

## Binding of the $\alpha$ -fodrin SH3 domain to the leading lamellae of locomoting chicken fibroblasts

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### SUMMARY

Fodrin (nonerythroid spectrin) is a membrane skeletal protein that plays an important role in the establishment and maintenance of the cell shape and polarity. We have identified in  $\alpha$ -fodrin an *src* homology 3 (SH3)-related region, a small domain that is present in a large number of proteins that are involved in signal transduction, cell polarization and membrane-cytoskeleton interactions. In this study we have explored the function of the  $\alpha$ -fodrin SH3 by incubating fixed and permeabilized cultured chicken fibroblasts with the  $\alpha$ -fodrin SH3 peptide, expressed in bacteria as a fusion protein with glutathione S-transferase. Immunofluorescence and immunoelectron microscopy showed that  $\alpha$ -fodrin SH3 binds to the cytoplasmic face of the plasma

membrane in the leading lamellae and the pseudopodial lobes of the spreading and locomoting cells. No, or only minimal, binding was seen in immotile cells, or in the stationary trailing ends of the locomoting cells. SH3 binding was also seen in cytochalasin-D-treated cells, suggesting that actin filaments are not responsible for the binding. These findings suggest that  $\alpha$ -fodrin SH3 interacts with plasma membrane components that are present in the leading lamellae exclusively or are modulated in a manner specific to the leading lamellae.

Key words: fodrin, leading lamellae, SH3, immunofluorescence, immunoelectron microscopy

### INTRODUCTION

The membrane skeleton is a meshwork of peripheral membrane proteins that underlies the plasma membrane of most types of cells (Bennett, 1990a). It takes part in the formation and delineation of the specialized cell surface domains and in more dynamic events such as ligand-induced receptor capping (Levine and Willard, 1983) and fusion of exocytotic vesicles to the plasma membrane (Perrin et al., 1987). The principal structural components of the membrane skeleton include fodrin (nonerythroid spectrin), actin oligomers, protein 4.1, ankyrin and adducin (Bennett, 1990a). Fodrin consists of  $\alpha$ - and  $\beta$ -subunits that form antiparallely aligned heterodimers. Heterodimers, in turn, form tetramers that are linked together via short actin oligomers. This meshwork is associated with the cytoplasmic face of the plasma membrane via ankyrin (Marchesi, 1985) and in an ankyrin-independent manner (Bennett, 1990b).

Protein and cDNA sequencing have shown that fodrin/spectrin subunits contain homologous, 106 amino acid residue repetitive domains, which probably account for the flexible, rod-like structure of the molecule (Speicher and Marchesi, 1984; Leto et al., 1988; Wasenius et al., 1989; Winkelmann et al., 1990). In the middle part of the  $\alpha$ -subunit there is a domain that is homologous to *src* homology

region 3 (SH3). SH3 is a 60 amino acid residue domain that was first identified in *src*-type protein tyrosine kinases as a conserved sequence N-terminal to the well-characterized SH2 (*src* homology region 2) domain (Pawson, 1988). A number of other signal transduction pathway proteins, such as phospholipase C (Stahl et al., 1988), phosphatidylinositol-3-kinase (Otsu et al., 1991), and p21<sup>ras</sup> GTP-ase activating protein (GAP; Trahey et al., 1988) also contain SH3 domains. It has also been found in a number of other, seemingly unrelated group of proteins, such as myosin 1A of *Acanthamoeba* (Jung et al., 1987), the p47 and p67 activators of the neutrophil respiratory-burst oxidase (Volpp et al., 1989; Leto et al., 1990) and the putative transcriptional factors vav and Hs1 (Kitamura et al., 1989; Bustelo et al., 1992; for a more extensive list of SH3-containing proteins, see Musacchio et al., 1992a). The crystal structure of  $\alpha$ -fodrin SH3 domain has been described recently (Musacchio et al., 1992b).

The function of SH3 is still a mystery. Its presence in proteins involved in cellular morphogenesis, cytoskeleton assembly and signal transduction strongly suggest, however, that it is involved in the protein-protein interactions that mediate the association of SH3-containing proteins with the plasma membrane or cytoskeleton (Lehto et al., 1988; Rodaway et al., 1989). In order to study the role of the  $\alpha$ -fodrin SH3 domain in cellular physiology, we used

a cell culture overlay technique that utilizes incubation of the monolayers of fixed, permeabilized cells with fodrin SH3 expressed in bacteria as a fusion protein with glutathione S-transferase (GT). Binding of the fusion protein was detected with the antibodies recognizing epitopes both in SH3 and in the GT tag, thus allowing the distinction between the exogenously added and the endogenous SH3 domains.

