Interactions of Tensin with Actin and Identification of Its Three Distinct Actin-binding Domains

Su Hao Lo, Paul A. Janmey,* John H. Hartwig,* and Lan Bo Chen
Dana-Farber Cancer Institute, *Brigham’s and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Abstract. Tensin, a 200-kD phosphoprotein of focal contacts, contains sequence homologies to Src (SH2 domain), and several actin-binding proteins. These features suggest that tensin may link the cell membrane to the cytoskeleton and respond directly to tyrosine kinase signalling pathways. Here we identify three distinct actin-binding domains within tensin. Recombinant tensin purified after overexpression by a baculovirus system binds to actin filaments with \(K_d = 0.1 \ \mu M\), cross-links actin filaments at a molar ratio of 1:10 (tensin/actin), and retards actin assembly by barbed end capping with \(K_d = 20 \ \text{nM}\). Tensin fragments were constructed and expressed as fusion proteins to map domains having these activities. Three regions from tensin interact with actin: two regions composed of amino acids 1 to 263 and 263 to 463, cosediment with F-actin but do not alter the kinetics of actin assembly; a region composed of amino acids 888-989, with sequence homology to insertin, retards actin polymerization. A claw-shaped tensin dimer would have six potential actin-binding sites and could embrace the ends of two actin filaments at focal contacts.

Focal contacts (adhesion plaques, focal adhesions) are specialized regions of plasma membranes where cultured cells such as fibroblasts adhere to the underlying substratum and where actin filament bundles terminate (Burridge et al., 1988; Geiger, 1989; Luna and Hitt, 1992). Focal contacts might be the sites where external signals such as mitogenic stimulations are transduced into the interior of cells (Lo and Chen, 1994). In the course of cell cycling or neoplastic transformation, major reorganizations of focal contacts are believed to be responsible for the loss of adhesion and concomitant morphological changes (Kellie, 1988). During cell migration, subtle but dynamic alterations in the structures and compositions of focal contacts are likely to play a role in both attachment and detachment of cells (Woods and Couchman, 1988). The supramolecular structure of the linkage between actin filaments and the plasma membrane at the focal contacts is, however, poorly understood.

A growing number of proteins have been identified at focal contacts. These include vinculin (Geiger, 1979; Feramisco and Burridge, 1980) \( \alpha \)-actinin (Lazarides and Burridge, 1975), talin (Burridge and Connell, 1983), paxillin (Turner et al., 1990), zyxin (Crawford and Beckerle, 1991), radixin (Sato et al., 1991), Src (Rohrschneider, 1980), focal adhesion kinase (FAK) \(^1\) (Schaller et al., 1992), tenuin (Tsukita et al., 1989), filamin/ABP-280 (Feramisco and Burridge, 1980; Pavalko et al., 1989), vasodilator-stimulated phosphoprotein (Reinhard et al., 1992), FAK-related nonkinase (Schaller et al., 1993), protease (Beckerle et al., 1987; Chen et al., 1984), fibronectin, and integrin (Chen et al., 1986; Horwitz et al., 1990). The role of each of these proteins in focal contacts is largely unknown, although, it appears that fibronectin and integrin are involved in the adhesion to the substratum (Horwitz et al., 1990), talin probably interacts with the cytoplasmic tail of integrin (Horwitz et al., 1986) and vinculin appears to bind talin (Burridge and Mangeat, 1984). A critical issue has been the identification of focal contact components involved in linking the actin filaments to the integral constituents of the plasma membranes. As reported by Wilkins and Lin (1986), tensin appears to be a candidate serving such a role. However, it should be noted that several alternatives or parallel linkages may exist in anchoring actin filaments to focal contacts (Muguruma et al., 1990; Otey et al., 1990; Turner and Burridge, 1991).

