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Talin contains three actin-binding sites each of which is adjacent to a vinculin-binding site

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SUMMARY

We have determined the sequence of chicken talin (2,541 amino acids, M_r 271,881) which is very similar (89% identity) to that of the mouse protein. Alignments with the *Caenorhabditis elegans* and *Dictyostelium discoideum* talin sequences show that the N- and C-terminal regions of the protein are conserved whereas the central part of the molecule is more divergent. By expressing overlapping talin polypeptides as fusion proteins, we have identified at least three regions of the protein which can bind F-actin: residues 102-497, 951-1,327 and 2,269-2,541. The N-terminal binding site contains a region with homology to the ERM family of actin-binding proteins, and the C-terminal site is homologous to the yeast actin-binding protein Sla2p. Each of the actin-binding sites is close to, but distinct from a binding site for vinculin, a protein which also binds actin. The Pro1176 to Thr substitution found in talin from Wistar-Furth rats does not destroy the capacity of this region of the protein to bind actin or vinculin. Microinjection studies showed that a fusion protein containing the N-terminal actin-binding site localised weakly to stress fibres, whereas one containing the C-terminal site initially localised predominantly to focal adhesions. The former was readily solubilised, and the latter was resistant to Triton extraction. The N-terminal talin polypeptide eventually disrupted actin stress fibres whereas the C-terminal polypeptide was without effect. However, a larger C-terminal fusion protein also containing a vinculin-binding site did disrupt stress fibres and focal adhesions. The results suggest that, although both the N- and C-terminal regions of talin bind actin, the properties of these two regions of the protein are distinct.

Key words: Chicken talin sequence, Actin-binding site, Vinculin, Focal adhesion

INTRODUCTION

Talin is a cytoskeletal protein localised specifically in adherens-type junctions with the extracellular matrix (Burridge and Connell, 1983; Geiger et al., 1985), although it is also found at points of cell contact between T-helper and antigen-presenting cells (Kupfer et al., 1987). It is thought to be one of a number of interacting proteins which link the cytoplasmic domains of integrins to the actin cytoskeleton (reviewed by Jockusch et al., 1995). The complete sequences of mouse (Rees et al., 1990) and *D. discoideum* (Kreitmeier et al., 1995) talin have been published, and the human gene has been assigned to chromosome 9p (Gilmore et al., 1995). Mouse talin contains 2,541 amino acids with a M_r of 269,854. Comparison with the *D. discoideum* sequence (2,491 amino acids) shows that the N- and C-terminal regions of the protein are conserved (Kreitmeier et al., 1995).

Calpain II cleaves chicken talin between residues 433 and 434 to produce a 47 kDa N-terminal fragment and a 190 kDa C-terminal fragment (Rees et al., 1990). Microinjection studies indicate that the N-terminal fragment is important in targeting talin to cell-matrix junctions (Nuckolls et al., 1990). This region of the protein can bind to charged lipids, and appears to contain a membrane binding domain (Niggli et al., 1994). Residues 165-373 show homology with the ezrin/radixin/moesin (ERM)/band 4.1 family of cytosketal proteins (Rees et al., 1990; Takeuchi et al., 1994), members of which also contain an N-terminal membrane-binding domain. The 190 kDa C-terminal polypeptide is composed of a large number of alanine-rich repeats of around 34 amino acids in length (McLachlan et al., 1994). This region is reported to contain a binding site for the β1 integrin cytoplasmic domain (Horwitz et al., 1986), and several binding sites for the cytoskeletal protein vinculin (Lee et al., 1992; Gilmore et al., 1993) which overlap sequences required for targeting the protein to focal adhesions in cultured cells (Gilmore et al., 1993). Talin is also an actin-binding protein reported to possess nucleating activity, and able to enhance F-

actin cross-linking by α-actinin (Muguruma et al., 1990, 1992; Kaufmann et al., 1991). The actin-binding sites in talin have not been mapped, but the 190 kDa fragment can bind to F-actin, and talin residues 2,300-2,541 show homology to a yeast actin-binding protein Sla2p (Holtzman et al., 1993). Interestingly, talin has recently been reported to bind to residues close to the C terminus of pp125 focal adhesion kinase (Chen et al., 1995; M. L. Borowsky and R. O. Hynes, unpublished data), a region which also binds paxillin (Hildebrand et al., 1995).

In *D. discoideum*, talin accumulates at the tips of filopodia formed in response to cAMP, consistent with the view that it plays a key role in linking actin to the membrane (Kreitmeier et al., 1995). Talin is recruited to newly forming focal adhesions before vinculin (DePasquale and Izzard, 1991) suggesting that it is involved in the early events leading to focal adhesion assembly. This conclusion is further supported by microinjection studies in which a polyclonal antibody to talin was found to inhibit both cell spreading and cell adhesion on fibronectin (Nuckolls et al., 1992). Down-regulation of talin in HeLa cells using antisense RNA also reduced the rate of cell spreading on fibronectin (Albiges-Rizo et al., 1995).

In the present study, we report the complete sequence of chicken talin. Alignment of this sequence with that of *C. elegans* (Moulder et al., 1996) and *D. discoideum* (Kreitmeier et al., 1995) talin sequences point to a number of blocks of conserved residues which may help to identify binding sites for proteins which interact with talin. We demonstrate that one of these conserved regions (residues 2,300-2,541) at the C-terminal end of the protein contains an F-actin binding site, although at least two other regions of the talin molecule also bind to F-actin. A second conserved region (residues 1,929-2,029) contains one of the several vinculin-binding sites in talin (Gilmore et al., 1993), each of which is adjacent to an actin-binding site. Finally, by microinjecting purified talin fusion proteins into chicken embryo fibroblasts (CEF), we provide evidence that the association between the N- and C-terminal regions of talin and the actin cytoskeleton show different characteristics.

MATERIALS AND METHODS

Isolation and sequencing of chicken talin cDNAs

The strategy used to isolate cDNAs encoding the complete sequence of chicken talin have been described previously (Gilmore et al., 1993). cDNAs were subcloned into either M13 or pBluescript and sequenced on both strands using the dideoxy chain termination method of Sanger et al. (1977). The complete nucleotide sequence was compiled and sequence alignments conducted using the UWGCG suite of programmes.

Purification of talin fusion proteins expressed in *E. coli*

The chicken talin cDNAs expressed as glutathione-S-transferase (GST) fusion proteins in *Escherichia coli* have been described previously (Gilmore et al., 1993). Fusion proteins were purified essentially as described by Smith and Johnson (1988) using glutathione coupled to agarose beads (Sigma) as an affinity matrix. The fusion proteins were eluted with 5 mM free glutathione. Concentrations of fusion proteins were determined using the Coomassie protein assay kit (Pierce, Rockford, Ill, USA), and the purity of the proteins analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Actin co-sedimentation assay

A fixed amount of rabbit muscle G-actin (20 μg) and increasing amounts of the appropriate GST-talin fusion protein were mixed in a total volume of 100 μl of buffer containing 10 mM Tris-HCl, pH 8.0, 0.2 mM ATP, 0.2 mM dithiothreitol, 0.2 mM $CaCl_2$ and 0.02% Triton X-100. Actin polymerisation was initiated by the addition of 100 mM NaCl, and the samples were incubated at room temperature for 60 minutes. Samples were centrifuged both with and without actin at 75,000 rpm (100,000 ***g***) for 15 minutes. The supernatants were removed, the pellet washed carefully with phosphate buffered saline (PBS), and resuspended in 100 μl PBS. Proteins present in equal volumes of both the supernatant and pellet were resolved by SDS-PAGE, the gels stained with Coomassie blue and analysed using a Molecular Dynamics scanning laser densitometer by volume integration. The ratio of the talin polypeptide:actin (arbitrary units) in the pellet was plotted against the concentration of the talin polypeptide added, and the binding affinity estimated from the concentration of talin polypeptide that produced 50% of maximal binding.

Binding of ^{125}I-vinculin to talin fusion proteins

Vinculin was purified from chicken gizzard by the method of Evans et al (1984), and ^{125}I-labelled with 0.5 mCi of Bolton and Hunter reagent to a specific activity of approximately 2×10^7 cpm per μg, as described previously (Gilmore et al., 1993). Purified talin fusion proteins (200 ng) in 25 mM Tris-HCl, pH 7.5/150 mM NaCl (TBS) were adsorbed onto plastic microtitre wells for 4 hours, and excess protein binding sites blocked with 2% BSA in TBS. ^{125}I-vinculin (4 nM) was added to the wells in 100 μl of TBS containing 4% BSA and 0.1% Tween-20 with or without unlabelled vinculin. Following an overnight incubation, wells were washed three times in TBS/0.1% Tween-20 and counted in a Beckman 5500 gamma counter.

