

Electrophoresis of proteins was performed on 15% SDS-polyacrylamide gels according to Laemmli (1970) except that the bisacrylamide concentration was 0.09%. Vinculin was iodinated using Iodogen (Pierce Chemical

Co., Rockford, IL) as has been described previously for talin (Turner and Burridge, 1989). For blot overlays, samples of digested α-actinin were transferred to nitrocellulose after electrophoresis. After incubating in buffer B containing 0.2% gelatin, 0.05% Tween-20 and 5% BSA (blocking buffer), the nitrocellulose was overlayed with 1.2×10^6 cpm/ml ^{125}I-vinculin in blocking buffer for 90 min and subsequently washed in this same buffer minus BSA with several changes over a 2-h period. Protein bands binding to the ^{125}I-vinculin were visualized by exposing the dried nitrocellulose sheet to x-ray film with an intensifying screen at −70°C for 5 d.

Actin Sedimentation Assays

To examine the binding of α-actinin, and the 27- and 53-kD fragments of α-actinin to F-actin, ~20 μg of each protein was added to 120 μg of G-actin and the actin was allowed to polymerize for 1 h by addition of KCl to a final concentration of 50 mM and $MgCl_2$ to 1 mM. F-actin and bound proteins were sedimented at 100,000 *g* for 45 min in a Beckman TL-100 ultracentrifuge (Beckman Instrument Co., Fullerton, CA) and equivalent amounts of the pellets and supernatants were analyzed by SDS-PAGE.

Results

Preparation of α-Actinin Fragments

α-Actinin was purified from chicken gizzard smooth muscle and treated with the enzyme thermolysin to generate two major proteolytic fragments of 27- and 53-kD that were separated by FPLC (Fig. 1). Fluorescent probes were conjugated to the two fragments of α-actinin or to intact α-actinin (Fig. 1) for microinjection into non-muscle cells.

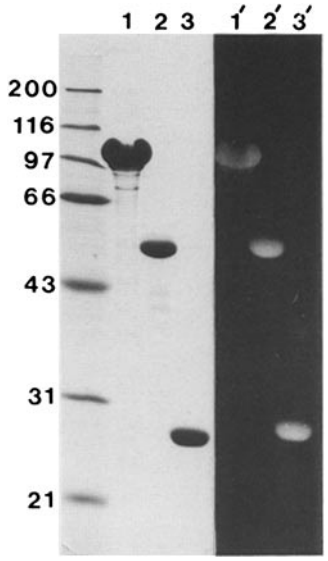

Figure 1. Purification and fluorescent labeling of intact α-actinin and its thermolysin fragments. α-Actinin was purified from chicken gizzard and cleaved using the enzyme thermolysin. The resulting two major fragments were separated from each other, intact α-actinin and thermolysin by FPLC Mono Q and HAP column chromatography. Intact α-actinin and the 27- and 53-kD fragments were fluorescently labeled as described in Materials and Methods. Shown here are intact α-actinin (lanes *1* and *1′*), the 53-kD (lanes *2* and *2′*) and the 27-kD (lanes *3* and *3′*) fragments. Lanes *1–3* show the Coomassie-blue stained gel of the purified proteins and lanes *1′–3′* show the fluorescence emitted under UV light by the labeled proteins. Molecular mass standards are shown at left in kilodaltons.

Localization of Microinjected α-Actinin and the 27- and 53-kD Fragments in Cells

Intact α-actinin that was microinjected into rat fibroblasts localized periodically along stress fibers (Fig. 2 *b*) and also at their ends in focal adhesions, as identified by staining with antibodies against talin (Fig. 2 *a*), consistent with previous observations (Feramisco, 1979; Kreis and Birchmeier, 1980; Sanger et al., 1984, 1986, 1987*a*; Meigs and Wang, 1986). The intensity of the fluorescent α-actinin incorporated into focal adhesions was variable. We have also found similar variability in the intensity of staining for α-actinin in focal adhesions when cells are stained with antisera against α-actinin (our unpublished observations). A similar distribution of injected α-actinin was obtained when the protein was labeled with IATR, FITC, or TRITC (data not shown). When the 53-kD fragment was injected into fibroblasts and the cells were fixed ~10 min after being returned to 37°C, the fragment localized predominantly in focal adhesions but not along stress fibers (Fig. 2 *d*). The β_1 subunit of integrin colocalized with the 53-kD fragment in focal adhesions (Fig. 2 *c*). The fluorescence intensity of the 53-kD fragment in focal adhesions was considerably weaker than what we have generally observed with injected talin or vinculin. The 27-kD fragment of α-actinin localized along the entire length of stress fibers including the focal adhesions when cells were fixed ~10 min after being injected (Fig. 2, *e* and *f*). This is consistent with the 27-kD fragment containing an actin-binding site. We noted that the injected 27-kD fragment stained stress fibers more uniformly than did intact α-actinin which is periodically distributed. The reason for this difference is not clear. Nuclear staining was also observed in cells that were injected with either the fluorescently labeled 27- or 53-kD fragment. Although it is not known why these labeled fragments concentrate in and around the nucleus, we have no reason to believe that it is due to the recognition of a physiologic nuclear binding site. Indeed, FITC-labeled vinculin and the 47-kD talin fragment have also been found to concentrate in the nucleus (Burridge and Feramisco, 1980; Nuckolls et al., 1990).

Disruption of the Actin Cytoskeleton by Microinjection of α-Actinin Fragments

Changes in the organization of the actin cytoskeleton were first observed when cells were microinjected with either the 27- or 53-kD fragment of α-actinin and returned to 37°C for ~30 min before fixation. In the examples shown in Fig. 3, the 53-kD fragment which had been microinjected 30 min before fixation was concentrated in most focal adhesions at the ends of actin bundles that were much thinner than normal (Fig. 3, *a* and *b*). 30 min after injection of the 53-kD fragment the focal adhesions of injected cells still contained talin (Fig. 3, *c* and *d*), vinculin, α-actinin, and β_1 integrin (not shown). 30 min after microinjection of the 27-kD fragment similar changes in stress fibers organization were seen and the 27-kD fragment continued to be detected along thin stress fibers (not shown) although with less fluorescence intensity.

When viewed by IRM, cells that had undergone more extensive disruption of the actin cytoskeleton after injection with either fragment for ~60 min before fixation still retained numerous focal adhesions, predominantly at the cell periphery (Fig. 4, *a* and *b*). These adhesions still contained talin (Fig. 4, *c* and *d*), vinculin and integrin (not shown), but did not appear to stain for endogenous α-actinin (Fig. 4, *e* and *f*). It should be noted, however, that detecting α-actinin in focal adhesions is more problematic than the detection of either talin or vinculin at these sites. In normal cells the level of α-actinin in focal adhesions appears to vary and in some adhesions it is difficult to detect. In case there was a variation with cell type, we have injected both fragments into multiple cell types, including rat embryo fibroblasts, chick embryo