## MATERIALS AND METHODS

### PCR amplification and expression of $\alpha$ -fodrin SH3 domain cDNA

The polymerase chain reaction (PCR) was used to amplify the sequence ranging from nucleotides 2863 to 3111 of a 3.7 kb long chicken brain fodrin cDNA clone (Wasenius et al., 1989). It encompasses the 10 domain of  $\alpha$ -fodrin, which corresponds to the SH3 region of, e.g., nonreceptor tyrosine kinases. The primers (upstream primer ATTGGATCCTGCAGGCAACAAGTT, downstream primer CCTGAATTCCAGAAGATTCTC) were designed to incorporate *Bam*HI and *Eco*RI sites in the 5' and the 3'-ends of the PCR product, respectively. The restriction sites are underlined. The PCR product was ligated into the pGEX-2T bacterial expression vector (Pharmacia, Uppsala, Sweden), which allows the expression of the cloned insert in fusion with GT (Smith and Johnson, 1988). The recombinant plasmid was introduced into *Escherichia coli* cells. Transformants were analyzed for the presence of the insert, and the flawless amplification and cloning were verified by sequencing. The fusion protein (GT-SH3) was expressed by induction with 0.1 mM isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG; Sigma Chemie GmbH, Diesenhofen, FRG) and purified by affinity chromatography on glutathione-agarose beads (Pharmacia). The bound protein was eluted by using 5 mM reduced glutathione, following the instructions laid out by the manufacturer. SH3 insert was cleaved off from the GT-SH3 by thrombin and further purified on HPLC by using Superdex 75HR 10/30 gel filtration column (Pharmacia).

### Preparation and characterization of antibodies to $\alpha$ -fodrin SH3

One hundred micrograms of affinity-purified GT-SH3 was emulsified with an equal volume of Freund's complete adjuvant and injected intradermally into a rabbit. Three booster injections with Freund's incomplete adjuvant were given at four-week intervals. The resulting antiserum K34 was affinity-purified on GT-SH3 immobilized on CNBr-activated Sepharose beads (Pharmacia).

Fodrin-enriched fraction from the chicken brain was made according to the method of Levine and Willard (1981). For western blotting, 0.5  $\mu$ g of the chicken brain extract enriched for fodrin, 25  $\mu$ g of the lysate of the cultured chicken embryo heart fibroblasts, 1.0  $\mu$ g of GT-SH3, 0.6  $\mu$ g of the purified GT and 0.5  $\mu$ g of the purified SH3 were subjected to polyacrylamide gel electrophoresis in the presence of 0.5% SDS (SDS-PAGE) (Laemmli, 1970), and blotted onto nitrocellulose filters (Towbin et al., 1979). Filters were incubated with antiserum K34 or anti-GT antibody mAb50-1 (Davern et al., 1987; obtained from Dr Kathy Davern, the Walter and Eliza Hall Institute of Medical Research, Victoria, Australia), followed by biotin-conjugated anti-rabbit or mouse immunoglobulins and AB-HRP-complex (DAKO A/S; Glostrup, Denmark). Immunoblots were developed with diaminobenzidine (0.5 mg/ml in PBS) with 0.01% H<sub>2</sub>O<sub>2</sub> and 0.03% NiCl<sub>2</sub>.

### Cell culture

Primary cultures of chicken embryo heart fibroblasts (CEHF) were

started from the explants of 13-day-old chicken embryos. They were cultured on glass coverslips (for fluorescence microscopy) or on collagen filters (for electron microscopy) in Eagle's minimal essential medium with Earle's salts (E-MEM; Gibco, Gaithersburg, MD, USA) supplemented with 2 mM glutamine, nonessential amino acids, 10% fetal calf serum and antibiotics. The cells were used for experiments between the 2nd and the 8th passage. Cytochalasin D was obtained from Sigma and was kept as a stock solution (1 mg/ml) in dimethyl sulfoxide at -20°C; it was applied to cell cultures at a final concentration of 10  $\mu$ g/ml for 15 min.

### Cell fixation, incubation with GT-SH3 and immunofluorescence microscopy

The cells were rinsed in phosphate-buffered saline (PBS: 0.01 M sodium phosphate, 145 mM NaCl, pH 7.2), and then fixed and permeabilized under cytoskeleton-stabilizing conditions (Vielkind and Swierenga, 1989). Briefly, the cells were first fixed in 4% formaldehyde in 100 mM PIPES, 5 mM EGTA, 2 mM MgCl<sub>2</sub>, pH 6.8, containing 0.2% Triton X-100. After washing with PBS, the cells were post-fixed in ice-cold methanol for 5 min. For some experiments the cells were first fixed with 4% formaldehyde (in 100 mM PIPES, 5 mM EGTA, 2 mM MgCl<sub>2</sub>, pH 6.8), permeabilized with 0.5% Triton X-100 in PBS, and then post-fixed in ice-cold methanol for 5 min.

For overlay experiments, the fixed and permeabilized cells were rinsed with PBS, and then incubated in 10% fetal calf serum to saturate the non-specific protein binding sites. Thereafter, they were overlaid with GT-SH3 (100  $\mu$ g/ml of PBS) at +4°C for 30 min. After washing with 0.02 M glycine in PBS, the cells were incubated with affinity-purified K34 or with affinity-purified anti-GT antibody (a kind gift from Dr Claudio Schneider, Trieste, Italy; Del Sal et al., 1992) at +4°C for 30 min, again washed with PBS-glycine, and then incubated with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulin (Caltag Laboratories, San Francisco, CA, USA). In experiments aimed to probe for the specificity of the fusion protein binding, the fixed and permeabilized cells were incubated with the bacterially expressed GT (100  $\mu$ g/ml of PBS) prior to incubation with GT-SH3 or staining with K34. Control experiments also included: first, overlaying with GT-SH3 and incubation with K34 and the secondary antibody of fixed, unpermeabilized cells (4% formaldehyde in 100 mM PIPES, 5 mM EGTA, 2 mM MgCl<sub>2</sub>, pH 6.8); and second, incubation with K34 and the secondary antibody of the fixed, permeabilized cells without prior incubation with the fusion protein.