We have undertaken the molecular cloning of tensin from chick embryo fibroblasts (CEF) (Davis et al., 1991) and chick cardiac muscle (Lo et al., 1994) (GenBank accession number M63606, M74165, M96624, and M96625). Unexpectedly, a database search revealed the presence of a "Src homology domain 2" (SH2 domain) between amino acid residues 1471 and 1580 of chick tensin. The SH2 domains bind phosphotyrosine and have now been found in a growing number of proteins involved in signal transduction (Koch et al., 1991; Pawson and Gish, 1992). Moreover, tensin shares significant sequence homology with several proteins which intriguingly fall into two categories: (a) those involved in cell...
adhesion and motility including actin, catenin-α, insertin, synapsin Ia, and F-actin-binding proteins; and (b) those involved in signal transduction including BCR, IL-2/EPO receptors, IL-3 receptor, and SH2 domains (Lo et al., 1994). If indeed tensin binds to actin filaments as has been proposed (Wilkins and Lin, 1986), it could potentially serve a critical role in integrating signal transduction with the cytoskeleton by binding to both tyrosine-phosphorylated proteins and actin filaments. Tensin might thus provide a nucleus around which signaling complexes can be assembled.

Here we report the interactions of recombinant chick cardiac muscle tensin with actin filaments and the identification of three distinct actin-binding regions in tensin. We propose a model that may permit treadmilling of membrane-attached actin filaments.

Materials and Methods

Construction and Expression of Epitope-tagged Tensin and Tensin Fragments as Fusion Proteins

Full-length epitope-tagged tensin was expressed and purified from a baculovirus expression system (Lo et al., 1994). A series of truncated tensin cDNAs was constructed and expressed as fusion proteins with glutathione-S-transferase (GST) using pGEX vectors (Pharmacia, Piscataway, NJ). Plasmid p52(263-741), encoding a 52-kD polypeptide of tensin (amino acids 263 to 741), was generated by subcloning theBgIII-EcoRI fragment (1,439 bp) restriction fragment of tensin cDNA into a pGEX-3X. Plasmid p25(263-292/543-741) was derived from p52 by deleting theSmal-PmlI fragment (753 bp) which encodes amino acid residues 293 to 543. Plasmid p32(263-463) was constructed by ligation of theBgIII-PmlI fragment (603 bp) into pGEX-3X. An EcoRI cloning site on Bluescript)-EcoRI fragment (1053 bp) fragment of clone HE9, a cDNA clone isolated from a chick cardiac muscle cDNA library, was ligated to a pGEX-2T to generate plasmid p37(393-741). Plasmid p29(1-263) and p51(1-463) were constructed by subeloning theBamHI-NsiI fragment into pGEX-3X and pGEX-3X respectively. A schematic representation of each truncated protein is shown in Fig. 4.

The fusion proteins were expressed and purified as described (Smith and Corcoran, 1990). In brief, the transformed cell culture (DH5α) was grown in LB/Ampicillin medium, diluted 1:10 into fresh LB for 1 h at 37°C, and induced with 1 mM IPTG for 2 h. Collected cells were resuspended in PBS. After sonication and centrifugation at 10,000 g for 10 min, the supernatant was incubated with glutathione (GSH)-conjugated beads. GSH-conjugated beads were washed with PBS and bound proteins eluted with 50 mM Tris HCl (pH 8) supplemented with 5 mM GSH.

Actin Filaments Codensation Assay

Rabbit skeletal muscle actin was purified as described previously (Spudich and Watt, 1971). Recombinant proteins and G-actin were first centrifuged for 20 min at 28,000 g in a Beckman Airfuge (Beckman Instruments, Fullerton, CA) to remove any aggregates. Tensin constructs were then mixed with rabbit skeletal G-actin at a molar ratio indicated in the figures in polymerization buffer (10 mM Tris, pH 7.4, 130 mM KCl, 2 mM MgCl2, 0.2 mM ATP, 1 mM β-mercaptoethanol, 0.1 mM EDTA, and 0.1% NaN3). The reaction mixture was incubated for 1 h at room temperature and ultracentrifuged for 20 min at 28,000 g. (about 100,000 g) in a Beckman Airfuge to pellet the F-actin and the associated proteins. The amounts of tensin in the supernatant and actin-containing pellet were determined by densitometric scanning of Coomassie blue-stained gels. Stained gels were digitized using a Hewlett Packard ScanJet Plus 256 grey scanner and a Macintosh Computer. Pixel densities of the tensin and actin polypeptides were counted from these digitized images and compared using the NIH image software. Using tensin as a standard, pixel counts were linear for <8 μg of protein per gel lane.