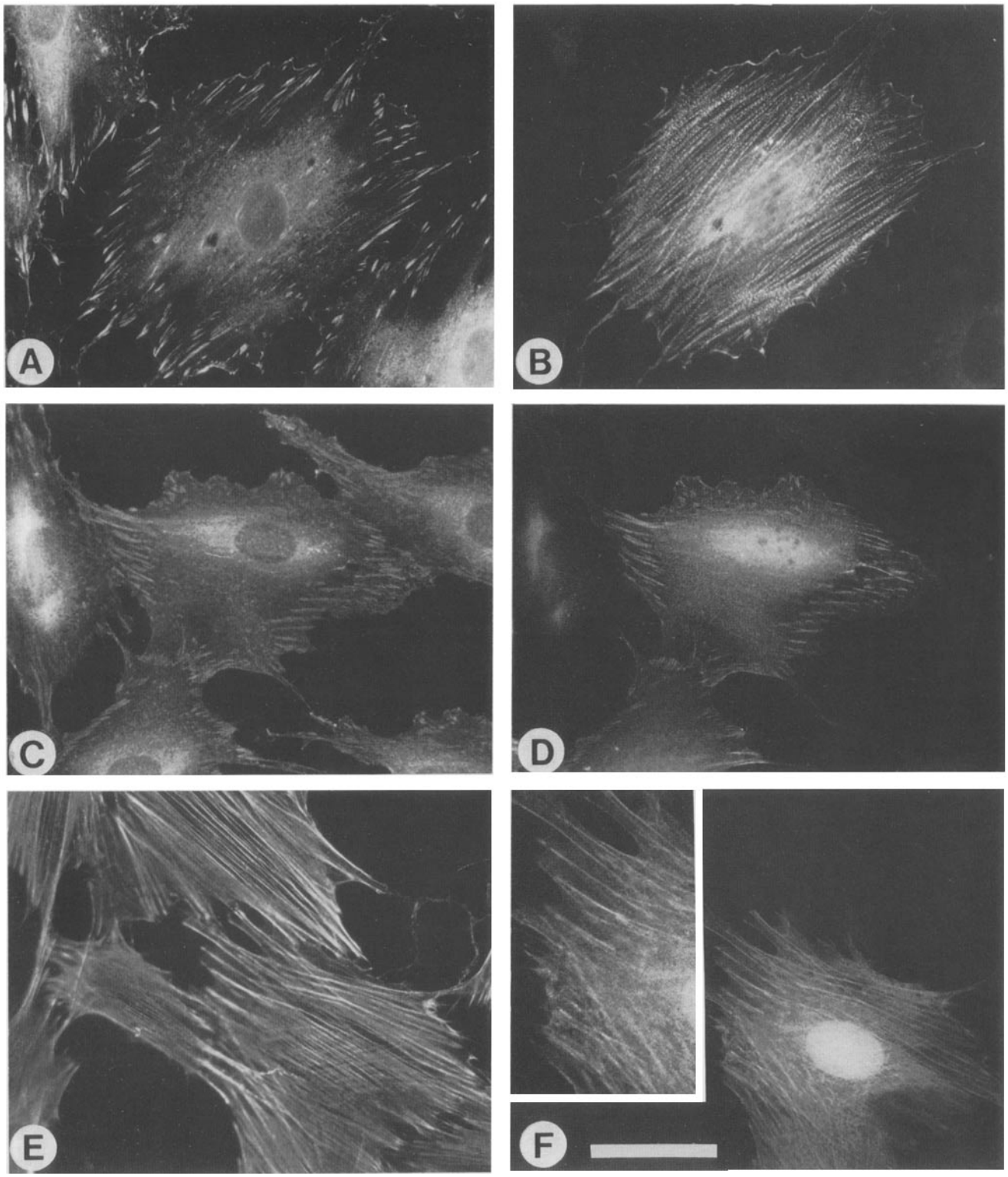

Figure 2. Microinjection of intact α-actinin and the 53- and 27-kD fragments of α-actinin into rat embryo fibroblasts (*REF-52*). IATR-α-actinin was microinjected into REF-52 cells and incubated for 2 h before fixation (*B*). The location of focal adhesions in the same field of cells is shown by immunostaining with antisera against talin, followed by FITC-anti-rabbit Ig (*A*). FITC-53-kD fragment was microinjected into cells and incubated for 10 min before fixation (*D*). The distribution of β_1 integrin in these cells is shown using β_1 antisera followed by RITC-anti-rabbit Ig (*C*). FITC-27-kD fragment was microinjected into cells and incubated for 10 min before fixation (*F*). Inset, higher magnification of the cell in *F*. The distribution of actin is shown by staining with rhodamine-phalloidin (*E*). Bar, 20 μm.

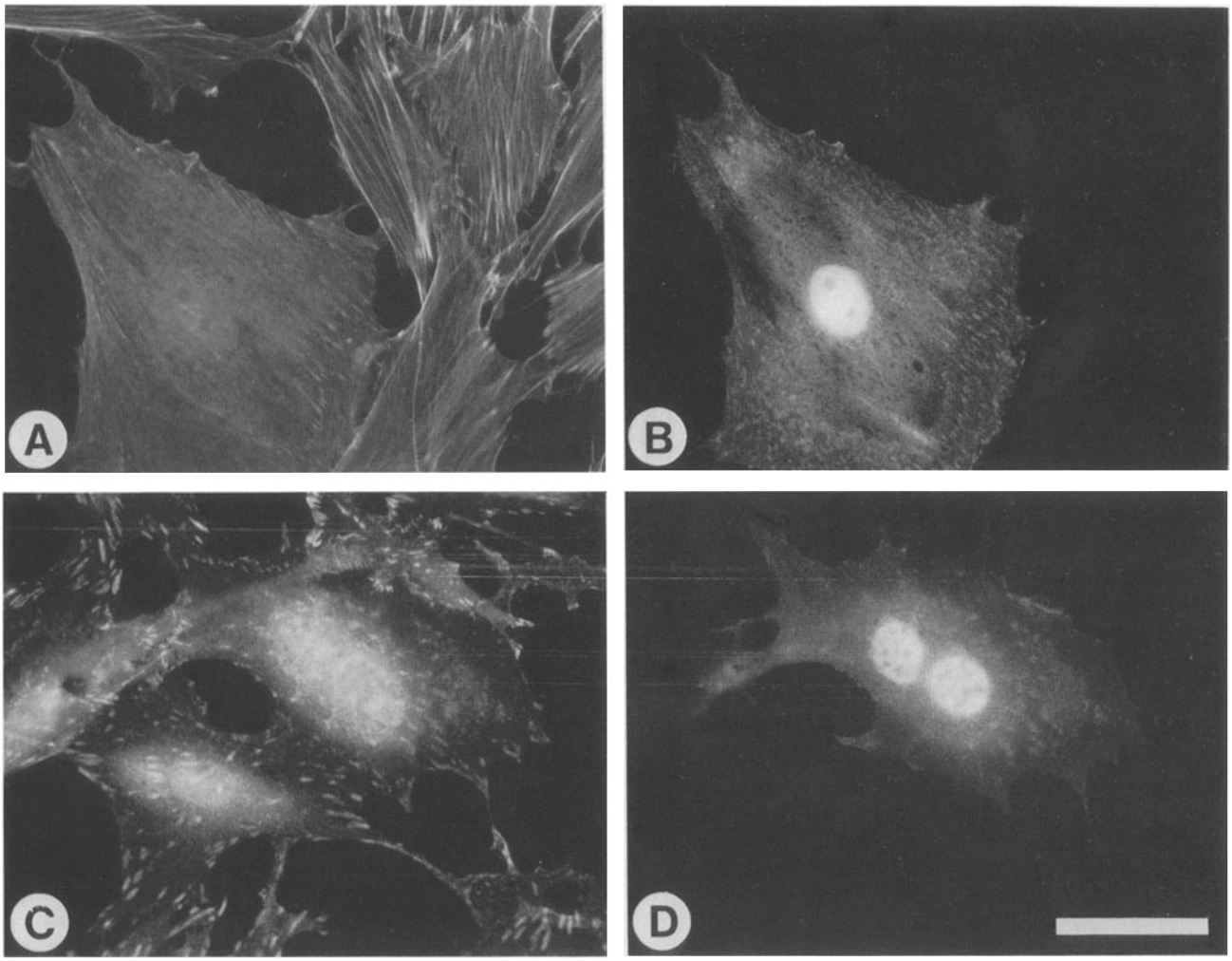

Figure 3. The distribution of the 53-kD α-actinin fragment in cells 30 min after microinjection. Cells injected with the TRITC-53-kD fragment (*B* and *D*) were fixed 30 min after injection and stained with fluorescein-phalloidin (*A*) or with antisera against talin followed by FITC-anti-rabbit Ig antibodies (*C*). The 53-kD fragment remains concentrated in many of the focal adhesions (*B* and *D*) despite considerable thinning of stress fibers. Bar, 20 μm.