For the visualization of actin, rhodamine-labelled phalloidin (Molecular Probes, Inc., Eugene, OR, USA) was used on cells post-fixed in cold ethanol instead of methanol. For fluorescence microscopic viewing, the samples were washed with PBS-glycine and water, mounted in Aquamount Mountant (BDH Chemicals, Poole, England) and observed under a Zeiss Axiovert 405M microscope. Images were recorded on p3200 Tmax film (Eastman Kodak Co., Rochester, NY, USA).

### Immunoelectron microscopy

The cells grown for 24 h on collagen filters (Transwell Col<sup>TM</sup>-wells, Costar, Cambridge, MA, USA) were fixed in 4% paraformaldehyde in 100 mM PIPES, 5 mM EGTA, 2 mM MgCl<sub>2</sub>, pH 6.8, containing 0.2% or Triton X-100. After washing in PBS, the cells were overlaid with GT-SH3 (100  $\mu$ g/ml of PBS) for 30 min, washed with PBS and postfixed with 8% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 0.5 h. The filters with the cells were removed and cryoprotected by immersing in 2.3 M sucrose, and then frozen in liquid nitrogen. Thin cryosections were cut with a Reichert Ultracut FC4-E ultramicrotome. The procedure for immunolabelling was as described elsewhere (Mäentausta

et al., 1991). Briefly, the sections were first incubated in 10% FCS with 0.02 M glycine in PBS and then labelled with affinity-purified K34 diluted in 5% FCS in PBS-glycine for 45 min, followed by incubation for 30 min with Protein A-gold complex (size 10 nm), made after Slot and Geuze (1985). The controls were prepared by incubating the cells with the bacterially expressed GT (100 µg/ml of PBS) or with PBS prior to staining with K34. The sections were embedded in methylcellulose and examined under a Philips 4120 LS transmission electron microscope, using an acceleration voltage of 60 kV.

**RESULTS**

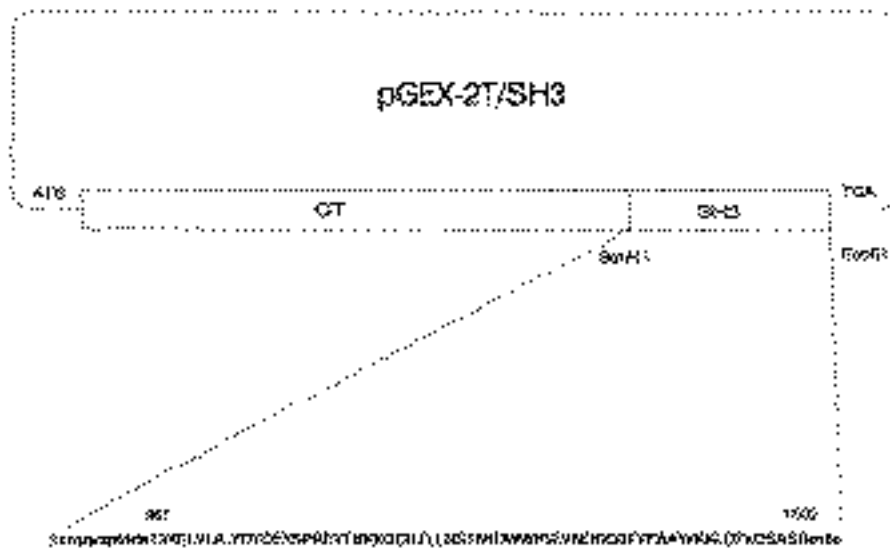
**Characterization of GT-SH3 antibodies**

Antibodies to -fodrin SH3 were raised by immunizing rab-

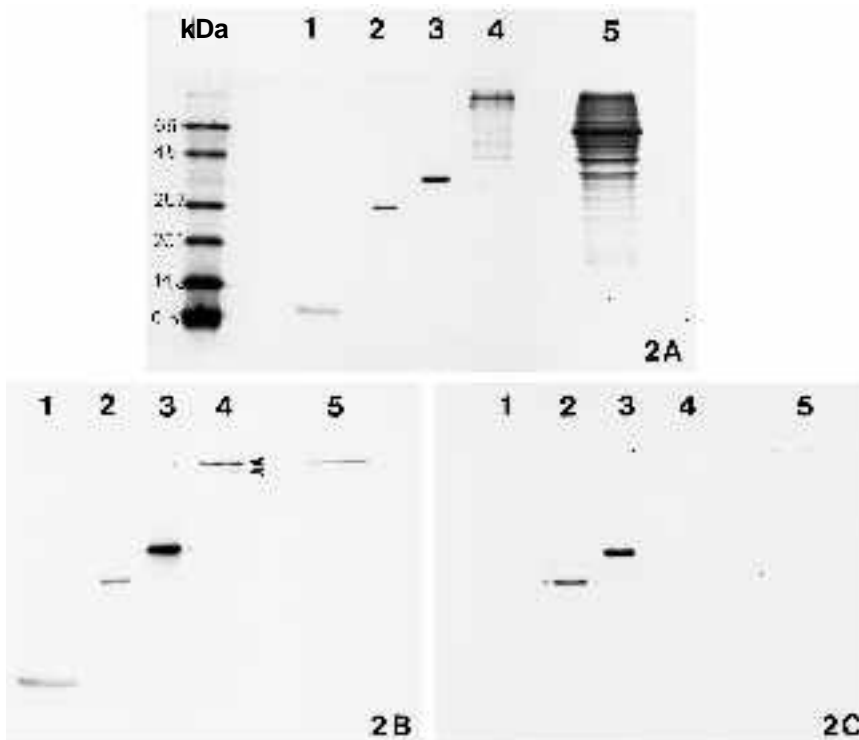
bits with the bacterially produced GT-SH3 fusion protein (Fig. 1). The same chimeric protein was used in the cell culture overlay experiments. Immunoblot analysis of the specificity of the resulting antiserum (K34) showed that K34 recognizes epitopes in both GT and SH3. It also labels intact -fodrin (Fig. 2).