Dynamic Light Scattering

The effect of tensin on actin filament structure in solution was measured by dynamic light scattering (DLS) sue by a Brookhaven Instruments BI20AT apparatus (Holtsville, NY) with a 12 channel autocorrelator and a 10 mW He-Ne laser emitting 633 nm light. Actin (3 μM) was polymerized for 4 h with various concentrations of tensin in siliconized glass tubes (3-mm inner dia). The total scattering intensity and the autocorrelation function of quasielastically scattered light were determined by standard methods (Hartwig et al., 1992). An increase in polymer mass/length ratio would retard the decay of the autocorrelation function and increase the total scattering intensity, if tensin caused lateral association of actin filaments. This method was used previously to document the bundling activity of MARCKS and its active domain (Hartwig et al., 1992).

Fluorescence Assays of Actin Polymerization

The ability of tensin fragments to influence actin assembly was determined by their effect on the rate and extent of the fluorescence increase caused by polymerization of pyrene iodoacetamide-labeled actin monomers. The basis of this assay, described in detail elsewhere (Koyama and Mihashi, 1981; Walsh et al., 1984), is that the fluorescence intensity of pyrene-actin is much greater for polymeric than monomeric actin. The rate of actin polymerization depends on the concentrations of free actin monomers and filament ends. Actin-binding proteins can alter polymerization kinetics or extent by numerous mechanisms including filament assembly, binding to actin monomers and preventing their incorporation into filaments, or binding to filament ends and blocking monomer addition. In typical polymerization assays, pyrene-labeled G-actin (3 μM) was added to tensin or its fragments already dissolved in 2 mM MgCl2, 150 mM KCl, and 0.5 mM ATP. The fluorescence intensity as a function of time was measured with a Perkin-Elmer LS-50 instrument (Norwalk, CT) using excitation at 365 nm and emission at 386 nm. All measurements were done at room temperature.

Kinetic Analysis

In a nucleated assembly experiment, the initial rate of polymerization d[F-actin]/dt is

\[ d[F-actin]/dt = k_{on} \text{[barbed ends]}[G-actin] + k_{off} \text{[pointed ends]}[G-actin] + \text{spontaneous polymerization} - \text{rates of disassembly} \]

where \( k_{on} \) is the rate constant of addition at the barbed end, \( k_{off} \) is the rate constant of addition at the pointed end. The back reactions (rates of disassembly) can be neglected if they are much smaller than the rates of nucleation. Under most ionic conditions, \( k_{on} \) is more than 10 times larger than \( k_{off} \).

d[F-actin]/dt can be calculated from the rate of fluorescence increase of pyrene-labeled G-actin from the relation

\[ d[F-actin]/dt = dF/dt \{[\text{total actin}]/[F-G]\} \]

where \( F \) is the fluorescence of the pyrene–actin when it is all polymerized, \( G \) is the fluorescence when all the pyrene–actin is monomeric, and \( f \) is the time-dependent fluorescence. The term \( k_{on} \text{[barbed ends]}[G-actin] \) can be determined from the difference in rates observed with nuclei in the absence and presence of tensin. The fluorescence intensity and the amount of tensin, which simplifies the estimate of the affinity of tensin for the barbed end.