fibroblasts, epithelial cells (BSC-1 and MDCK) and gerbil fibroma cells (IMR-33), and found that they all respond by disassembling their stress fibers beginning ~30 min after injection of these fragments. We generally were unable to detect α-actinin associated with focal adhesions beyond 30 min after injection of either α-actinin fragment, even though talin and vinculin were still present until ~60 min post-injection. Disruption of the cytoskeleton was not observed when cells were injected with fluorescently labeled intact α-actinin, vinculin or with a control protein, TRITC-labeled ovalbumin.

At 2 h after injection, the injected cells had lost essentially all of their stress fibers (Fig. 5). Microtubule organization, however, appeared unaltered by injection of cells with either fragment (not shown). At this advanced stage of stress fiber disruption, cells injected with either fragment had few focal adhesions as determined by IRM (Fig. 6, *b* and *d*) or by immunofluorescence with antibodies against vinculin (Fig. 6 *c*), talin, or β_1 integrin (not shown). Those focal adhesions that were present tended to be small and confined to the periphery of the cell.

We estimated that the maximum final concentration of labeled fragments injected into cells was ~20 μM based on a needle concentration of 200 μM and assuming the volume of injected protein to be ~10% of the total cell volume. Needle concentrations of α-actinin fragments that ranged from ~50-200 μm (final concentrations ~5–20 μM in cells) resulted in stress fiber and focal adhesion disruption in all cells. We estimated an α-actinin concentration in cells of 1.5–7.5 μM based on our measurement by RIA of ~1.5 pg α-actinin/fibroblast and a cell volume of 1–5 pl (Pavalko, unpublished results). When needle concentrations of either α-actinin fragment were reduced below ~50 μM (~5 μM final concentration in cells), stress fiber and focal adhesion disassembly were no longer observed suggesting that a molar concentration of α-actinin fragment approximately equal to or greater than the endogenous α-actinin concentration was required for cytoskeletal disassembly.

Cytoskeletal Disruption after Microinjection of α-Actinin Fragments Was Reversible

When REF-52 cells were injected with ~200 μM concentrations of either fragment of α-actinin and then returned to

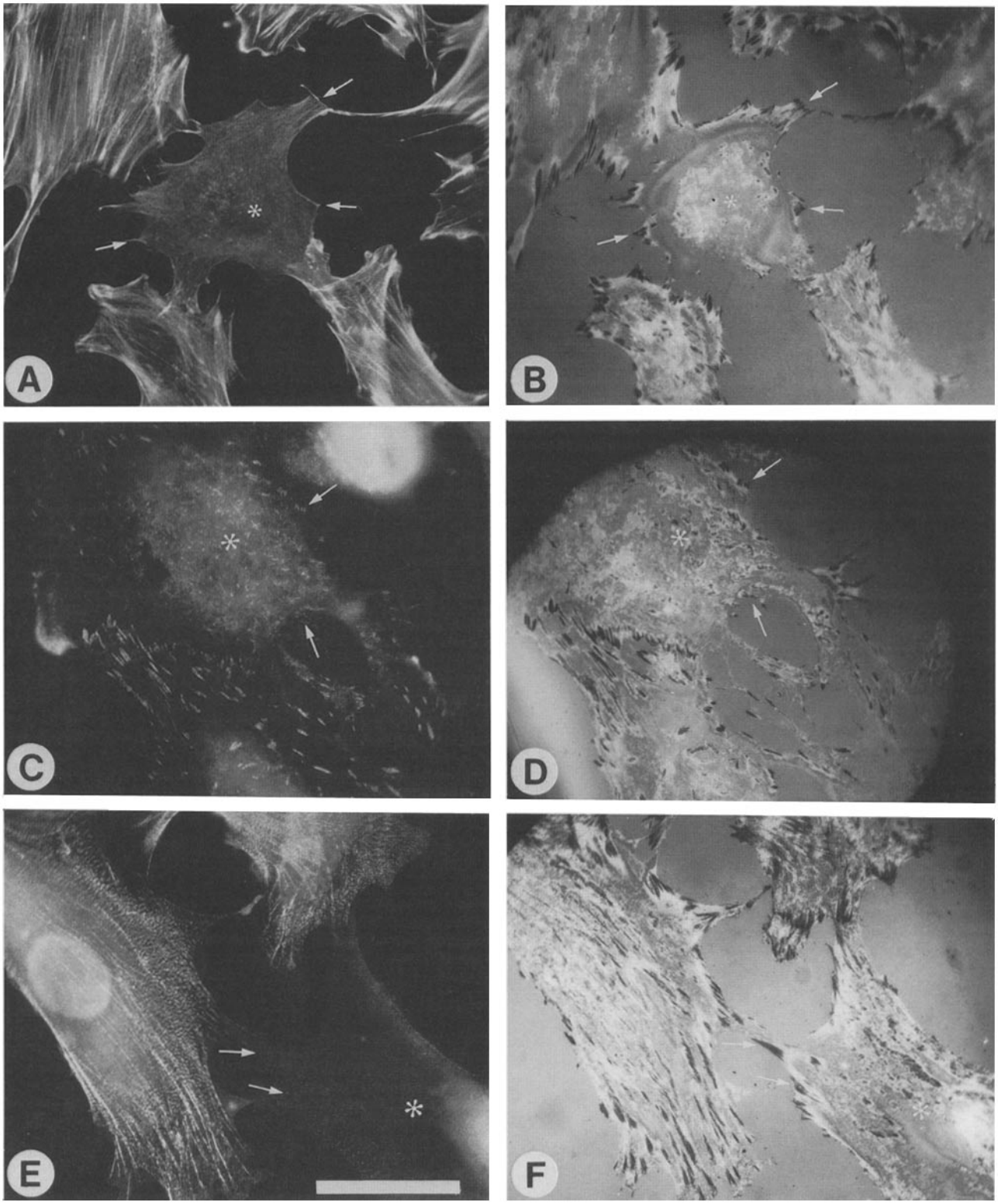

Figure 4. Focal adhesions, detected by IRM and antibody staining, persist in cells 60 min after injection with the 53-kD fragment of α-actinin although most stress fibers have been disrupted. Cells injected with the TRITC-53-kD fragment (indicated by *) were fixed after 60 min and stained with fluorescein-phalloidin (*A*), talin antisera (*C*), or α-actinin antisera (*E*) followed by FITC-anti-rabbit Ig antisera. (The α-actinin antisera was raised against mammalian α-actinin and cross-reacts poorly with chicken α-actinin 53-kD fragment.) (*B*, *D*, and *F*) The corresponding IRM images. Note that numerous small focal adhesions (*arrows*) are still present in the injected cells, especially around the cell margin. Note also that the focal adhesions remaining in the injected cell in *C* and *D* also stain for talin. These adhesions are generally smaller than their counterparts in uninjected cells such as the cell at the bottom of *C* and *D*. The injected cell in *E* and *F* has no detectable α-actinin in its remaining focal adhesions (*arrows*) although the uninjected cell at the left of the field stains normally for α-actinin. Bar, 20 μm.