Microinjection of GST-fusion proteins into chicken embryo fibroblasts (CEF)

CEF were grown in Dulbecco's modified Eagle's medium (Life Technologies, Paisley, UK) containing 10% tryptose phosphate broth plus 5% newborn calf serum and 1% chick serum (Advanced Protein Products, West Midlands, UK). Cells were seeded onto glass coverslips (etched with a cross to aid relocation of the injected cells) at least 2 days prior to injection. Approximately 50 cells were microinjected, and the cells returned to the incubator for between 5 and 60 minutes prior to fixing and staining. All injections were carried out with the aid of a Leitz Diavert microscope using a Leitz micromanipulator connected to a Narishige microinjector unit. Microinjection needles were pulled from glass capillaries (Clarke Biomedical Ltd, Slough, Berks., UK) on a microelectrode puller (Campden Instruments Ltd, Sileby, Leics., UK).

Indirect immunofluorescence

The intracellular localisation of the injected GST-fusion proteins was determined as follows. Injected cells were incubated for 5 minutes at 37°C, and cells were then extracted (2 minutes) with 0.5% Triton X-100 in MES buffer (50 mM MES buffer, pH 6.0, 3 mM EGTA, 5 mM $MgCl_2$) to remove the cytoplasmic pool of injected protein. Extracted cells were fixed in 3.8% formaldehyde in PBS, and the injected GST-fusion proteins were detected with a polyclonal rabbit anti-GST antibody (a generous gift from Dr E. Moiseeva, Department of Biochemistry, University of Leicester) and a Texas Red-labelled anti-rabbit IgG (Amersham). The effects of microinjected proteins on the actin cytoskeleton were determined after incubating injected cells for 30-60 minutes at 37°C. Cells were then fixed in 3.8% formaldehyde in PBS, permeabilised with 0.2% Triton X-100 in PBS, and stained with FITC-labelled phalloidin (Sigma) according to the manufacturers' instructions. Cells were stained for vinculin using a mouse monoclonal antibody V284 (Asijee et al., 1990) (Serotec, Kidlington, Oxford, UK) followed by a Texas Red-labelled sheep anti-mouse IgG.

RESULTS

The complete sequence of chicken talin. Comparison of the mouse, chicken, *C. elegans* and *D. discoideum* talin amino acid sequences

Analysis of the complete deduced amino acid sequence of chicken talin shows the protein to contain 2,541 amino acids with a M_r of 271,881. The chicken and mouse (Rees et al., 1990) proteins are very similar throughout the entire sequence (Table 1). Comparison with the *C. elegans* (Moulder et al., 1996) and *D. discoideum* (Kreitmeier et al., 1995) sequences indicates that talin is highly conserved (Table 1), the N- and C-terminal regions of the protein being the most similar (Fig. 1A). Residues 1-433, which are N-terminal to the calpain II cleavage site in chicken talin, show 57% identity (79% similarity) with *C. elegans* talin, and 44.4% identity (65.9% similarity) with *D. discoideum* talin. There are two blocks of conserved sequences in the C-terminal region. Residues 1,960-2,150 in chicken show 59.2% identity (71.7% similarity) with the *C. elegans*, and 27% identity (42% similarity) with the *D. discoideum* protein. Residues 2,300-2,541 in chicken show 50.8% identity (68.5% similarity) with the *C. elegans*, and 35.2% identity (51.5% similarity) with the *D. discoideum* protein. This latter region is therefore slightly more conserved, and displays homology with the C-terminal region of the yeast actin-binding protein Sla2p (Holtzman et al., 1993) (Fig. 1B), suggesting that this region of talin may also bind to actin. The central region of the protein (residues 434-1,960), which is predicted to be highly α-helical in both mouse and chicken talin and contains multiple alanine-rich repeats (McLachlan et al., 1994), is significantly less conserved in primary sequence (chicken to *C. elegans*, 30.6% identity, 51.2% similarity; chicken to *D. discoideum* 19.4% identity, 40.9% similarity).

Table 1. Comparison of the amino acid sequences of mouse, chicken, *C. elegans* and *D. discoideum* talin

		Sequence identity (%)			
		Mouse	Chicken	C.e.	D.d.
Similarity (%)	Mouse	100	89.0	38.8	24.4
	Chicken	93.5	100	38.9	24.6
	C.e.	58.7	58.8	100	24.7
	D.d.	45.5	45.2	47.8	100

Identification of actin-binding sites in talin

To establish which regions of the talin molecule bind to F-actin, overlapping talin fusion proteins spanning nearly the entire sequence of the protein were purified and analysed for their ability to co-sediment with F-actin. Five fusion proteins extending through residues 102-1,327 were all found to co-sediment with F-actin, as did a fusion protein spanning residues 1,646-2,541, whereas little if any of the various fusion proteins were able to sediment in the absence of F-actin (Fig. 2). In contrast, fusion proteins spanning residues 1,304-2,023 (Fig. 2) and 1,554-2,124 (data not shown) did not co-sediment with F-actin. The smallest fusion proteins with binding activity were those spanning residues 102-497, 951-1,327 and 2,269-2,541. We conclude that at least three distinct regions of the talin molecule are able to bind to F-actin.

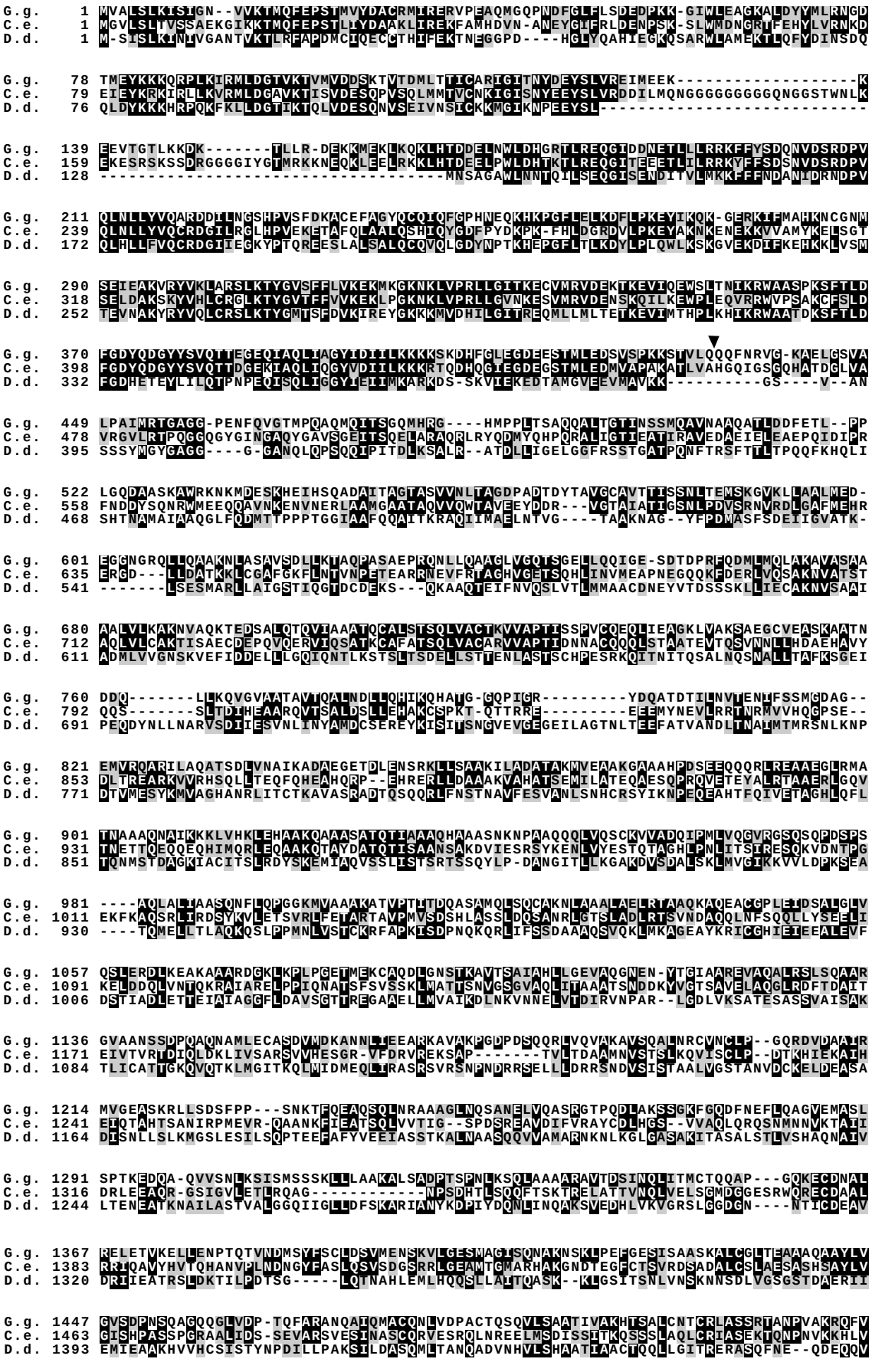
To assess the relative affinities of these regions of the talin molecule for F-actin, increasing amounts of the talin polypeptides were incubated with a fixed quantity of F-actin, and the amount of the talin polypeptide co-sedimenting with F-actin determined by SDS-PAGE and densitometry. The ratio of the talin polypeptide:actin found in the pellet was then plotted against the concentration of talin polypeptide added, and the relative affinities of the fusion proteins estimated from the concentrations of the proteins giving 50% of maximal binding (Fig. 3). The values for talin polypeptides spanning residues 102-497 and 2,269-2,541 were 5 μM and 17 μM, respectively. Unfortunately, the talin fusion protein spanning residues 951-1,327 tended to aggregate at higher concentrations and was therefore replaced by one spanning residues 852-1,328. The relative affinity of this talin polypeptide for F-actin was 8 μM. The affinities of the recombinant talin polypeptides for F-actin are lower than that of intact talin for G-actin (K_d 0.25 μM; Kaufmann et al., 1991), but are similar to those determined for other recombinant actin-binding domains from proteins such as α-actinin, utrophin and dystrophin (Winder et al., 1995).