From Eq. 1 it is evident that the rate of addition to the barbed end is sim-
ply proportional to the concentration of free ends, so that the rate depends on the concentration of the end-binding protein by the relation
\[ \frac{dF}{dt} = k_{ba} [\text{free barbed ends}] [\text{G-actin}] + B \]  
and
\[ [\text{free barbed ends}] = \frac{[\text{total barbed ends}] \times K_a}{[\text{Tensin}] + K_a} \]  
where \( K_a \) is the equilibrium dissociation constant for binding of tensin to the barbed end, and \( B \) is the baseline rate when all ends are capped.

The effect of barbed end-binding proteins on the critical actin monomer concentration has been analyzed by Selve and Wegner (1986). Rather than the simple proportionality between initial polymerization rate and fraction of ends capped, the relation between the fraction of capped ends and the amount of unpolymerized actin is given by the relation (Selve and Wegner, 1986)
\[ C' - C = \frac{(k_b + [C] - k_b^-) K [\text{Tensin}]}{k_b + + k_p + + K [\text{Tensin}]} \]
or the equivalent relation (38)
\[ C' - C = \frac{(C_p - C)(CsK [\text{Tensin}])}{C_p - C + (CsK [\text{Tensin}])} \]  
where \( C' \) is the concentration of unpolymerized actin in the presence of the binding protein, \( C_p \) and \( C \) are the corresponding values when all barbed ends are capped or both filament ends are free, respectively, \( K \) is the association constant for binding of the tensin to the barbed filament end, and \( s \) is the so-called treadmilling efficiency parameter, which depends on the relative values of the rate constants, determined to be between 0.1 and 0.25.

**Electron Microscopy**

Rabbit skeletal muscle F-actin, 1.5 \( \mu \)M, incubated with or without tensin for at least 30 min was visualized by negative staining with 2% uranyl acetate. Protein was adhered to a carbon film by the carbon-flotation technique (Lake, 1979). All specimens were viewed and photographed at 80 kV in a JEOL 1200-EX transmission electron microscope.

**Results**

**Interactions of Recombinant Tensin with Actin**

Purified recombinant tensin binds to F-actin. Fig. 1 shows that tensin cosediments with actin filament. Tensin alone did not sediment under these conditions. Binding was saturated at a stoichiometry of 1 tensin per 11 filament subunit as calculated from Scatchard analysis. The \( K_a \), estimated from these binding data, is 0.1 \( \mu \)M. To examine the effect of tensin on the structure of actin filaments, mixtures of tensin and F-actin were negatively stained with uranyl acetate and analyzed by transmission electron microscopy. Fig. 2 C shows that tensin promotes the aggregation of F-actin in solution at a molar ratio of 1:10 (tensin/actin). The ability of tensin to aggregate actin filaments was confirmed by light scattering measurements. Molar ratios as low as 1:400 (tensin/actin) increased the scattering intensity and retarded the diffusion motions responsible for the decay in intensity autocorrelation. Higher molar ratios up to 1:25 resulted in a large increase in the light scattering (Fig. 2 A). These observations are consistent with a previous report indicating that the tensin fragment, HA1, has F-actin bundling activity (Wilkins and Lin, 1986).

We next determined the effect of recombinant tensin on the kinetics of actin assembly. As shown in Fig. 3 A, the rate of actin polymerization is reduced by full length tensin. A 50% reduction in the rate of actin polymerization (3 \( \mu \)M actin) is achieved by 10–100 nM tensin, suggesting that the dissociation constant for the binding of tensin to the barbed filament end is around 10–100 nM. This effect of purified recombinant tensin on the actin assembly rate was first observed in the cosedimentation assay (Fig. 1) where high concentrations of tensin increased the amount of actin remaining in the supernatant when the mixture was centrifuged one hour after the start of polymerization.
Figure 3. The effects and affinities of tensin and gelsolin on actin assembly. Pyrene-labeled G-actin (3 μM in A and 2.5 μM in B and C) was polymerized in the presence of various concentrations of recombinant tensin or purified plasma gelsolin as indicated. In B and C, the polymerization of actin was initiated by addition of unlabeled F-actin seeds (0.8 μM). The inset to B shows initial rates calculated by linear fits to curves such as those in B measured for various concentrations of gelsolin or tensin as shown. The solid lines are theoretical predictions based on Eqs. 3 and 4 using $K_d$ values of 0.1 and 20 nM.