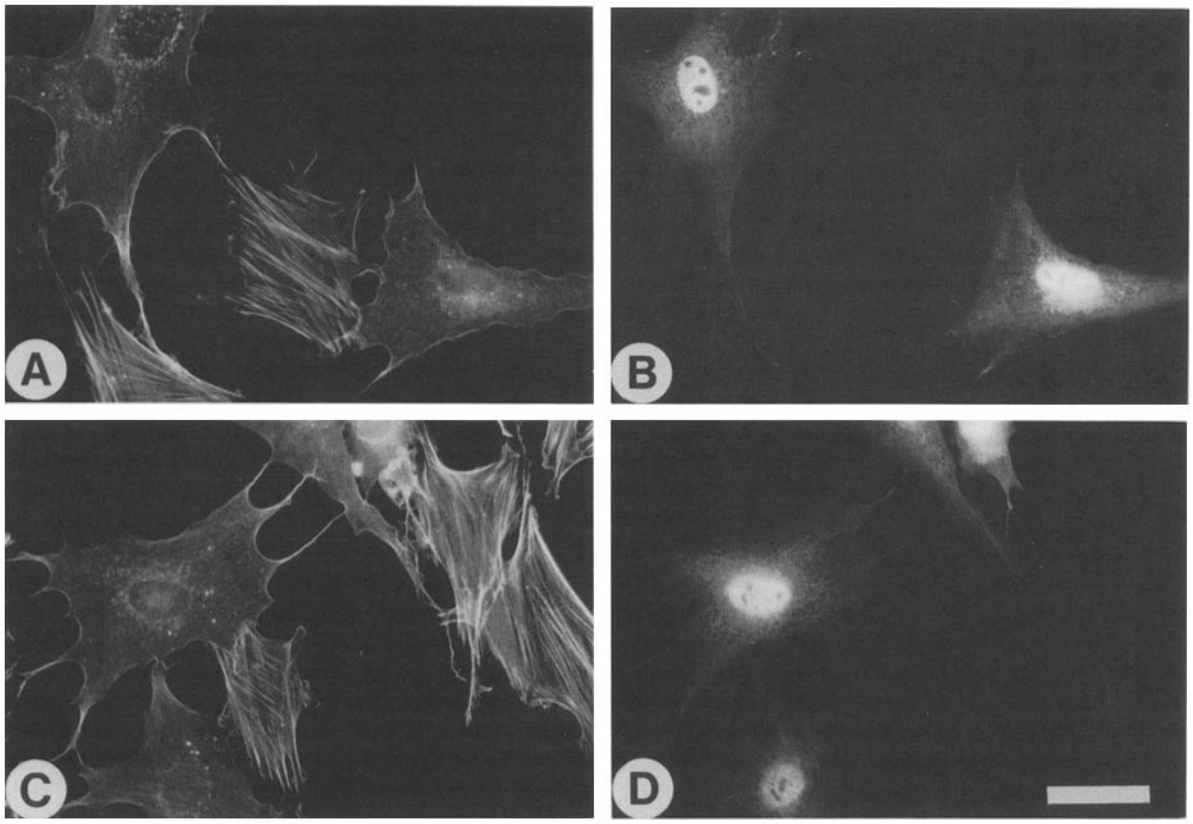

Figure 5. 2 h after injection of α-actinin fragments stress fibers are completely disassembled. REF-52 cells were injected with FITC-27-kD (*B*) or FITC-53-kD (*D*) fragment of α-actinin are returned to 37°C for 2 h before fixation. *A* and *C* show rhodamine-phalloidin staining demonstrating the complete lack of stress fibers in injected cells. Bar, 20 μm.

37°C for 18 h before fixation and staining with phalloidin, ~70% of the cells had redeveloped stress fibers (35 of 49 cells injected with 53 kD; 26 of 38 cells injected with 27 kD). The remaining 30% of cells showed little or no stress fiber reformation although the cells remained spread. Since injection with these fragments resulted in a complete loss of stress fibers in all cells after 2 h, the ability of a majority of cells to recover indicates that in most cases the disruption was reversible and was not the result of cell death.

Analysis of the Actin-binding Properties of α-Actinin Fragments

The 27-kD fragment has previously been shown to contain the binding site for actin (whereas the 53-kD fragment does not bind to actin) (Mimura and Asano, 1986). This was confirmed in actin sedimentation assays (Fig. 7). Additionally, we found no evidence that 53-kD dimers could undergo an exchange of subunits with intact α-actinin (Fig. 7, lane *19*). If such an exchange of subunits had occurred we would have expected to find a proportion of the 53-kD fragment sedimenting with actin/α-actinin complexes. Thus, it seemed unlikely that the 53-kD fragment could directly affect cross-linking of actin filaments by α-actinin or associate with soluble α-actinin to alter the exchange of α-actinin between stress fibers and the cytoplasm.

Binding of Vinculin to Fragments of α-Actinin

The binding domain on α-actinin for vinculin was determined by using ^{125}I-labeled vinculin in a protein overlay assay of α-actinin fragments that had been separated by SDS-gel electrophoresis and transferred to nitrocellulose. Fig. 8 shows a Coomassie blue–stained gel of α-actinin digested with thermolysin for increasing lengths of time (*A*). ^{125}I-vinculin binds to intact α-actinin, to a band just below intact α-actinin of ~90 kD, and to the 27-kD α-actinin fragment. Vinculin also bound to higher molecular weight bands from the crude gizzard extract which probably correspond to talin and to its 190-kD proteolytic fragment, which are both present in this extract and are known to bind vinculin (Burridge and Mangeat, 1984). The band at ~38 kD in lanes *5–9* of Fig. 8 is the enzyme thermolysin, which may bind vinculin since vinculin is a substrate for thermolysin (data not shown). Neither the 53-kD fragment nor its apparent precursors were recognized by vinculin.