Further definition of the C-terminal vinculin-binding site in talin

We have previously provided evidence that talin contains three vinculin-binding sites spanning residues 498-656, 852-950 and 1,554-2,268 (Gilmore et al., 1993). To define further the boundaries of the C-terminal vinculin-binding site in relation to the neighbouring actin-binding site, a series of cDNAs encoding this region were made by PCR following standard procedures (Gilmore et al., 1993), and the expressed fusion proteins assayed for their ability to bind ^{125}I-vinculin in a solid phase binding assay. ^{125}I-vinculin bound to talin polypeptides spanning residues 1,554-2,124, 1,554-2,060 and 1,554-2,029 in a manner that was inhibited by unlabelled vinculin (Fig. 4). However, a talin polypeptide spanning residues 1,554-1,929 was unable to bind ^{125}I-vinculin above background levels, and activity was not regained by a further deletion to residue 1,829. We conclude that residues 1,929-2,029 are essential for vinculin binding to this region of the talin molecule. However, the fact that there was a significant reduction in vinculin binding when residues 2,029-2,124 were deleted raises the possibility that this region contains more than one vinculin-binding site.

To determine how many vinculin-binding sites are contained within the C-terminal region of talin, binding of ^{125}I-vinculin to a talin fusion protein spanning residues 1,646-2,541 was studied in the presence of increasing concentrations of unlabelled vinculin. Scatchard analysis of the data indicated a high and lower affinity interaction between vinculin and the talin polypeptide, with K_d values of 4×10^{-7} M and 3.5×10^{-6} M (Fig. 5), a characteristic previously observed using talin purified from chicken gizzard (Gilmore et al., 1993). We have shown that the biphasic nature of the binding curve is due to the presence of two classes of labelled vinculin molecules with a high and lower affinity for talin (A. P. Gilmore, unpublished data), a property which results from the intramolecular association between the head and tail regions of the vinculin molecule (Johnson and Craig, 1994). The stoichiometry of binding for the high affinity interaction was 0.2 moles of vinculin bound per mole of talin whereas that for the lower affinity interaction, which measures the total number of vinculin binding sites in talin, was close to 1:1. The results