**Overlay experiments and immunofluorescence microscopy**

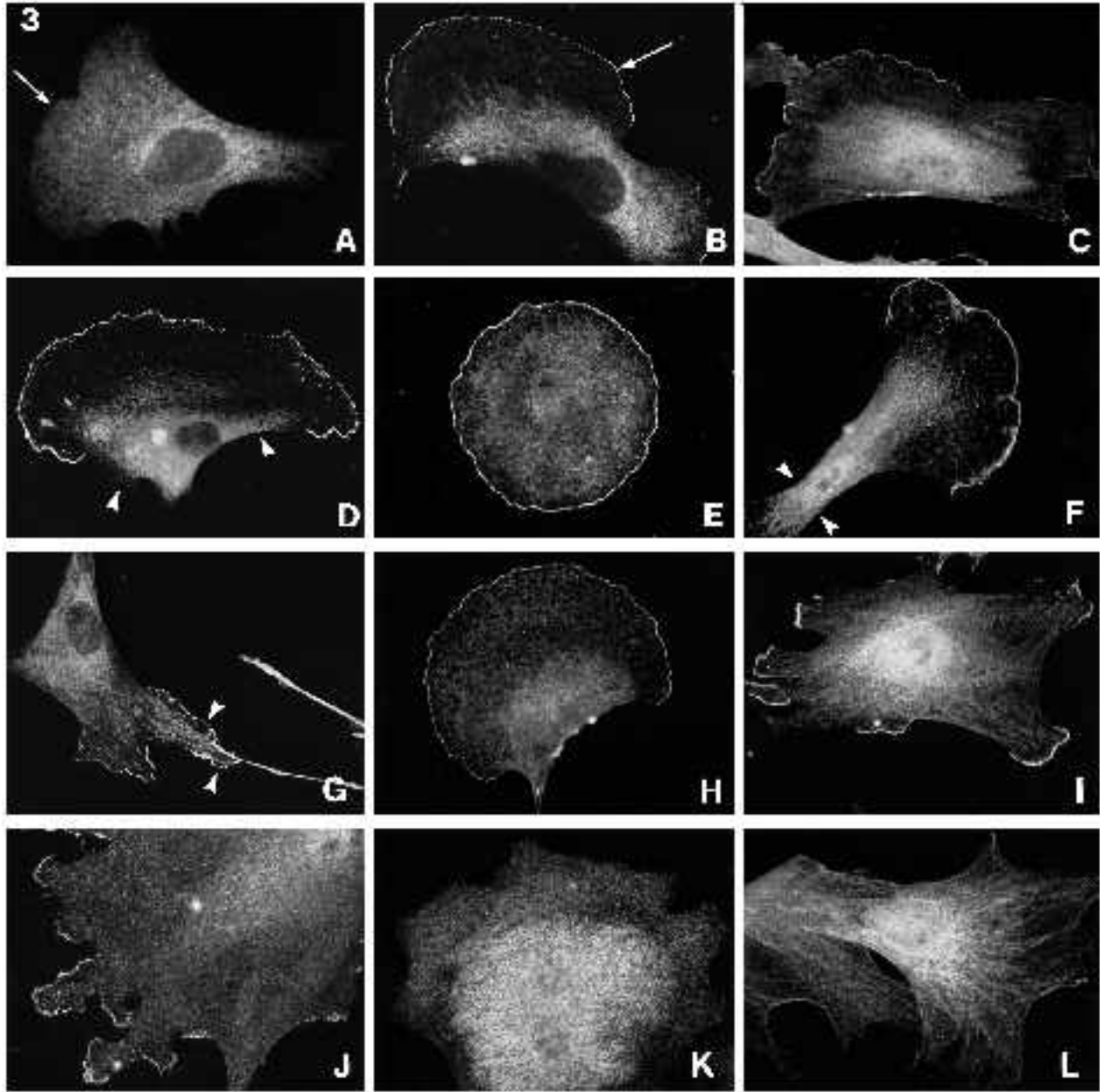
Fig. 3 shows the immunofluorescence images of the fixed and permeabilized fibroblasts. In the control (K34 without a prior incubation with GT-SH3), homogeneous plasma membrane staining was seen (Fig. 3A), similar to that found with anti-fodrin antibodies in other studies (Lazarides and Nelson, 1982; Lehto and Virtanen, 1983). Also in cells



**Fig. 1.** Schematic representation of the GT-SH3 fusion protein expression system. Fodrin SH3 cDNA (the corresponding amino acid sequence shown in capital letters) and part of the flanking sequence (small letters) were fused in-frame to the glutathione S-transferase cDNA in pGEX-2T. Numbering refers to the sequence given by Wasenius et al. (1989). ATG denotes the initiation and TGA the stop codon.