Discussion

An interaction between purified α-actinin and the cytoplasmic domain of integrin was recently reported (Otey et al., 1990). The binding site on α-actinin for integrin was found

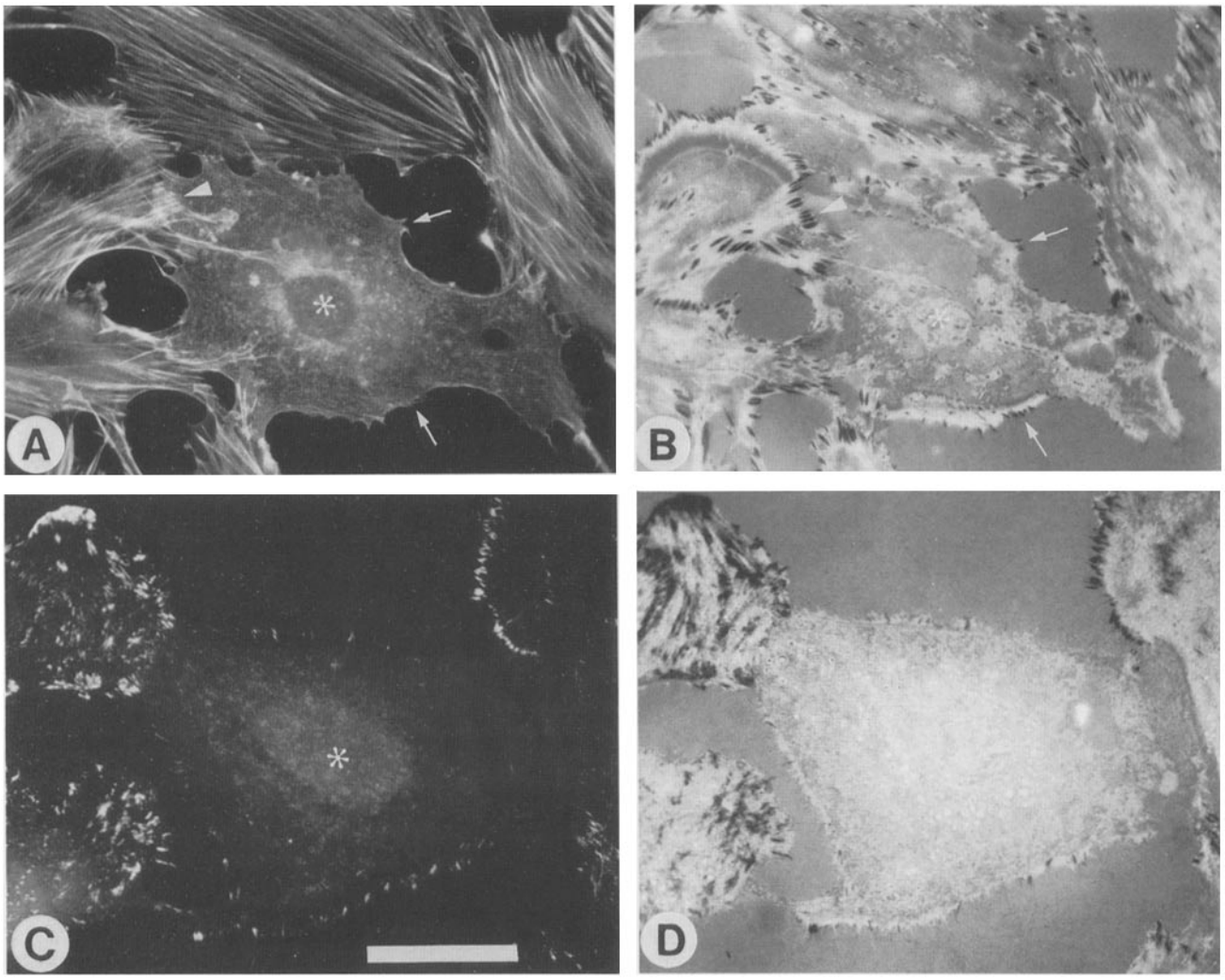

Figure 6. Microinjection of α-actinin fragments causes focal adhesions to disassemble 2 h after injection. REF-52 cells were microinjected with the TRITC-53-kD fragment (*A* and *B*) or FITC-27-kD fragment (*C* and *D*) of α-actinin and fixed 2 h after injection. The same field of cells shown in *A* stained with fluorescein-phalloidin are seen by IRM (*B*) demonstrating that the injected cell (indicated by *), which has no stress fibers, has only a few small adhesions around the cell margin (*arrows*). Uninjected cells have normal stress fibers that terminate in large black focal adhesions (*arrowheads*). Note that in *A* and *B* some of the normal adhesions in uninjected cells overlap with the injected cell. *C* and *D* show a cell injected with the TRITC-27-kD fragment that has been stained with antibodies against vinculin (*C*) and shown by IRM (*D*). This cell almost completely lacks focal adhesion staining for vinculin (*C*) and has only very small focal adhesions around the cell margin as seen by IRM (*D*). Bar, 20 μm.

to lie within the 53-kD rod domain of α-actinin. This work depended on in vitro assays, but raised the possibility that α-actinin may provide a direct link between integrins and actin within cells. We have explored this possibility further by asking whether the integrin-binding fragment of α-actinin would colocalize with integrin in focal adhesions following microinjection of this fragment into cells. We found that this was the case. Additionally, we found that the 27-kD fragment of α-actinin initially bound along stress fibers following its microinjection into cells, consistent with its containing an actin-binding site. Somewhat surprisingly, disassembly of actin stress fibers and focal adhesions began to be detected ~30 min after introduction of either fragment into cells.

Disruption of Actin Organization by the 53-kD Fragment

The initial localization of the microinjected 53-kD fragment of α-actinin in focal adhesions but not along stress fibers is consistent with the previously described interaction of this fragment with integrin cytoplasmic domains, but it could also result from other interactions. For example, it has been reported that α-actinin binds to vinculin, another protein concentrated in focal adhesions (Wachsstock et al., 1987). Using a blot assay we have failed to detect an interaction between the 53-kD fragment and vinculin but have shown that this apparent interaction occurs in the 27-kD domain of α-actinin. Alternatively, the 53-kD fragment may localize to fo-

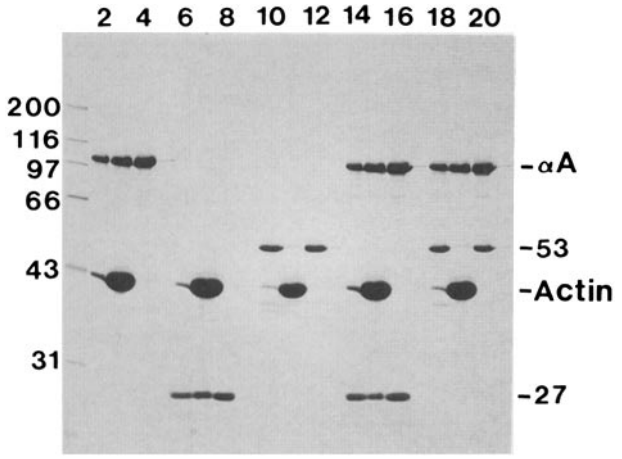

Figure 7. Actin sedimentation assays with α-actinin and the 27- and 53-kD fragments of α-actinin. Supernatants are shown in all even numbered lanes (*2–20*) and pellets in all odd numbered lanes (*3–21*). Molecular mass standards are shown in lane *1*, mass in kilodalton is shown at left. Intact α-actinin sediments with actin (lanes *2* and *3*) but not alone (lanes *4* and *5*). the 27-kD fragment sediments with actin (lanes *6* and *7*) but not alone (lanes *8* and *9*). The 53-kD fragment does not sediment either with actin (lanes *10* and *11*) or alone (lanes *12* and *13*). When mixed together, intact α-actinin and the 27-kD fragment sediment with actin (lanes *14* and *15*) but not alone (lanes *16* and *17*). When intact α-actinin and the 53-kD fragment are mixed together, only intact α-actinin sediments with actin (lanes *18* and *19*) demonstrating that the 53-kD fragment does not undergo significant subunit exchange with intact α-actinin dimers. Intact α-actinin does not sediment when mixed with the 53-kD fragment in the absence of actin (lanes *20* and *21*).