A


G.g. 1 MVALSLKKSIGN--VVKTMQFEPSTMVYDACRMIRERVPEAQMGQPNDFGLFLSDEDPKK-GIVLEAGKALDYYMLRNGD
C.e. 1 MGVLSLTVSSAEKGIKKTMQFEPSTLLYDAAKLIREKFAMHDVN-ANEYGIFRLDENPSK-SLWMDNGRTFEHYLVRNKD
D.d. 1 M-SISLKKNIVGANTVKTLRFAPDMCIQECCTHIFEKTNEGGPD----HGLYQAHIEGKQSARWLAMEKTLQFYDINSDQ
G.g. 78 TMEYKKKQRPLKIRMLDGTVKTVMVDDSKTVTDMLTTICARIGITNYDEYSLVREIMEEK-------------------K
C.e. 79 EIEYKRKIRLLKVRMLDGAVKTISVDESQPVSQLMMIVCNKIGISNYEEYSLVRDDILMQNGGGGGGGGGQNGGSTWNLK
D.d. 76 QLDYKKKHRPQKFKLLDGTIKTQLVDESQNVSEIVNSICKKMGIKNPEEYSL-------------------------
G.g. 139 EEVTGTLKKDK-------TLLR-DEKKMEKLKQKLHTDDELNWLDHGRTLREQGIDDNETLLLRRKFFYSDQNVDSRDPV
C.e. 159 EKESRSKSSDRGGGGIYGTMRKKNEQKLEELRKKLHTDEELPWLDHTKTLREQGITEEETLILRRKYFFSDSNVDSRDPV
D.d. 128 ----------------------------------MNSAGAWLNNTQILSEQGISENDITVLMKKFFFNDANIDRNDPV
G.g. 211 QLNLLYVQARDDILNGSHPVSFDKACEFAGYQCQIQFGPHNEQKHKPGFLELKDFLPKEYIKQK-GERKIFMAHKNCGNW
C.e. 239 QLNLLYVQCRDGILRGLHPVEKETAFQLAALQSHIQYGDFPYDKPK-FHLDGRDVLPKEYAKNKENEKKVVAMYKELSGT
D.d. 172 QLHLLFVQCRDGIIEGKYPTQREESLALSALQCQVQLGDYNPTKHEPGFLTLKDYLPLQWLKSKGVEKDIFKEHKKLVSM
G.g. 290 SEIEAKVRYVKLARSLKTYGVSFFLVKEKMKGKNKLVPRLLGITKECVMRVDEKTKEVIQEWSLTNIKRWAASPKSFTLD
C.e. 318 SELDAKSKYVHLCRGLKTYGVTFFVVKEKLPGKNKLVPRLLGVNKESVMRVDENSKQILKEWPLEQVRRWVPSAKCFSLD
D.d. 252 TEVNAKYRYVQLCRSLKTYGMTSFDVKIREYGKKKMVDHILGITREQMLLMLTEIKEVIMTHPLKHIKRWAATDKSFTLD
G.g. 370 FGDYQDGYYSVQTTEGEQIAQLIAGYIDIILKKKKSKDHFGLEGDEESTMLEDSVSPKKSTVLQQQFNRVG-KAELGSVA
C.e. 398 FGDYQDGYYSVQTTDGEKIAQLIQGYVDIILKKKRTQDHQGIEGDEGSTMLEDMVAPAKATLVAHGQIGSGQHATDGLVA
D.d. 332 FGDHETEYLILQTPNPEQISQLIGGYIEIIMKARKDS-SKVIEKEDTAMGVEEVMAVKK----------GS----V--AN
G.g. 449 LPAIMRTGAGG-PENFQVGTMPQAQMQITSGQMHRG----HMPPLTSAQQALTGTINSSMQAVNAAQATLDDFETL--PP
C.e. 478 VRGVLRTPQGGQGYGINGAQYGAVSGEITSQELARAQRLRYQDMYQHPQRALIGTIEATIRAVEDAEIELEAEPQIDIPR
D.d. 395 SSSYWGYGAGG----G-GANQLQPSQQIPITDLKSALR--ATDLLIGELGGFRSSTGATPQNFTRSFTTLTPQQFKHQLI
G.g. 522 LGQDAASKAWRKNKMDESKHEIHSQADAITAGTASVVNLTAGDPADTDYTAVGCAVTTISSNLTEMSKGVKLLAALMED-
C.e. 558 FNDDYSQNRWMEEQQAVNKENVNERLAAMGAATAQVVQWTAVEEYDDR---VGTAIATIGSNLPDVSRNVRDLGAFMEHR
D.d. 468 SHTNAMAIAAQGLFQDMTTPPPTGGIAAFQQAITKRAQIIMAELNTVG----TAAKNAG--YFPDMASFSDEIIGVATK-
G.g. 601 EGGNGRQLLQAAKNLASAVSDLLKTAQPASAEPRQNLLQAAGLVGQISGELLQQIGE-SDTDPRFQDMLMQLAKAVASAA
C.e. 635 ERGD---LLDATKKLCGAFGKFLNTVNPETEARRNEVFRTAGHVGETSQHLINVMEAPNEGQQKFDERLVQSAKNVATST
D.d. 541 -------LSESMARLLATGSTIQGTDCDEKS---QKAAQTEIFNVQSLVTLMMAACDNEYVTDSSSKLLIECAKNVSAAI
G.g. 680 AALVLKAKNVAQKTEDSALQTQVIAAATQCALSTSQLVACTKVVAPTISSPVCQEQLIEAGKLVAKSAEGCVEASKAATN
C.e. 712 AQLVLCAKTISAECDEPQVQERVIQSATKCAFATSQLVACARVVAPTIDNNACQQQLSTAATEVTQSVNNLLHDAEHAVY
D.d. 611 ADMLVVGNSKVEFIDDELLLGQIQNTLKSTSLTSDELLSTTENLASTSCHPESRKQITNITQSALNQSNALLTAFKSGEI
G.g. 760 DDQ-------LLKQVGVAATAVTQALNDLLQHIKQHAIG-GQPIGR---------YDQATDTILNVTENTFSSMGDAG--
C.e. 792 QQS-------SLTDIHEAARQVTSALDSLLEHAKCSPKT-QTTRRE---------EEEMYNEVLRRTNRMVVHQGPSE--
D.d. 691 PEQDYNLLNARVSDIIESVNLINYAMDCSEREYKISITSNGVEVGEGEILAGTNLTEEFATVANDLTNALMTMRSNLKNP
G.g. 821 EMVRQARILAQATSDLVNAIKADAEGETDLENSRKLLSAAKILADATAKMVEAAKGAAAHPDSEEQQQRLREAAEGLRMA
C.e. 853 DLTREARKVVRHSQLLTEQFQHEAHQRP--EHRERLLDAAAKVAHATSEMILATEQAESQPRQVETEYALRTAAERLGQV
D.d. 771 DTVMESYKMVAGHANRLITCTKAVASRADTQSQQRLFNSTNAVFESVANLSNHCRSYIKNPEQEAHTFQIVETAGHLQFL
G.g. 901 TNAAAQNAIKKKLVHKLEHAAKQAAASATQTIAAAQHAAASNKNPAAQQQLVQSCKVVADQIPMLVQGVRGSQSQPDSPS
C.e. 931 TNETTQEQQEQHIMQRLEQAAKQTAYDATQTISAANSAKDVIESRSYKENLVYESTQTAGHLPNLITSIRESQKVDNTPG
D.d. 851 TQNMSTDAGKIACITSLRDYSKEMIAQVSSLISTSRTSSQYLP-DANGITLLKGAKDVSDALSKLMVGIKKVVLDPKSEA
G.g. 981 ----AQLALIAASQNFLQPGGKMVAAAKATVPTLTDQASAMQLSQCAKNLAAALAELRTAAQKAQEACGPLEIDSALGLV
C.e. 1011 EKFKAQSRLIRDSYKVLETSVRLFETARTAVPMVSDSHLASSLDQSANRLGTSLADLRTSVNDAQQLNFSQQLLYSEELI
D.d. 930 ----TQMELLTLAQKQSLPPMNLVSTCKRFAPKISDPNQKQRLIFSSDAAAQSVQKLMKAGEAYKRICGHIEIEEALEVF
G.g. 1057 QSLERDLKEAKAAARDGKLKPLPGETMEKCAQDLGNSTKAVTSAIAHLLGEVAQGNEN-YTGIAAREVAQALRSLSQAAR
C.e. 1091 KELDDQLVNTQKRAIARELPPIQNATSFSVSSKLMATLSNVGSGVAQLITAAATSNDDKYVGTSAVELAQGLRDFTDAIT
D.d. 1006 DSTIADLETTEIAIAGGFLDAVSGTIREGAAELLMVAIKDLNKVNNELVTDIRVNPAR--LGDLVKSATESASSVAISAK
G.g. 1136 GVAANSSDPQAQNAMLECASDVWDKANNLIEEARKAVAKPGDPDSQQRLVQVAKAVSQALNRCVNCLP--GQRDVDAAIR
C.e. 1171 EIVTVRTDIQLDKLIVSARSVVHESGR-VFDRVREKSAP-------TVLTDAAMNVSTSLKQVISCLP--DTKHIEKAIH
D.d. 1084 TLICATTGKQVQTKLMGITKQLWIDMEQLIRASRSVRSNPNDRRSELLLDRRSNDVSISTAALVGSTANVDCKELDEASA
G.g. 1214 MVGEASKRLLSDSFPP---SNKTFQEAQSQLNRAAAGLNQSANELVQASRGTPQDLAKSSGKFGQDFNEFLQAGVEMASL
C.e. 1241 EIQTAHTSANIRPMEVR-QAANKFIEATSQLVVTIG--SPDSREAVDIFVRAYCDLHGS--VVAQLQRQSNMNNVKTAII
D.d. 1164 DISNLLSLKMGSLESILSQPTEEFAFYVEEIASSTKALNAASQQVVAMARNKNLKGLGASAKITASALSTLVSHAQNAIV
G.g. 1291 SPTKEDQA-QVVSNLKSISMSSSKLLLAAKALSADPTSPNLKSQLAAAARAVTDSINQLITMCTQQAP---GQKECDNAL
C.e. 1316 DRLEEAQR-GSIGVLETLRQAG----------NPSDHTLSQQFTSKTRELATTVNQLVELSGMDGGESRWQRECDAAL
D.d. 1244 LTENEATKNAILASTVALGGQIIGLLDFSKARIANYKDPIYDQNLINQAKSVEDHLVKVGRSLGGDGN----NTICDEAV
G.g. 1367 RELETVKELLENPTQTVNDMSYFSCLDSVMENSKVLGESMAGISQNAKNSKLPEFGESISAASKALCGLTEAAAQAAYLV
C.e. 1383 RRIQAVYHVTQHANVPLNDNGYFASLQSVSDGSRRLGEAMTGMARHAKGNDTEGFCTSVRDSADALCSLAESASHSAYLV
D.d. 1320 DRIIEATRSLDKTILPDTSG-----LQTNAHLEMLHQQSLLALTQASK--KLGSITSNLVNSKNNSDLVGSGSTDAERII
G.g. 1447 GVSDPNSQAGQQQGLVDP-TQFARANQAFQMACQNLVDPACTQSQVLSAATIVAKHTSALCNTCRLASSRTANPVAKRQFV
C.e. 1463 GISHPASSPGRAALIDS-SEVARSVESINASCQRVESRQLNREELMSDISSITKQSSSLAQLCRIASEKTQNPNVKKHLV
D.d. 1393 EMIEAAKHVVHCSISTYNPDILLPAKSILDASQMLTANQADVNHVLSHAATIAACTQQLLGITRERASQFNE--QDEQQV