**Fig. 2.** (A-C) Analysis of the specificity of the antiserum K34. Samples applied in lanes 1-5 in (A-C) were as follows: HPLC-purified SH3 (lane 1); GT (lane 2); GT-SH3 (lane 3); chicken brain fodrin (lane 4) and CEHF lysate (lane 5). Molecular mass markers: bovine serum albumin (66 kDa); ovalbumin (45 kDa); chymotrypsinogen A (25.7 kDa); trypsin inhibitor (20.1 kDa); lysozyme (14.3 kDa) and aprotinin (6.5 kDa). (A) Coomassie blue-staining. (B) Immunoblotting with K34. Specific binding of K34 to chicken brain -fodrin (lane 4 upper arrowhead), and to -fodrin of the CEHFs (lane 5) is seen. The lower band in lane 4 corresponds to the degradation product of -fodrin. K34 also labels purified SH3 (lane 1), GT (lane 2) and GT-SH3 (lane 3). (C) Immunoblotting with monoclonal antibody to *Schistosoma japonicum* GT (anti-GT antibody Ab50-1). Specific binding to GT (lane 2) and GT-SH3 (lane 3) is seen.



**Fig. 3.** Demonstration by immunofluorescence microscopy of the binding of GT-SH3 to the leading lamellae and pseudopods of the fixed and permeabilized CEHF, and of the specificity of the binding of GT-SH3 for the SH3 domain. Fixation was with 4% formaldehyde plus 0.2% Triton X-100 (A-G and I-L) or with 4% formaldehyde followed by 0.5% Triton X-100 (H). (A) Immunofluorescence staining with K34 (dilution 1:30) of the control cells (no incubation with GT-SH3). (B-G) Immunofluorescence staining with K34 (dilution 1:50) of the cells incubated with GT-SH3. (H) Immunofluorescence staining with K34 (dilution 1:50) of the cells incubated with GT-SH3, fixation with 4% formaldehyde followed by 0.5% Triton X-100. (I) Immunofluorescence staining with anti-GT antibody of the cells incubated with GT-SH3. (J) Immunofluorescence staining with K34 (dilution 1:50) of the cells incubated first with the bacterially expressed GT and then with GT-SH3. (K) Immunofluorescence staining with K34 (dilution 1:50) of the cells incubated with bacterially expressed GT. (L) Immunofluorescence staining with anti-GT antibody of the cells incubated with bacterially expressed GT. See the text for explanation of arrows and arrowheads. A-C,E,F,H-L,  $\times 1000$ ; D,G,  $\times 500$ .

overlaid with GT-SH3, a weak, membrane-associated staining was seen. Moreover, a distinct, dotted decoration was observable. It was only seen in cells incubated with the fusion protein, not in control stainings (compare arrows in Fig. 3A and B). It was restricted to the leading lamellae and the pseudopods, areas representing the highly mobile,

actin-rich compartments of the cell (Fig. 3B-G). More specifically, the speckled pattern was most pronounced in the leading edges, the narrow rims located at the tips of the lamellar veils (Small and Rinnerthaler, 1985) (Fig. 3B-D). In symmetrically spreading cells, the entire circumference of the cell was decorated in a regular fashion (Fig. 3E).

Often the decoration was also seen in minute pouchings along the extended, pseudopod-like projections of the lamellae (Fig. 3G, arrowheads). The more stationary regions, such as the trailing ends, remained negative (Fig. 3D and F, arrowheads). A similar decoration was also seen in cells that were first fixed with formaldehyde and only then extracted with 0.5 % Triton X-100 (Fig. 3H).

A similar staining pattern was obtained when cells overlaid with GT-SH3 were incubated with anti-GT antibody followed by FITC-conjugated anti-rabbit immunoglobulin (Fig. 3I). Decoration of the peripheral rim, indicative of GT-SH3 binding to leading lamellae, was only seen in the freshly seeded cells (5-12 hours in culture), which exhibit a growth pattern characteristic of spreading and motile cells. Only a minimal decoration of the cell periphery was seen in confluent, stationary cells that had been in culture for several days (not shown). No staining was seen in fixed, unpermeabilized cells, indicating that GT-SH3 binding occurs at the cytoplasmic side of the lamellar structures (not shown).