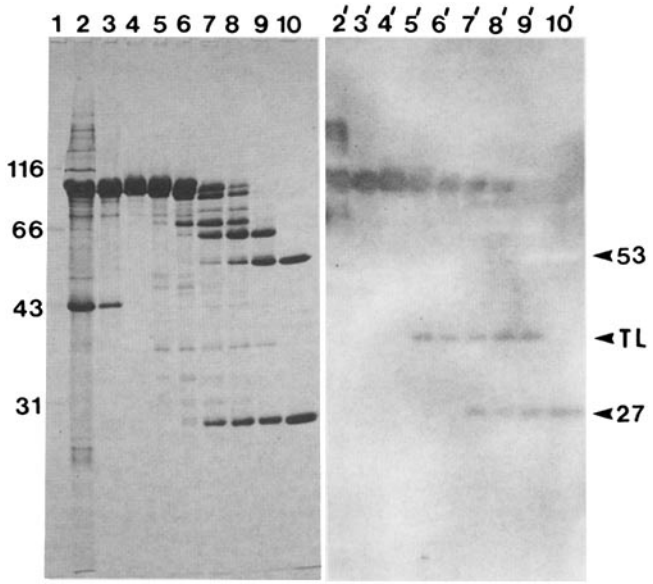

Figure 8. ^{125}I-vinculin overlay of α-actinin and α-actinin fragments generated by thermolysin cleavage. Lanes *1–10* show a polyacrylamide gel stained with Coomassie blue. Lane *1*, molecular mass standards. Lane *2*, fraction of chicken gizzard proteins enriched for α-actinin after DE52 anion exchange chromatography. Lane *3*, fraction of chicken gizzard proteins after an additional purification step on a CL-6B gel filtration column. Lane *4*, purified α-actinin after Mono Q anion exchange chromatography. Lanes *5–9*, time course of digestion of purified α-actinin with thermolysin (lane *5* = 10 min, *6* = 30 min, *7* = 45 min, *8* = 90 min, and *9* = 120 min) at 37°C. Lane *10*, FPLC-purified 27- and 53-kD fragments mixed back together, i.e., no thermolysin is present in this lane. Lanes *2'–10'*, samples identical to those in lanes *2–10* transferred to nitrocellulose and overlayed with ^{125}I-vinculin. Note that the vinculin binds to intact α-actinin and to the 27-kD fragment, but not to the 53-kD fragment. In lane *2'*, vinculin binds to several proteins of ~190–220 kD (probably talin and its fragments which are known to be present in this DE52 fraction) and to unidentified proteins ~80 kD. Vinculin also binds to thermolysin (*TL*) at 38 kD. Molecular masses are shown at left in kilodaltons.

cal adhesions because of an association with some other as yet unidentified focal adhesion component. At the moment, targeting of the 53-kD α-actinin fragment to focal adhesions via an interaction with integrin cytoplasmic domains is a likely explanation, but we will continue to look for other cytoskeletal proteins that bind to this region of α-actinin.

Disruption of stress fibers was complete 2 h after injection of the 53-kD fragment. The number of focal adhesions was also reduced although the time course of focal adhesion loss lagged slightly behind the disassembly of stress fibers. By 2 h after injection only a few small adhesions remained around the cell periphery. Stress fiber and focal adhesion disassembly occurred when the concentration of injected 53-kD fragment exceeded ~5 μM. We had not anticipated this result since microinjection of vinculin (Burridge and Feramisco, 1980), talin or talin fragments (Hock et al., 1989; Nuckolls et al., 1990) does not noticeably disrupt focal adhesions or stress fibers. After their injection into cells, vinculin, talin, and talin fragments accumulate in focal adhesions, either by direct addition to these structures or by exchange with the proteins already present. In contrast, reversible disruption of stress fibers in nonmuscle cells, and in some cases of focal adhesions as well, has been observed following microinjection of several actin-binding proteins that sever or cap actin filaments (Fuchtbauer et al., 1983; Jockusch et al., 1985; Weeds et al., 1985; Cooper et al., 1987; Huckriede et al., 1990; Franck et al., 1990). Myofibrils in cardiac myocytes, however, were resistant to disassembly by the actin capping/severing protein gelsolin (Sanger et al., 1987*b*) and gelsolin from some sources had no affect on stress fibers in nonmuscle cells (Cooper et al., 1987; Huckriede et al., 1990). Stress fibers have also been disrupted by microinjection of vitamin D binding protein and DNase I, two other proteins that bind monomeric actin (Sanger et al., 1990).

Our results differ from these previous studies in that the 53-kD fragment of α-actinin does not bind actin directly (Mimura and Asano, 1986). We have confirmed this lack of interaction with actin and have presented evidence indicating that there is no detectable subunit exchange between the 53-kD fragment and intact α-actinin dimers, which would have resulted in defective molecules unable to cross-link actin filaments. We can envisage at least three explanations for why the microinjected 53-kD fragment disrupts stress fibers. In the first case, the fragment may compete with endogenous intact α-actinin for binding to integrin cytoplasmic domains and thereby dislocate one of the attachments of stress fibers to focal adhesions. The detachment of the stress fiber may result in its disassembly. A second possible explanation is that the 53-kD fragment may recognize and bind to a

presently unidentified focal adhesion component that is competed from focal adhesions and is necessary for maintaining actin attachments. Despite the lack of binding of the 53-kD fragment to stress fibers, a third possible explanation is that the 53-kD fragment competes with intact α-actinin for binding to some other protein along the length of stress fibers whose displacement results in an unbundling of actin filaments. Although our data do not yet allow us to distinguish among these possibilities, they suggest a role for protein binding sites within the rod domain of α-actinin in maintaining actin–integrin interactions and stress fiber organization in cells.

Disruption of Actin Organization by the 27-kD Fragment

The 27-kD fragment of α-actinin contains the binding site for actin and initially localizes along actin stress fibers and focal adhesions after its microinjection into nonmuscle cells. The actin-binding activity of this fragment presumably accounts for its ability to bind along stress fibers including at their ends where they terminate in focal adhesions. One possible explanation for the subsequent disassembly of stress fibers after introduction of this fragment into cells is that it is displacing endogenous α-actinin molecules along stress fibers that are necessary for bundling actin filaments. An alternative explanation for the loss of stress fibers after microinjection of the 27-kD fragment may lie in the ability of this fragment to bind to vinculin or another, unidentified, focal adhesion component. Interference of a link between vinculin or some other protein and α-actinin at focal adhesions could potentially lead to a weakening of stress fiber-membrane attachments sufficient to disassemble stress fibers. The time course and concentration dependence of stress fiber and focal adhesion disruption was indistinguishable from that seen after injection of the 53-kD fragment. Neither fragment of α-actinin resulted in detachment of cells from the substrate despite the disruption of stress fibers and focal adhesions.

Stress Fiber Disassembly Precedes Loss of Focal Adhesions

At intermediate time points (~60 min) after microinjection of α-actinin fragments, cells that lacked stress fibers often revealed small focal adhesions, particularly at their peripheries. These residual focal adhesions continued to stain for vinculin, talin, and integrin but not α-actinin. Evidence consistent with the loss of focal adhesions being secondary to the loss of stress fibers comes from the studies of Meigs and Wang (1986) who noted that loss of α-actinin from stress fibers and focal adhesions preceded the loss of vinculin from focal adhesions in BSC-1 cells treated with phorbol esters. Stress fibers can also be disrupted by microinjection into cells of a number of agents which appear to decrease or inhibit the interaction of myosin with actin, such as the catalytic subunit of the cAMP-dependent protein kinase, or antibodies to the myosin light chain kinase (Lamb et al., 1988) or type 1 protein phosphatase (Fernandez et al., 1990). Under these conditions it would be interesting to determine whether focal adhesions subsequently disassemble.