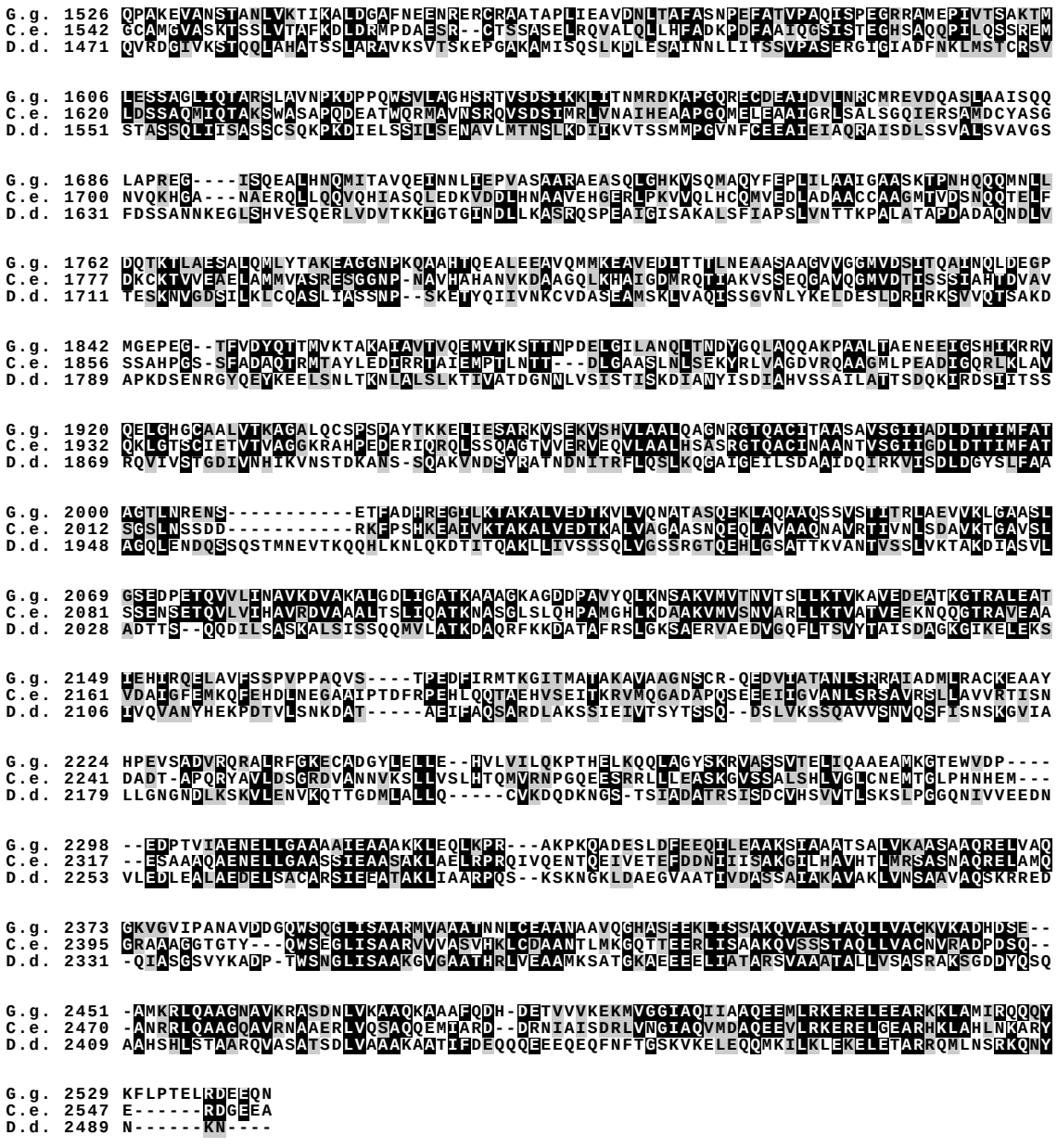


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Fig. 1. Sequence alignment of chicken talin. (A) Talin sequences from chicken (*Gallus gallus*) (this study), *C. elegans* (Moulder et al., 1996) and *D. discoideum* (Kreitmeier et al., 1995) were aligned using the Clustal programme within the UWGCG package. Identities (black) and conservative substitutions (grey) were highlighted using the programme Boxshade. The arrowhead indicates the calpain II cleavage site between residues 433 and 434 inferred from N-terminal sequencing of the 190 kDa talin polypeptide liberated from chicken gizzard talin by this protease (Rees et al., 1990). (B) Alignment of the C-terminal sequences of chicken, nematode and slime mould talin with the C-terminal region of the *S. cerevisiae* actin binding protein Sla2p (Holtzman et al., 1993). A noteable feature is the sequence GLISAA which is present in all four proteins.

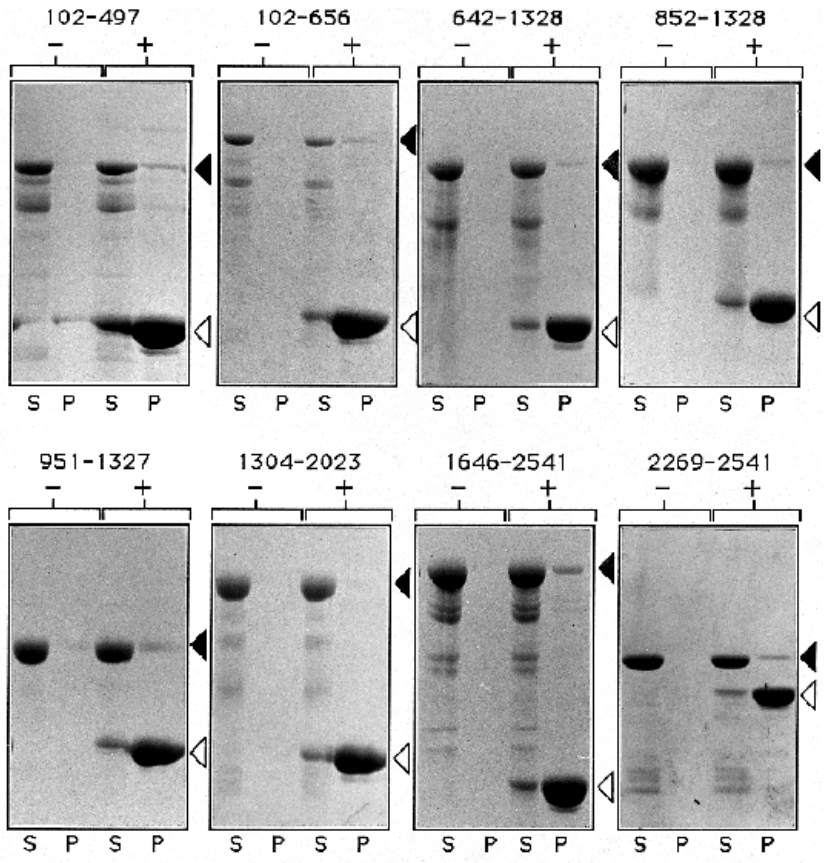

Fig. 2. Binding of talin fusion proteins to F-actin. Chicken talin fusion proteins were incubated in the presence (+) and absence (–) of G-actin, and 100 mM NaCl added to initiate actin polymerisation. The samples were then centrifuged at 100,000 ***g*** for 15 minutes to pellet F-actin; samples of the supernatant (S) and the pellet (P) were analysed by SDS-PAGE, and the proteins visualised by staining with Coomassie blue. The positions of the fusion proteins (filled arrowheads) and actin (open arrowheads) are shown to the right of each panel.

indicate that the C-terminal region of talin contains a single vinculin-binding site. Binding of ^{125}I-vinculin to a fusion protein spanning residues 565-1,328 was also biphasic (K_d values 1×10^{-7} M and 4×10^{-6} M) with Bmax values of 0.1 and 2:1, respectively. These results are consistent with previous results indicating that there are two additional independent vinculin-binding sites in talin between residues 498 and 656 and 852-950 (Gilmore et al., 1993).

Analysis of the potential effects of a Pro 1,176 to Thr substitution in talin on binding to actin

A point mutation has been identified in talin in the Wistar-Furth strain of rat which results in a Pro to Thr substitution at residue 1,176 (Jackson et al., 1993). Interestingly, these rats show an heriditary defect in platelet formation, and the distribution of talin in megakaryocytes and platelets is abnormal (Jackson et al., 1992; Pestina et al., 1995). Proline residues in the talin rod domain are predicted to be clustered in the so called leader sequence at the beginning of each of the multiple 34 residue repeats (McLachlan et al., 1994). The substitution could therefore destroy the folding of talin resulting in a non-functional protein, or it could lead to inactivation of a binding site in the talin molecule. This region of talin contains an actin-binding site (residues 951-1,327), and is close to a vinculin-binding site (residues 852-950), and we therefore investigated the effect of the substitution on the relative binding affinity of a fusion protein spanning residues 852-1328 for F-actin. Both the mutant and the wild-type fusion proteins bound to F- actin (Fig. 6A,B), and the susbstitution had no effect on the affinity of the talin polypeptide for F-actin (Fig. 6C). Neither did the substitution have any obvious affect on the ability of a talin fusion protein spanning residues 565-1,328 to bind to vinculin or to localise to focal adhesions when microinjected into CEF (data not shown). These results are consistent with recent findings that the substitution does not segregate with the platelet defect in Wistar-Furth rats (C. W. Jackson, personal communication) and may indeed be silent, although we do not exclude the possibility that it might inactivate an as yet uncharacterised binding site. The talin rod domain is thought to contain a binding site for β1 integrins (Horwitz et al., 1986), although the site has yet to be defined.