Since retardation of polymerization can be caused by inhibiting either nucleation or monomer addition, the monomer addition rate was measured directly by seeding the polymerization with preformed filament ends. When unlabeled F-actin seeds (1.0 μM) are added to pyrene-labeled monomeric actin (3.8 μM), the barbed ends of the filament seeds serve as nucleation sites for monomer addition, thus accelerating the rate of actin polymerization. Preincubation of the F-actin seeds with tensin weakened their ability to accelerate actin assembly (Fig. 3 B). We have compared the effects of tensin and gelsolin, another capping protein, on the elongation of F-actin. The inset of Fig. 3 B shows the dependence of the initial rate of polymerization on the amount of gelsolin or tensin added to the filament seeds. A fit of Eqs. 3 and 4 to the data confirm that gelsolin binds the barbed filament end with an apparent $K_d$ no greater that 0.1 nM, and that tensin binds the barbed end much more weakly, with an apparent $K_d$ of ~20 nM. Tensin at concentrations as high as 50 nM did not lower the final fluorescence of 3 μM polymerized pyrene-actin, suggesting that the critical concentration of actin is not significantly altered by tensin (Fig. 3 C). In contrast, 2.5 nM gelsolin increased the critical concentration of F-actin to the extent expected, assuming nearly all of the barbed ends are blocked. These findings suggest that tensin is able to retard, but not diminish the extent of actin polymerization, and this limited inhibition results from the moderate affinity of tensin for the barbed filament end.

Figure 2. Effect of tensin on F-actin structure. (A) Effect of increasing concentrations of recombinant tensin on the amount of light scattered by a 3 μM F-actin solution. (B and C) Structure of 1.5 μM F-actin in the absence (B) or presence of 0.15 μM tensin (C). Actin was incubated with or without tensin in 0.1 M KCl, 0.2 mM CaCl$_2$, 2 mM MgCl$_2$, 0.5 mM ATP, and 10 mM Tris, pH 7.0 for 30 min at 37°C then negatively stained with 1% uranyl acetate. In the presence of tensin, aggregates of actin filaments are formed. Bar, 0.1 μm.
Figure 4. Summary of truncated fusion proteins of tensin and their actin-binding activities. The various tensin fragments were expressed as GST-fusion proteins. Their relative locations within tensin are indicated as well as the predicted size of the GST-tensin fragments. The ability of the various fusion proteins to interact with actin is summarized.

Expression and Purification of Tensin Fragments as Fusion Proteins in Bacteria

To identify the actin-binding domains of tensin, a series of tensin fragments fused with (GST) was constructed and expressed in Escherichia coli (Fig. 4). The location and the length of fragments studied were based on: (a) clues from preliminary experiments where longer fragments had been expressed and found to have actin-binding activities; (b) sequence homology with other actin-binding proteins; (c) the locations of convenient restriction enzyme sites; and (d) the solubility of fragments expressed. All of the constructed clones were confirmed by DNA sequencing for accuracy. Every clone, when expressed in E. coli, gave rise to a fusion protein of the size expected from the insert plus the GST portion. All of them bound to glutathione (GSH)-conjugated beads. In addition to the correct molecular mass, some purified fusion proteins such as p51(1-463) also contained smaller polypeptides. These are most likely breakdown products, since they are recognized by tensin antibodies (data not shown).