The specificity of the binding of the fusion protein for the SH3 domain was studied by incubating the permeabilized cells first with the bacterially produced GT and then with GT-SH3 (Fig. 3J). No diminution of the lamellar decoration was seen in immunofluorescence staining with K34, indicating that the fusion protein is not displaced by GT and that the binding is not due to the GT tag. On the other hand, incubation of the cells with the bacterially produced GT, followed by staining with K34, revealed a homogeneous, weak membrane staining but no lamellar decoration (Fig. 3K). Neither was lamellar decoration seen in cells overlaid with GT and then incubated with anti-GT antibody

and FITC-anti-rabbit immunoglobulins (Fig. 3L), also reinforcing the conclusion that the SH3 portion of the fusion protein is responsible for the binding to the lamellar region (compare also Fig. 3I and L).

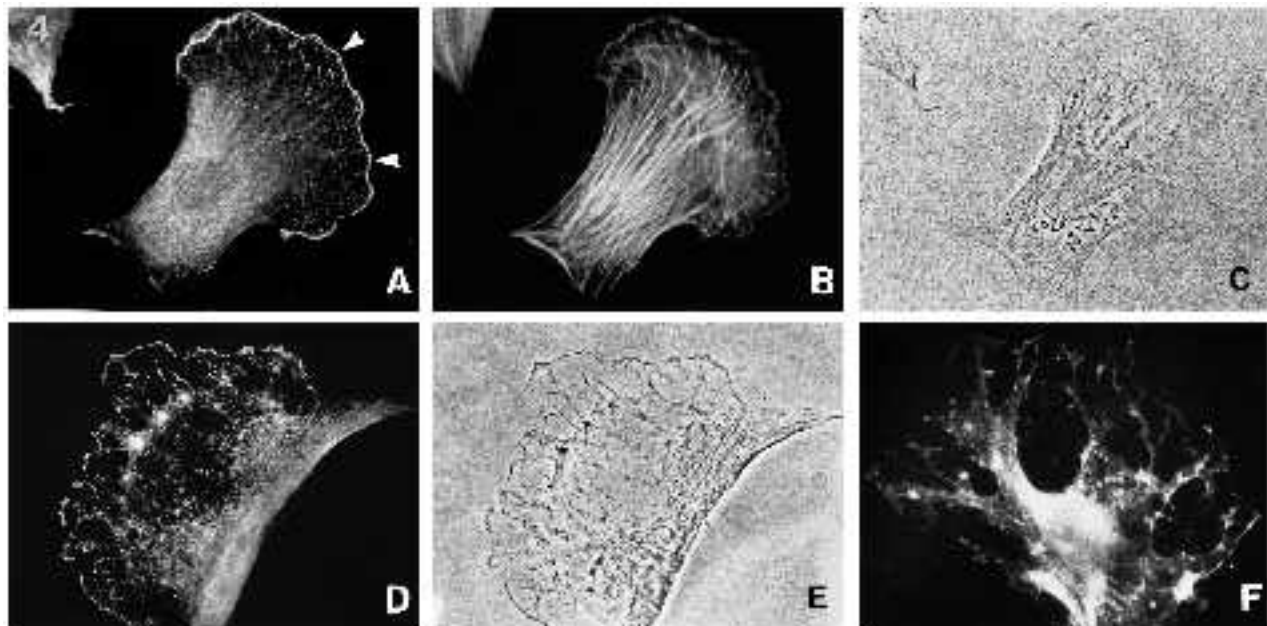
In view of the prominence of the actin-based structures in the leading lamellae (Small et al., 1978; Small and Rinnerthaler, 1985), we decided to investigate whether there are any signs of interaction between GT-SH3 and actin. To this end, we used double-labelling fluorescence microscopy and treatment of the cells with the microfilament-disrupting drug cytochalasin D (Cooper, 1987).

Fig. 4 shows double-labelling for the bound GT-SH3 and F-actin (labelling with rhodamine-phalloidin). Fusion protein appeared to bind to the lamellar edges where no prominent rhodamine-decorated actin filaments could be discerned (Fig. 4A-C). There was, however, a faint rhodamine-positive rim-like staining along the lamellar veils, which seemed to overlap partially with the GT-SH3 binding (Fig. 4A, arrowheads).