What Is the Role of α-Actinin in Focal Adhesions?

α-Actinin is normally included in lists of focal adhesion proteins (e.g., Burridge et al., 1988). However, we have frequently observed that the intensity of staining for α-actinin varies among different cell types. Even within a single cell, adhesions may vary in their intensity of staining with α-actinin antibodies. This raises the critical question of the relative role of α-actinin in focal adhesions. We suspect that multiple modes of attachment between actin filaments and the plasma membrane occur at focal adhesions, one of which may involve talin (Burridge et al., 1990) and another of which may involve α-actinin bridging directly between actin and integrin (Otey et al., 1990). Attachments involving different proteins may reflect different stages in the formation of focal adhesions. It could be, for example, that α-actinin is not important in the initial formation of a focal adhesion but is involved in the attachment of mature stress fibers to the focal adhesion. Consistent with this idea, in response to microinjection of the 53-kD fragment, we have observed focal adhesions lacking detectable α-actinin that stain for both vinculin and talin. In general these structures did not support prominent stress fibers. Future experiments will be aimed at testing this idea that the level of α-actinin in a focal adhesion varies with its maturity.

We are grateful to Dr. Glen Nuckolls for extensive scientific discussions and for expert technical advice on microinjection technique. We also thank Drs. Carol Otey and Christopher Turner for advice and critical reading of the manuscript; Dr. Richard Hynes for his gift of antibody against integrin and Ms. Betty Stewart for help with preparation of the manuscript.

This work was supported by National Institutes of Health (NIH) grant GM29860 to K. Burridge and a Muscular Dystrophy Association grant to K. Burridge. F. M. Pavalko was supported by an NIH National Research Service Award CA-08493.

Received for publication 10 October 1990 and in revised form 26 April 1991.

References

Baron, M. D., M. D. Davison, P. Jones, and D. R. Critchley. 1987. The sequence of chick α-actinin reveals homologies to spectrin and calmodulin. *J. Biol. Chem.* 262:17623–17629.

Belkin, A. M., and V. E. Koteliansky. 1987. Interaction of iodinated vinculin, metavinculin and α-actinin with cytoskeletal proteins. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 220:291–294.

Bennett, J. P., K. S. Zaner, and T. P. Stossel. 1984. Isolation and some properties of macrophage α-actinin: evidence that it is not an actin gelling protein. *Biochemistry.* 23:5081–5086.

Bretscher, A., J. Vandekerckhove, and K. Weber. 1979. α-actinins from chicken skeletal muscle and smooth muscle show considerable chemical and immunological differences. *Eur. J. Biochem.* 100:237–243.

Burridge, K., and J. R. Feramisco. 1980. Microinjection and localization of a 130K protein in living fibroblasts: a relationship to actin and fibronectin. *Cell.* 19:587–595.

Burridge, K., and L. McCullough. 1980. The association of α-actinin with the plasma membrane. *J. Supramol. Res.* 13:53–65.

Burridge, K., and J. R. Feramisco. 1981. Non-muscle α-actinins are calcium-sensitive actin-binding proteins. *Nature (Lond.).* 294:565–567.

Burridge, K., and L. Connell. 1983. A new protein of adhesion plaques and ruffling membranes. *J. Cell Biol.* 97:359–367.

Burridge, K., and P. Mangeat. 1984. An interaction between vinculin and talin. *Nature (Lond.).* 308:744–746.

Burridge, K., K. Fath, T. Kelly, G. Nuckolls, and C. Turner. 1988. Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu. Rev. Cell Biol.* 4:487–525.

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.

Chen, W. T., and S. J. Singer. 1982. Immunoelectron microscopic studies of the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. *J. Cell Biol.* 95:205–222.

Chen, W. -T., E. Hasegawa, T. Hasegawa, C. Weinstock, and K. M. Yamada. 1985. Development of cell surface linkage complexes in cultured fibroblasts. *J. Cell Biol.* 100:1103–1114.

Cooper, J. A., J. Bryan, B. Schwab III, C. Frieden, D.J. Loftus, and E. Elson.