Interactions of Tensin Fragments with Actin

The actin-binding activity of each tensin fragment was assessed by cosedimentation with F-actin. Fig. 5 shows that only fragments derived from the NH2-terminal half of tensin bind significantly to F-actin. Fusion proteins including p51(residues 1-463), p29 (residues 1-263), p52 (residues 263-741), and p23 (residues 263-463) bound to F-actin. The location of each fragment within tensin is shown in Fig. 4. The $K_a$ determined for fusion proteins for actin filaments were 0.25 $\mu$M for p51, 0.33 $\mu$M for p29, and 0.91 $\mu$M for p23 (Fig. 7). The GST core protein, or fragments containing the SH2 domain of tensin, p25 (residues 263-292/543-741), p37 (residues 393-741) or p40 (residues 366-741), did not bind to F-actin filaments (Fig. 6).

The domain responsible for retarding actin polymerization was determined by the effects of various tensin fragments on pyrene-labeled actin. Fig. 7 A shows that p27(888-1142) and pl(888-989) retard polymerization of actin from barbed end filament seeds in a manner similar to that of full-length tensin (see also Fig. 3 B). Increasing p27 concentration leads to a saturation of retardation shown in Fig. 7 B, consistent with barbed end capping rather than monomer sequestration as being the mechanism for slowing polymerization. Moreover, the final extent of polymerization is not affected by p27, suggesting that p27 does not form an irreversible cap on the barbed end. Other tensin fragments, including p29, which contains an F-actin-binding site did not alter the kinetics of pyrene–actin polymerization under these conditions (data not shown).

Discussion

Based on mapping studies of tensin fragments expressed in bacteria described above, monomeric tensin contains three independent actin-binding domains as summarized in a schematic structure shown in Fig. 4. Recombinant tensin slows the initial rate of actin polymerization and greatly diminishes the rate of F-actin-nucleated elongation. The interaction of tensin with the ends of actin filaments was detected by its effects on the kinetics and extent of actin polymerization under conditions where polymerization was accelerated by preformed filament nuclei so that polymerization was

97, 66, and 43 kD). (B) The GST portion of the fusion protein or the SH2 domain of tensin (after removing GST) bind poorly to actin (o). Binding was assayed by cosedimentation with F-actin as above. Supernatants (S) and pellets (P) were displayed by 12% SDS-PAGE. Molecular mass markers of 80, 49.5, 32.5, 27.5, and 18.5 kD are in the first lane. Arrowheads indicate the GST, the SH2 domain, or fusion proteins.
Figure 7. Effect of tensin and tensin fragments on actin polymerization. The polymerization of pyrene G-actin (2.8 μM) in the presence of various concentrations of tensin p27(888-1142), or pl(888-989) was initiated by addition of unlabeled F-actin seeds (0.9 μM).

Prolonged polymerization rates were determined by monomer addition to filament ends rather than by the kinetics of nucleus formation. As a guide to evaluating the efficiency of tensin binding to the filament ends, identical experiments were also made using gelsolin, a barbed-end binding protein with an affinity of 10^11 M^-1, which forms a practically irreversible bond with the filament end (Selve and Wegner, 1986).

Proteins that bind to the ends of actin filaments are often called capping proteins, but this is an ambiguous term, because the effects of such barbed-end binding proteins can be very different depending on the affinity and rates of exchange at the filament end. Fig. 3 B shows that the rate of addition of monomers to filaments was strongly decreased by low molar ratios of tensin to actin, suggesting that this effect was the result of blocking sites of addition rather than sequestering monomers. Tensin at 50 nM decreased the initial rate of nucleated assembly nearly as strongly as gelsolin did at 25 nM. Fitting Eq. 3 (see Kinetic Analysis in Materials and Methods) to the data in Fig. 3 B shows that the dissociation constant of tensin for the barbed filament end is ~20 nM, and that the corresponding value for gelsolin is at most 0.1 nM (Fig. 3 B).