Microinjection of talin polypeptides containing N- and C-terminal sequences into CEF

Talin is related to the ERM family of cytoskeletal proteins by virtue of a low level sequence similarity between the N-terminal region of the proteins (Rees et al., 1990; Takeuchi et al., 1994). Studies on ezrin have shown that both the N- and C-terminal regions of the protein associate with the actin cytoskeleton, although only the latter interaction was stable to extraction with Triton X-100 (Algrain et al., 1993). To establish whether the N- and C-terminal regions of talin showed similar characteristics, purified talin fusion proteins spanning residues 102-497 (which includes the region of homology with the ERM family of proteins) and 2,269-2,541 (homologous with a yeast actin-binding protein; Fig. 1B) were microinjected into CEF, and their subcellular distribution and resistance to Triton X-100 extraction determined. Cells injected with the fusion proteins were incubated at 37°C for 5 minutes, and either extracted with Triton X-100 to remove the soluble pool of injected protein prior to fixation and staining, or fixed first then extracted with Triton X-100. The fusion

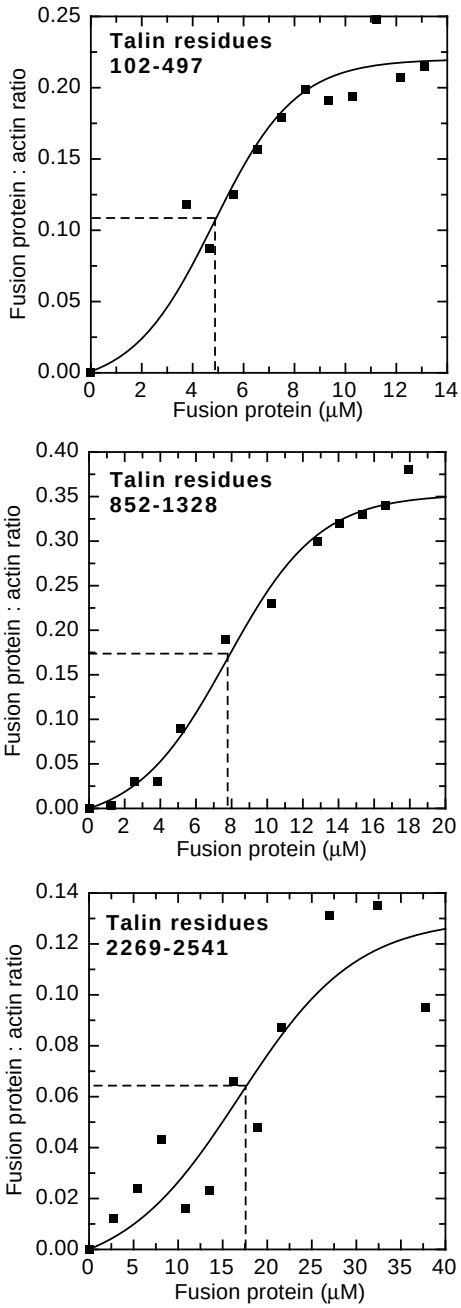

Fig. 3. Determination of the relative affinities of talin polypeptides for F-actin. Increasing amounts of the talin fusion proteins indicated were incubated with a fixed concentration of F-actin (5 μM), and binding analysed by co-sedimentation, SDS-PAGE and scanning densitometry as described in Materials and Methods. The ratio of the talin polypeptide:actin (arbitrary absorbance units) in the pellet were plotted against the amount of talin polypeptide added using the Profit package (Quantum Soft, Zurich, Switzerland).

proteins were localised using an anti-GST antibody, and actin was visualised with FITC-phalloidin. The intracellular localisation of the fusion protein GST/102-497 proved difficult to establish. If the cells were extracted with Triton X-100 prior to fixation, staining was completely abolished (data not shown). In cells fixed then stained, the large pool of cytosolic fusion protein made it impossible to visualise any discrete

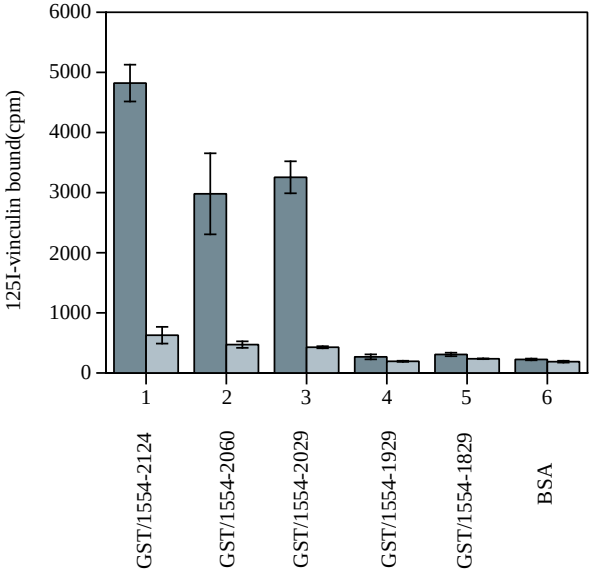

Fig. 4. Binding of ^{125}I-vinculin to talin fusion proteins in the region of residues 1,554-2,124. Microtitre wells coated with the talin fusion proteins (200 ng) indicated were incubated with ^{125}I-vinculin (4 nM) in the absence or presence of 500 nM unlabelled vinculin, and the amount of ^{125}I-vinculin bound determined as described in Materials and Methods. Assays were carried out in triplicate and the bars show the standard error about the mean.

Table 2. Effect of microinjecting talin fusion proteins into CEF on actin stress fibres

Effect on actin stress fibres	Talin fusion proteins (amino acid co-ordinates)		
	102-497	2,269-2,541	1,646-2,541
No disruption	28±3	90±6	11±1
Partial disruption	49±5	10±6	51±1
Total disruption	23±4	0	38±1

Talin polypeptides expressed as GST-fusion proteins were injected into CEF, the cells incubated at 37°C for 30 minutes, then fixed and stained for F-actin as described in Materials and Methods. The effect of the fusion protein on the organisation of actin stress fibres is expressed as a percentage (± s.d.) of the total number of cells injected.

localisation of the protein within the cell body. However, the protein appeared to co-align with actin filaments extending from focal adhesions at the cell periphery (Fig. 7A,B). Interestingly, when injected cells were incubated for 30 minutes at 37°C prior to fixation and staining, the GST/102-497 fusion protein caused disruption of actin stress fibres in most though not all cells (Fig. 7C,D). Vinculin-containing focal adhesions were similarly disrupted (data not shown). Quantitative analysis showed that the fusion protein caused either a partial or total disruption of stress fibres in >70% cells (Table 2), whereas injection of GST alone produced little or no effect (Fig. 7E,F).

Microinjection of the fusion protein GST/2,269-2,541 resulted in significantly different results. The fusion protein localised predominantly to focal adhesions within 5 minutes of microinjection (Fig. 8A,B), with weak co-localisation with actin filaments in some cells (data not shown). Staining was

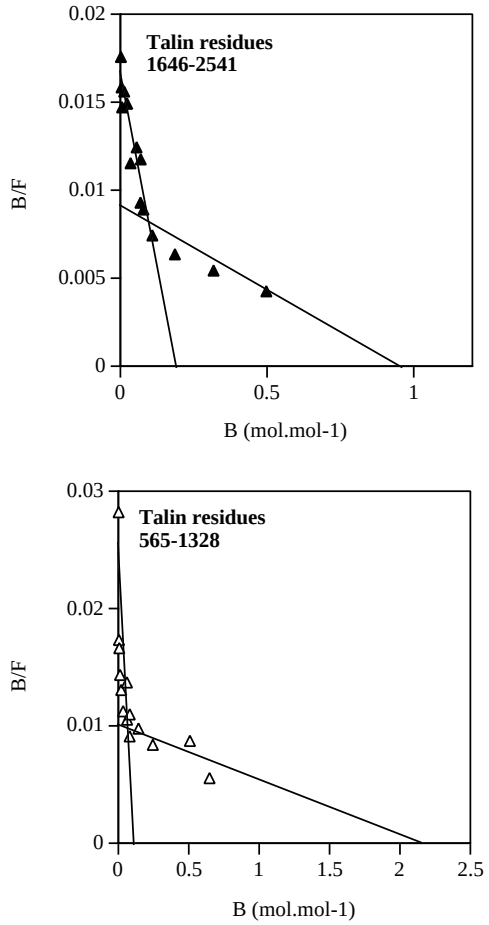

Fig. 5. Stoichiometry of binding of vinculin to talin fusion proteins spanning residues 565-1,328 and 1,646-2,541. Microtitre wells coated with the talin fusion proteins (200 ng) indicated were incubated with ^{125}I-vinculin (4 nM) in the presence of increasing concentrations (5 nM - 4,000 nM) of unlabelled vinculin, and the amount of ^{125}I-vinculin bound determined as described in Materials and Methods. The means of triplicate assays were corrected for non-specific binding to wells coated with BSA. The data are presented as Scatchard plots.