1987. Microinjection of gelsolin into living cells. *J. Cell Biol.* 104:491–501.
Damsky, C. H., K. A. Knudsen, D. Bradley, C. A. Buck, and A. F. Horwitz. 1985. Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. *J. Cell Biol.* 100:1528–1539.
Davison, M. D., and D. R. Critchley. 1988 α-actinins and the DMD protein contain spectrin-like repeats. *Cell.* 52:159–160.
Davison, M. D., M. D. Baron, D. R. Critchley, and J. C. Wootton. 1989. Structural analysis of homologous repeated domains in α-actinin and spectrin. *Int. J. Biol. Macromol.* 11:81–90.
Duhaiman, A. S., and J. R. Bamburg. 1984. Isolation of brain α-actinin. Its characterization and comparison of its properties with those of muscle α-actinins. *Biochem.* 23:1600–1608.
Endo, T., and T. Masaki. 1982. Molecular properties and functions in vitro of chicken smooth muscle α-actinin in comparison with those of striated muscle α-actinins. *J. Biochem. (Tokyo).* 92:1457–1468.
Feramisco, J. R. 1979. Microinjection of fluorescently labeled α-actinin into living fibroblasts. *Proc. Natl. Acad. Sci. USA.* 76:3967–3971.
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.
Fernandez, A., D. L. Brautigan, M. Mumby, and N. J. C. Lamb. 1990. Protein phosphatase type-1, not type-2A, modulates actin microfilament integrity and myosin light chain phosphorylation in living nonmuscle cells. *J. Cell Biol.* 111:103–112.
Franck, Z., M. Footer, and A. Bretscher. 1990. Microinjection of villin into cultured cells induces rapid and long-lasting changes in cell morphology but does not inhibit cytokinesis, cell motility or membrane ruffling. *J. Cell Biol.* 111:2475–2485.
Fuchtbauer, A., B. M. Jockusch, H. Maruta, M. W. Kilimann, and G. Isenberg. 1983. Disruption of microfilament organization after injection of F-actin capping proteins into living tissue culture cells. *Nature (Lond.).* 304:361–364.
Geiger, B. 1979. A 130-K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells. *Cell.* 18:193–205.
Geiger, B., A. J. Dutton, K. T. Tokuyasu, and S. J. Singer. 1981. Immunoelectron microscope studies of membrane-microfilament organizations: 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., W. F. H. M. Mommaerts, M. K. Reedy, and K. Seraydarian. 1969. Studies on α-actinin-like proteins liberated during trypsin digestion of α-actinin and myofibrils. *Biochim. Biophys. Acta.* 175:174–194.
Hammond, R. G. 1987. Protein sequence of DMD gene is related to actin-binding domain of α-actinin. *Cell.* 51:1.
Hock, R. S., J. M. Sanger, and J. W. Sanger. 1989. Talin dynamics in living microinjected non-muscle cells. *Cell Motil. Cytoskel.* 14:271–287.
Horwitz, A. F., K. Duggan, C. Buck, M. C. Beckerle, and K. Burridge. 1986. Interactions of plasma membrane fibronectin receptor with talin: a transmembrane linkage. *Nature (Lond.).* 320:531–533.
Huckriede, A., A. Fuchtbauer, H. Hinssen, C. Chaponnier, A. Weeds, and B. M. Jockusch. 1990. Differential effects of gelsolins on tissue culture cells. *Cell. Motil. Cytoskel.* 16:229–238.
Imamura, M., T. Endo, M. Kuroda, T. Tanaka, and T. Masaki. 1988. Substructure and higher structure of chicken smooth muscle α-actinin molecule. *J. Biol. Chem.* 263:7800–7805.
Jockusch, B. M., A. Fuchtbauer, C. Wiegand, and B. Honer. 1985. Dynamics of microfilament organization and membrane anchorage. *In* J. E. Dumont, B. Hamprecht, and J. Nunez, editors. Elsevier North Holland, Amsterdam. 9:135–142.
Kelly, T., L. Molony, and K. Burridge. 1987. Purification of two smooth muscle glycoproteins related to integrin: distribution in cultured chicken embryo fibroblasts. *J. Biol. Chem.* 262:17189–17199.
Koenig, M., A. P. Monaco, and L. M. Kunkel. 1988. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell.* 53:219–228.
Kreis, T. E., and W. Birchmeier. 1980. Stress fiber sarcomeres of fibroblasts are contractile. *Cell.* 22:555–561.
Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
Lamb, N. J. C., A. Fernandez, M. A. Conti, R. Adelstein, D. B. Glass, W. J. Welch, and F. R. Feramisco. 1988. Regulation of actin microfilament integrity in living nonmuscle cells by the cAMP-dependent protein kinase and the myosin light chain kinase. *J. Cell Biol.* 106:1955–1971.
Landon, F., Y. Gache, J. Touitou, and A. Olomucki. 1985. Properties of two isoforms of human blood platelet α-actinins. *Eur. J. Biochem.* 153:231–237.
Lazarides, E., and K. Burridge. 1975. α-Actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. *Cell.* 6:289–298.
Maruyama, K., and S. Ebashi. 1965. α-Actinin, a new structural protein from striated muscle. II. Action on actin. *J. Biochem. (Tokyo).* 58:13–19.
Masaki, T., M. Endo, and S. Ebashi. 1967. Localization of α-actinin at Z-bands. *J. Biochem. (Tokyo).* 62:630–632.
Meigs, J. B., and Y. Wang. 1986. Reorganization of α-actinin and vinculin induced by a phorbol ester in living cells. *J. Cell Biol.* 102:1430–1438.
Mimura, N., and A. Asano. 1979. Ca^{2+}-sensitive gelation of actin filaments by a new protein factor. *Nature (Lond.).* 282:44–48.
Mimura, N., and A. Asano. 1986. Isolation and characterization of a conserved actin-binding domain from rat hepatic actinogelin, rat skeletal muscle and chicken gizzard α-actinins. *J. Biol. Chem.* 261:10680–10687.
Mimura, N., and A. Asano. 1987. Further characterization of a conserved actin-binding 27 kDa fragment of actinogelin and α-actinins and mapping of their binding sites on the actin molecule by chemical cross-linking. *J. Biol. Chem.* 262:4717–4723.
Noegel, A., W. Witke, and M. Schleicher. 1987. Calcium sensitive non-muscle α-actinin contains EF-hand structures and highly conserved regions. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 221:391–396.
Nuckolls, G. H., C. E. Turner, and K. Burridge. 1990. Functional studies of the domains of talin. *J. Cell Biol.* 110:1635–1644.
Otey, C. A., F. M. Pavalko, and K. Burridge. 1990. An interaction between α-actinin and the β_1 integrin subunit in vitro. *J. Cell Biol.* 111:721–729.
Otto, J. J. 1983. Detection of vinculin-binding proteins with an ^{125}I-vinculin gel overlay technique. *J. Cell Biol.* 97:1283–1287.
Pavalko, F. M., C. A. Otey, and K. Burridge. 1989. Identification of a filamin isoform enriched at the ends of stress fibers in chicken embryo fibroblasts. *J. Cell Sci.* 94:109–118.
Podlubnaya, Z. A., L. A. Tskhovnebova, M. M. Zaalishvili, and G. A. Stefanenko. 1975. Electron microscopic study of α-actinin. *J. Mol. Biol.* 92: 357–359.
Pollard, T. D. 1981. Purification of a calcium-sensitive actin gelation protein from *Acanthamoeba*. *J. Biol. Chem.* 256:7666–7670.
Sanger, J. W., B. Mittal, and J. M. Sanger. 1984. Interaction of fluorescently labeled contractile proteins with the cytoskeleton in cell models. *J. Cell Biol.* 99:918–928.
Sanger, J. M., B. Mittal, M. Pochapin, and J. W. Sanger. 1986. Observations of microfilament bundles in living cells microinjected with fluorescently labeled contractile proteins. *J. Cell Sci. Suppl.* 5:17–44.
Sanger, J. M., B. Mittal, M. B. Pochapin, and J. W. Sanger. 1987*a*. Stress fiber and cleavage furrow formation in living cells microinjected with fluorescently labeled α-actinin. *Cell Motil. Cytoskel.* 7:209–220.
Sanger, J. M., B. Mittal, A. Wegner, B. M. Jockusch, and J. W. Sanger. 1987*b*. Differential response of stress fibers and myofibrils to gelsolin. *Eur. J. Cell Biol.* 43:421–428.
Sanger, J. M., G. Dabiri, B. Mittal, M. A. Kowalski, J. G. Haddad, and J. W. Sanger. 1990. Disruption of microfilament organization in living nonmuscle cells by microinjection of plasma vitamin D-binding protein or DNase I. *Proc. Natl. Acad. Sci. USA.* 87:5474–5478.
Singh, I., D. E. Goll, F. M. Robson, and M. H. Stromer. 1977. N- and C-terminal amino acids of purified α-actinin. *Biochim. Biophys. Acta.* 491:29–45.
Spudich, J. A., and S. Watt. 1971. The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* 246:4866–4871.
Suzuki, A., D. E. Goll, I. Singh, R. E. Allen, R. M. Robson, and M. J. Stromer. 1976. Some properties of purified skeletal muscle α-actinin. *J. Biol. Chem.* 251:6860–6870.
Turner, C. E., and K. Burridge. 1989. Detection of metavinculin in human platelets using a modified talin overlay assay. *Eur. J. Cell Biol.* 49:202–206.
Wachsstock, D. J., J. A. Wilkins, and S. Lin. 1987. Specific interaction of vinculin with α-actinin. *Biochim. Biophys. Res. Commun.* 146:554–560.
Weeds, A. G., H. E. Harris, J. Gooch, and P. Pope. 1985. Actin capping proteins. *In* Hormones and Cell Regulation. J. E. Dumont, B. Hamprecht, and J. Nunez, editors. Elsevier North Holland, Amsterdam. 9:143–165.
Wehland, J., M. Osborn, and K. Weber. 1979. Cell to substratum contacts in living cells: a direct correlation between interference reflection and indirect immunofluorescence microscopy using antibodies against α-actinin. *J. Cell Sci.* 37:257–273.
Wilkins, J. A., K. Y. Chen, and S. Lin. 1983. Detection of high molecular weight vinculin binding proteins in muscle and nonmuscle tissues with an electroblot-overlay technique. *Biochem. Biophys. Res. Commun.* 116:1026–1032.