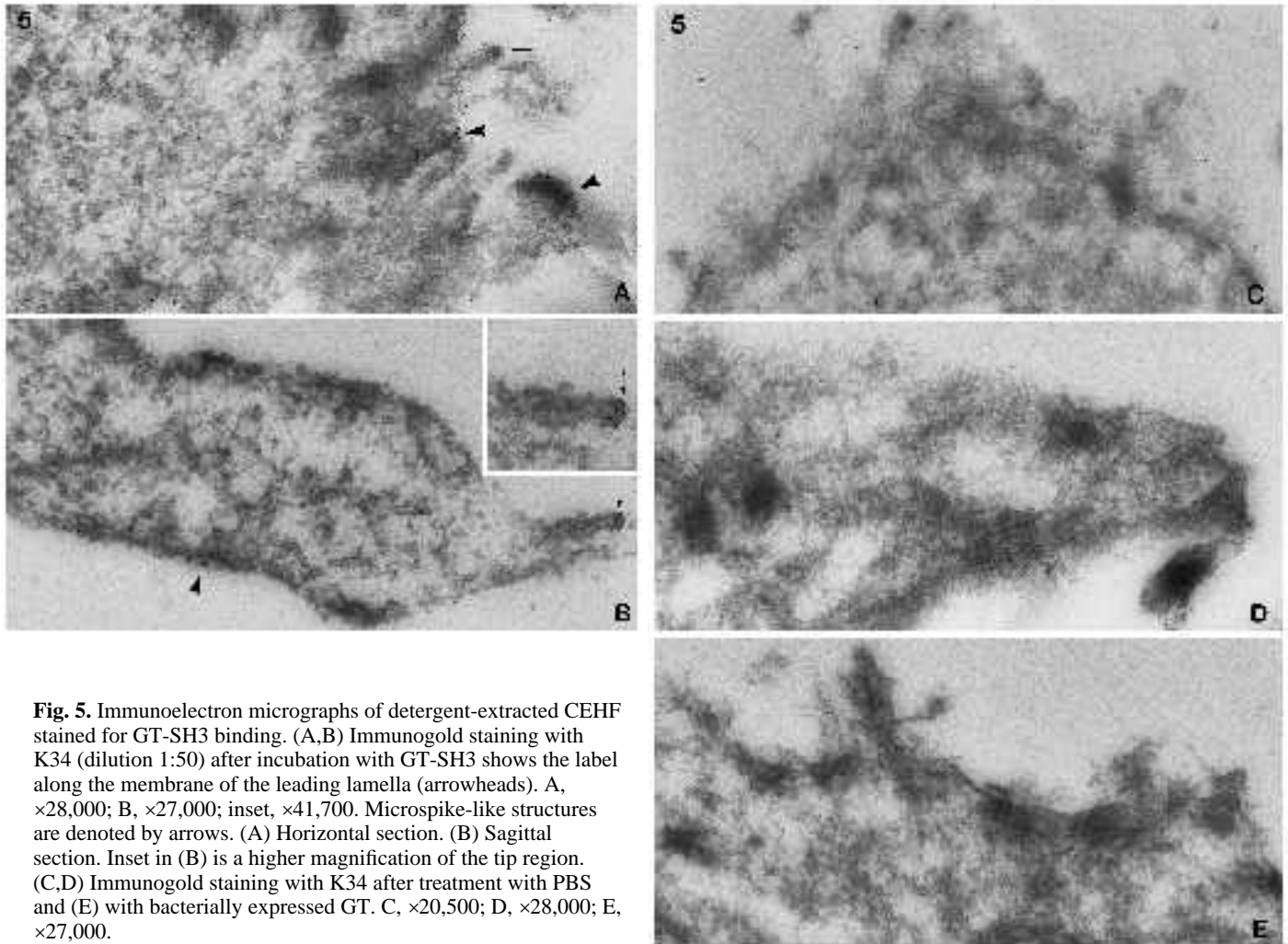
In cytochalasin-D-treated cells, a typical arborization, indicative of the disruption of the actin filaments (Schliwa, 1982), was seen at a concentration of 10  $\mu\text{g/ml}$  (Fig. 4D,E). Binding of the fusion protein to the lamellar membrane regions was evident in these cells when compared with the control cells (Fig. 4F). These findings indicate that intact actin filaments are not needed for the binding of GT-SH3 to the lamellar structures.

#### Overlay experiments and immunoelectron microscopy

Fig. 5 depicts immunoelectron microscopic images of the cultured CEHFs overlaid with GT-SH3, incubated with K34



**Fig. 4.** Immunofluorescence micrographs of CEHF stained for GT-SH3 binding and for actin. (A and B) Immunofluorescence staining with: (A) K34 (dilution 1:50); and (B) for actin of the cells incubated with GT-SH3. (C) Phase-contrast micrograph of the same cell as in (A) and (B). (D) Immunofluorescence staining with K34 (dilution 1:50) of the cells treated with cytochalasin D and incubated with GT-SH3. (E) Phase-contrast micrograph of the same cell as in (D). (F) Immunofluorescence staining with K34 (dilution 1:50) of the cells treated with cytochalasin D (no incubation with GT-SH3). For arrowheads, see text. A-F,  $\times 1000$ .



**Fig. 5.** Immunoelectron micrographs of detergent-extracted CEHF stained for GT-SH3 binding. (A,B) Immunogold staining with K34 (dilution 1:50) after incubation with GT-SH3 shows the label along the membrane of the leading lamella (arrowheads). A,  $\times 28,000$ ; B,  $\times 27,000$ ; inset,  $\times 41,700$ . Microspike-like structures are denoted by arrows. (A) Horizontal section. (B) Sagittal section. Inset in (B) is a higher magnification of the tip region. (C,D) Immunogold staining with K34 after treatment with PBS and (E) with bacterially expressed GT. C,  $\times 20,500$ ; D,  $\times 28,000$ ; E,  $\times 27,000$ .

and gold-labelled Protein A. A distinct decoration of the membranous structures in the lamellae regions could be seen in both horizontal (Fig. 5A) and sagittal (Fig. 5B) sections of the cells (arrowheads). Prominent labelling was also seen in microspike-like structures in the protruding ends of the cells (arrows). Almost no, or only a minimal, labelling was seen in the corresponding structures in cells incubated with K34 and gold-conjugated Protein A without prior overlay with GT-SH3 (Fig. 5C-D) or in cells overlaid first with bacterially produced GT and then incubated with K34 and gold-conjugated Protein A (Fig. 5E).