In contrast to the effect of tensin on polymerization rates, the final extent of polymerization was not affected by 50 nM tensin whereas 2.5 nM gelsolin was sufficient to produce the maximal decrease in polymerization, an indication of nearly complete capping of the barbed end. The difference in affinities explains why gelsolin, but not tensin, increases the critical concentration for actin polymerization under experimental conditions. By use of measured values for C and Cp (0.15

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and 1.0, respectively) and a value of $s = 0.16$, the predictions of Eq. 6 agree with the experimental finding that gelsolin increases the critical concentration for actin polymerization, but that tensin does not, at the concentrations tested under these conditions. Analysis of such experiments by equilibrium thermodynamics provides at least a relative measure of the affinity of tensin for the filament end, but a rigorous analysis is not yet possible since tensin contains multiple actin-binding domains which may compete with the p27 domain to bind the full-length protein either to the side or the end of the filament.

The barbed-end binding activity of tensin is conferred by a small domain located between residues 888-989 in the middle of the protein, since a bacterially expressed protein that contains only these 102 residues shows the same inhibitory effect on the actin assembly as that of full-length protein.

Fragments most likely derived from tensin have previously been reported to possess barbed end-binding activity (Wilkins and Lin, 1986). Moreover, Schröer and Wegner (1985) reported that a small protein (20–80 kD), later named insertin (Ruhnau et al., 1989), retards actin assembly at high ratios to actin. Insertin was copurified with vinculin on DEAE-cellulose and gel filtration columns and separated from vinculin by hydroxylapatite chromatography. Sequencing studies of insertin reveal this protein to have a nearly identical amino acid sequence from residues 862 to 1224 of tensin (Weigt et al., 1992). This region contains tensin's barbed end binding site identified here. Northern blot analysis with tensin cDNA probes, however, detect only a single mRNA species of 11 kb in chick embryo fibroblasts (Davis et al., 1991). These findings strongly suggest that insertin is a proteolytic fragment of tensin. All activities previously described for insertin are likely to be present in tensin. Insertin has been shown to cap inefficiently actin filaments and to allow monomers to be "inserted" onto the barbed ends of filaments (Gaertner and Wegner, 1991).

Tensin not only binds to the barbed ends of filaments, but also interacts with it along the length of the filaments. Fig. 2A demonstrates that when actin is incubated in higher molar ratio to tensin (1 tensin/25 actin), the light scattering from actin filaments is increased and by the electron microscopy, pairs of aligned filament are detected. In many cases, the filaments interacting in this fashion are quite short. Since short fibers do not spontaneously align at these concentrations (Janney et al., 1986), the pairing of filaments might require stabilization by tensin molecules along their lengths, in addition to attachment at their ends. Tensin, however, is not detected at high levels within microfilament bundles (stress fibers) in situ, instead being restricted to the stress fiber termination sites at focal contacts (Davis et al., 1991; Lo et al., 1994). Therefore, the significance of tensin's actin-cross-linking capacity might be confined to the focal contacts.

Interactions of tensin with the sides of the filaments are not unexpected based on the primary structure in which two stretches of amino acid sequences have been noted (Lo et al., 1994). One has an actin-like sequence, the other has an actin-binding consensus. However, two sequences do not share homology to each other. The actin related domain is situated at the very amino terminus of tensin. Residues 1-30 of tensin are highly homologous to residues 223 to 251 of actin. These residues in actin are exposed on the surface of subdomain 4 in the molecule (Kabsch et al., 1990), are involved in the longitudinal bonds between subunits (Holmes et al., 1990), and correspond to the pointed end of the subunit (relative to decoration by S1). Although this region of tensin might be expected to bind to the barbed end of actin filaments, our studies fail to detect capping activity in vitro. Rather, this domain of tensin binds to the side of actin filaments with high stoichiometry (1:2–4 actin subunits in filaments). Residues 223-251 of actin have also been proposed as a tropomyosin-binding site because Tetrathylena actin, poorly conserved in this region, does not bind tropomyosin (Milligan et al., 1990). If indeed these sequences in tensin also bind tropomyosin, one can imagine an intriguing competition between tropomyosin and actin for binding with this region of tensin, particularly when focal contacts are in the flux of attachment and detachment during cell migration.