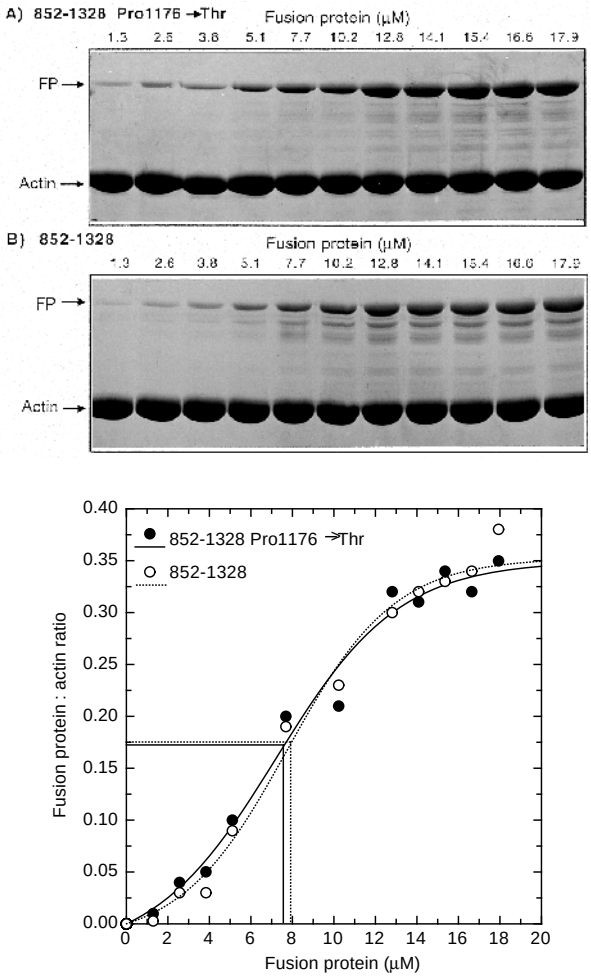

Fig. 6. The effect of a Pro 1,176 to Thr substitution on binding of a talin polypeptide spanning residues 852-1,328 to F-actin. Platelet talin isolated from the Wistar-Furth (WF) strain of rat contains a Pro 1,176 to Thr substitution (Jackson et al., 1993). (A,B) The effect of this substitution on binding of increasing concentrations of a chicken talin fusion protein (residues 852-1,328) to a fixed concentration of F-actin (5 μM) was analysed using a co-sedimentation and SDS-PAGE as described in Materials and Methods. (C) The ratio of the talin polypeptide:actin (arbitrary absorbance units) in the pellet was plotted against the amount of talin polypeptide added using the Profit package (Quantum Soft, Zurich, Switzerland).

resistant to extraction with Triton X-100. After 30 minutes, very few cells (<20%) showed any signs of stress fibre disruption (Fig. 8C) or loss of vinculin-containing focal adhesions (data not shown). However, the fusion protein was no longer localised specifically in focal adhesions and appeared to be more uniformly distributed throughout the cell with some evidence of filamentous staining (Fig. 8D). A larger C-terminal talin polypeptide (residues 1,646-2,541) was also seen to localise to the ends of actin stress fibres in focal adhesions within 5 minutes of injection (Fig. 9A,B), and the protein was again resistant to extraction with Triton X-100. However, >80% of cells which were microinjected with GST/1,646-2,541 and incubated for 30 minutes at 37°C showed partial or total disruption of actin stress fibres (Fig. 9C,D and Table 2), and loss of vinculin-containing focal adhesions (Fig. 9E,F).

DISCUSSION

Comparisons of the chicken talin sequence reported here with those of *C. elegans* (Moulder et al., 1996) and *D. discoideum* (Kreitmeier et al., 1995) talin sequences show the N- and C-terminal regions of the protein to be conserved. The region N-terminal to the calpain II cleavage site in chicken talin contains two blocks of conserved sequence; residues 4-129 and 166-422. The intervening region contains an insert in *C. elegans* and a deletion in *D. discoideum* talin. There are also two conserved regions toward the C terminus of the protein, one spanning residues 1,960-2,150, the other residues 2,334-2,524 (Fig. 1A,B). We show here that the former contains a vinculin-binding site, and the latter an actin-binding site which displays homology to the yeast actin-binding protein Sla2p (Holtzman et al., 1993). However, two other regions of talin also bind to F-actin, namely residues 102-497 and 951-1,327. Interestingly,

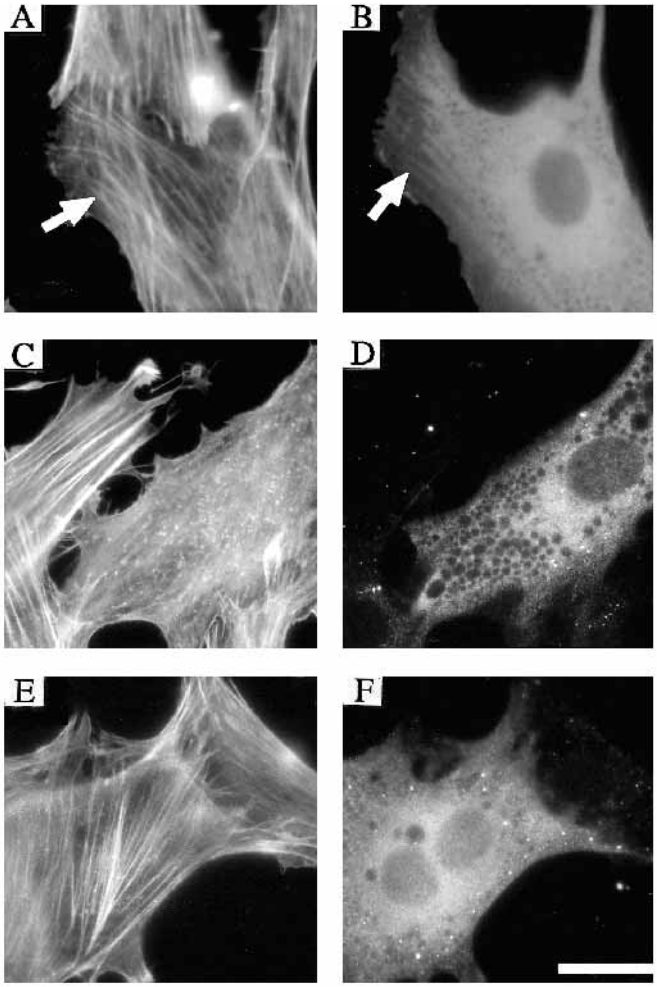

Fig. 7. Microinjection of CEF with a talin fusion protein containing residues 102-497. CEF were microinjected with the talin fusion protein GST/102-497 and incubated at 37°C for 5 minutes (A,B) prior to fixation and permeabilisation. Cells were stained for F-actin using FITC-phalloidin (A) and for the injected fusion protein using a rabbit polyclonal antibody to GST and a Texas red-labelled anti-rabbit IgG (B). The fusion protein was weakly associated with actin filaments at the periphery of the cell (arrows). Injected cells were also incubated at 37°C for 30 minutes (C,D) and fixed then stained for F-actin (C) and the GST/102-497 (D). Under these conditions, the actin cytoskeleton in most injected cells was completely disrupted, whereas uninjected cells contained numerous actin stress fibres. Cells injected with GST alone (E,F) and stained for F-actin (E) and GST (F) were unaffected. Bar, 5 μm.