## DISCUSSION

The present results suggest that there are binding sites for -fodrin SH3 domain in the leading lamellae of the motile fibroblasts. Fodrin SH3 is a representative of a family of similar but non-identical protein domains that have been found in more than 40 different proteins, most of which are involved in signal transduction, cell polarization and membrane-cytoskeleton interactions (Musacchio et al., 1992a). However, a characteristic feature of the various SH3 domains is the relatively low similarity between the

sequences. In fact, a high degree of conservation is only seen within short stretches at the N and C termini of the sequence (Musacchio et al., 1992a). Furthermore, the crystal structure of the fodrin SH3 domain reveals that there is considerable variation between various SH3 domains even in the amino acid positions, which seem to form a smooth patch on the protein surface, probably representing a binding site for a large molecule. This variability suggests that each SH3 domain shows unique binding properties (Musacchio et al., 1992b). Thus, it is likely that the lamellar decoration seen in this study is based on specific binding between fodrin SH3 and its cognate protein. Indirect evidence for this also comes from the observations that K34 antibody, raised against GT-fodrin SH3 fusion protein, does not show any cross reactivity with other SH3-containing proteins.

The binding of fodrin SH3 to the leading lamellae of cultured fibroblasts was inferred from the characteristic decoration pattern seen in the fixed and permeabilized cells. We used a formaldehyde-based fixation mixture with low concentrations of anionic detergent and ions, which, due to its cytoskeleton-stabilizing properties and the preservation of the cellular morphology, has proved especially well-suited for the studies of cytoskeletal organization (Vielkind and

Swierenga, 1989). No binding of GT-SH3 was seen in control stainings and in unpermeabilized cells, indicating that the lamellar staining is due to specific interactions and that the binding sites are intracellular. The lack of binding of GT-SH3 to, e.g., trailing ends of the cells could also be explained by the differential extractability of the putative binding sites in different cell compartments. This was tested by permeabilizing the cells only after extensive treatment with the cross-linking fixative formaldehyde. There was no change in the GT-SH3 binding pattern under these conditions, strongly suggesting that the differential decoration of the lamellar areas is not an artifact. Moreover, we do not see plasma membrane binding of GT-SH3 in cells that do not form distinct leading lamellae, such as many epithelial cells (data not shown).

The biochemical nature of the SH3-interacting components in the leading lamellae remains to be established. They could be molecules that are only present in the leading lamellae, due to de novo synthesis, or to a translocation from the cytoplasm. On the other hand, they may represent more widely occurring proteins, which undergo conformational changes or post-translational modifications specific for the motile parts of the cytoplasm or the plasma membrane. Biochemically, the most characteristic feature of the leading lamella is its high content of actin (Bretscher, 1991). However, we failed to show any association between SH3 and actin. The binding of fodrin SH3 to actin is unlikely also, on the basis that actin-binding sites in fodrin are located in the N terminus of the  $\alpha$ -chain (Morrow, 1989) and that the SH3 domain of myosin I C of *Acanthamoeba* does not show affinity for actin (Doberstein and Pollard, 1992). We cannot rule out, however, the possibility that  $\alpha$ -fodrin SH3 binds actin indirectly via an actin-associated protein, such as talin,  $\alpha$ -actinin or vinculin, which are preferentially expressed in the lamellar regions (Burrige et al., 1988).

Many of the characteristics of the SH3-plasma membrane interaction and the transient and dynamic nature of the lamellipodial organization can be accounted for if we assume that fodrin SH3 binds to conformationally altered or post-translationally modified proteins. These could be extracellular signal-relaying proteins, such as integrins and various actin-associated proteins that become phosphorylated in the lamellar regions (Glenney, 1992). This mechanism is an intuitively attractive one on the basis that SH3-binding, e.g. to post-translationally modified protein domains that show sequence diversity, would be concomitant with the presence of a variety of cytoskeletal and signalling proteins that lack an extensive homology among their different SH3 sequences (Musacchio et al., 1992a). Another intriguing possibility is that fodrin SH3 is involved in a GTPase-driven assembly of a plasma membrane complex that regulates actin polymerization (Hall, 1992). Indirect evidence for the feasibility of such a model comes from two sources. First, microinjection of p21<sup>ras</sup>, a member of the ras GTPase superfamily, into living cells produces a dramatic increase in actin fibers (Paterson et al., 1990). Second, a recently identified protein that binds to the SH3 region of abl, shows similarity with GAP-rho and, thus, may link SH3 to ras-related proteins (Cicchetti et al., 1992).

The SH3 domain has been recognized in at least 40 pro-

teins (Musacchio et al., 1992a). Many of them also possess SH2 domain(s), which have a role in targeting tyrosine kinase substrates to activated kinases, such as growth factor receptors (Matsuda et al., 1990; Koch et al., 1991). The binding of fodrin SH3 to the leading lamellae is intriguing, especially since many of the signal transduction pathway proteins operate in these specialized membrane regions (Lassing and Lindberg, 1985; Burrige et al., 1988). Thus, fodrin SH3 may function in coupling the highly dynamic signal transduction system to the structural components of the cytoskeleton that undergo rapid and extensive changes upon locomotion, growth stimulation and malignant transformation (Boschek et al., 1981; David-Pfeuty and Singer, 1980; Mellström et al., 1988).

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