Tensin also contains a stretch of sequence from residues 371 to 395 homologous to the consensus actin-binding sites of spectrin, dystrophin, α-actinin, plakin/timbrin, ABP-280, and ABP-120 (Bresnick et al., 1990, 1991; Hartwig and Kwiatowski, 1991; Matsudaira, 1991; Lo et al., 1994). As predicted, a bacterially expressed fragment of tensin containing amino acids 263-463 cosedimented with actin filaments. Curiously, a second bacterially expressed tensin fragment, p40(366-741) which contains residues 366-741 of tensin but not the amino-terminal region of p23(263-463) did not bind actin in our cosedimentation assay. This observation suggests that the actin-binding consensus alone is not sufficient for actin binding. Alternatively, p40(366-741) may not refold into a native conformation for actin-binding.

The amino terminus of tensin, from amino acids 32 to 76 within the first actin-binding domain, contains a region with homology to catenin-α (Nagafuchi et al., 1991; Lo et al., 1994). Catenin-α, a vinculin homologue, might bind vinculin as is the case for tensin (Wilkins, J. A., M. A. Risinger, E. Coffey, and S. Lin. 1987. J. Cell Biol. 104:130a). This homologous sequence in tensin could be involved in tensin-vinculin interactions. Because this sequence immediately follows the presumptive tropomyosin binding site, by steric hindrance, one might envision a three-way competition among vinculin, tropomyosin, and actin at the NH₂-terminal region of tensin.

The insertin domain of tensin also overlaps with sequences homologous to the β chain of mouse IL-3 receptor (Lo et al., 1994). The homology covers most of the cytoplasmic tail of the β chain of mouse IL-3 receptor. This portion of the IL-3 receptor is known to interact with tyrosine kinase and is directly involved in the transduction of the IL-3 signal (Kitamura et al., 1991). This homology is consistent with the following notions. (a) This portion of tensin may also interact with tyrosine kinase (indeed there are four potential tyrosine phosphorylation sites in this region). (b) Tyrosine phosphorylation at this site might regulate tensin's actin-binding activity. Actin-binding activities of several proteins including caldesmon (Yamashiro et al., 1990), synapsin I (Bahler et al., 1987), and MARCKS (Hartwig et al., 1992) are known to be influenced by phosphorylation. (c) Tyrosine phosphorylation of tensin might trigger the interactions of tensin with other SH2 domain-containing proteins. (d) The homologous domain in the IL-3 receptor might have actin-interacting activity or even an insertin-like activity. It is known that IL-3 treatment does lead to a rapid reorganization of actin filaments (Sabe et al., 1991).
Fig. 8 suggests a model for the interaction of tensin with actin filaments at focal contacts. This model is based on three actin-binding sites per subunit, the apparent dimerization of subunits (Lo et al., 1994), and on the assumption that subunit self-association occurs near their carboxyl ends as is the case for other actin filament cross-linking proteins (Matsudaira, 1991), which is supported by the fact that the amino termini of tensin are occupied by actin-binding domains and are unlikely to be the dimerization sites. In this model, two actin-binding sites bind fibers some distance away from filament ends to hold the filaments, whereas the third actin-binding domain with barbed end binding activity weakly caps the terminal subunit at one strand of the double-helical actin filament. This would allow the addition of actin monomer to the other strand, as previously proposed by Wegner's group for insertin (Gaertner and Wegner, 1991). The dimeric tensin would, therefore, cross-link two illa-monomer to the other strand, as previously proposed by Wegner's group for insertin (Gaertner and Wegner, 1991). The dimeric tensin would, therefore, cross-link two illa-monomer to the other strand, as previously proposed by Wegner's group for insertin (Gaertner and Wegner, 1991). The dimeric tensin would, therefore, cross-link two illa-monomer to the other strand, as previously proposed by Wegner's group for insertin (Gaertner and Wegner, 1991). The dimeric tensin would, therefore, cross-link two...