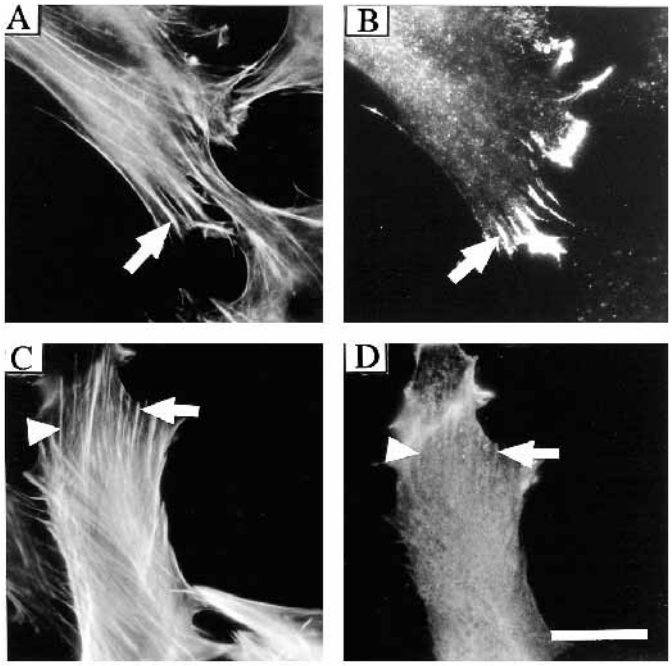

Fig. 8. Microinjection of CEF with a talin fusion protein containing residues 2,269-2,541. Injected cells were incubated at 37°C for 5 minutes (A,B), permeabilised with Triton X-100 to extract the cytoplasmic pool of injected protein, then fixed and stained for F-actin (A) and injected fusion protein (B) as described in Materials and Methods. Under these conditions, the fusion protein was localised to focal adhesions (arrows) with little or no staining along actin stress fibres. Injected cells were also incubated at 37°C for 30 minutes (C-D), fixed first and then stained for F-actin (C) and the injected GST-fusion protein (D). The injected fusion protein (D) was now localised in focal adhesions (arrow) and along the actin stress fibres extending from focal adhesions (arrowhead), and the actin cytoskeleton remained unaffected (C). Bar, 5 μm.

each of the actin-binding sites in talin is distinct from, but adjacent to a binding site for vinculin, a protein which can also bind to F-actin (Menkel et al., 1994; Johnson and Craig, 1994). Microinjection studies show that those regions of talin containing the N- and C-terminal actin-binding sites have distinctive properties in terms of their localisation, resistance to detergent extraction and ability to disrupt focal adhesions and actin stress fibres. A schematic representation of the domain structure of talin which incorporates this new information is shown in Fig. 10.

The microinjection studies of Nuckolls et al. (1990) indicate that the N- terminal region of talin contains key determinants responsible for specific localisation of the protein to cell-matrix junctions. However, binding partners for this region of talin which might account for the localisation of the protein have remained elusive. The fact that the N-terminal region contains two blocks of conserved residues separated by a non-conserved region suggests that it is composed of separate domains with distinct functions. Talin residues 165-373 share homologies with band 4.1 and the ERM family of cytoskeletal proteins (Rees et al., 1990; Takeuchi et al., 1994). This region of the band 4.1 protein contains a binding site for the cytoplasmic domain of the transmembrane protein glycophorin C as well as the peripheral membrane protein p55 (Marfatia et al., 1994), evidence that it is important in localising band 4.1 to the membrane/cytoskeletal interface. Whether the ERM homology region in talin contains a binding site for an integral membrane protein remains to be clarified, although the available evidence suggests that integrins bind to the C-terminal rod domain of the molecule (Horwitz et al., 1986). However, we demonstrate here that the ERM homology region in talin does contain or is adjacent to an actin-binding site, as a fusion protein spanning residues 102-497 binds actin in vitro, and localises (albeit weakly) to actin stress fibres when microinjected into cultured cells. Interestingly, the 47 kDa N-terminal talin polypeptide liberated by calpain II cleavage (residues 1-433) localized to

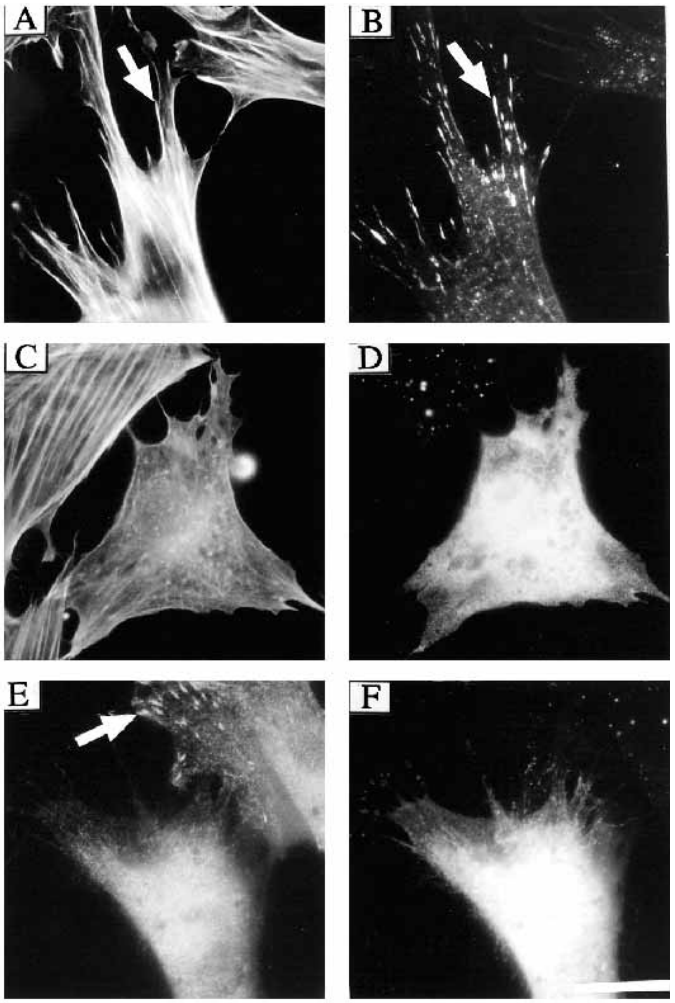

Fig. 9. Microinjection of CEF with a talin fusion protein containing residues 1,646-2,541. Injected cells were incubated at 37°C for 5 minutes (A,B), permeabilised with Triton X-100 to extract the cytoplasmic pool of injected protein, then fixed and stained for F-actin (A) and injected fusion protein (B) as described in Materials and Methods. The fusion protein localised to focal adhesions (arrow) with little or no staining along actin stress fibres. Injected cells were also incubated at 37°C for 30 minutes (C-F) and fixed first, and then stained for F-actin (C), vinculin (E) and the injected GST-fusion protein (D,F). Under these conditions, the actin cytoskeleton and vinculin-containing focal adhesions were completely disrupted in most of the injected cells, although they were unaffected in neighbouring cells which had not been injected with the talin fusion protein (arrows). Bar, 5 μm.

focal adhesions rather than actin filaments when microinjected into cells (Nuckolls et al., 1990). These results support the notion that the conserved residues at the extreme N-terminal region of talin play a role in targeting the protein to focal adhesions, whereas the ERM homology region provides a link to the actin cytoskeleton. This link would appear to be relatively labile as the talin fusion protein spanning residues 102-497 was quantitatively extracted from microinjected cells treated with Triton X-100 prior to fixation. More C-terminal regions of the protein which localised to focal adhesions, were resistant to detergent extraction. These observations are similar to those made with cells stably expressing various talin polypeptides (Gilmore et al., 1993), and are reminiscent of studies on ezrin (Algrain et al., 1993). The expressed N-terminal region of ezrin, which localized to the sub-membranous actin cytoskeleton, was solubilised by Triton X-100, whereas the C-terminal region of the protein, which localised to actin stress fibres, was resistant to detergent extraction. The N-terminal region of talin has also been shown to bind charged lipids (Niggli et al., 1994) and might therefore interact with membranes in a manner independent of protein-protein interactions. Platelet talin has been shown to translocate to the membrane following activation even in the absence of integrin GPIIb-IIIa (Bertagnolli et al., 1993). These various observations suggest a model in which the N-terminal head region of talin binds to one or more proteins responsible for localising talin specifically to cell-matrix junctions, to membrane lipids, and to the actin cytoskeleton via interactions which are readily disrupted by non-ionic detergents.

The C-terminal rod domain of talin contains two additional actin-binding sites neither of which displays any obvious sequence homology to the other, or to those mapped to the C-terminal regions of ezrin (Turunen et al., 1994) and moesin (Pestonjamasp et al., 1995). Talin binds to both G- and F-actin, and is reported both to nucleate actin filament assembly (Muguruma et al., 1990; Kaufmann et al., 1991), and to augment the ability of α-actinin to cross-link F-actin (Muguruma et al., 1992). The actin cross-linking activity may be explained by the fact that talin contains more than one actin-binding site, or by the observation that it can form dimers under certain conditions (Molony et al., 1987). What role each of the actin-binding sites in talin fulfills remains to be explored. The C-terminal site (residues 2,300-2,541) is homologous to a region of the *S. cerevisiae* protein Sla2p which appears to be important in controlling the assembly of the cortical actin cytoskeleton at bud sites (Holtzman et al., 1993). By analogy, the C-terminal actin-binding site in talin may play a key role in the assembly of actin filaments associated with focal adhesions. The fact that a fusion protein (residues 2,269-2,541) containing this site localised to focal adhesions when microinjected into CEF may be relevant in this regard. The three actin-binding sites in talin are each close to regions of the protein that bind vinculin (Fig. 10), a protein which can also bind to actin (Menkel et al., 1994; Johnson and Craig, 1994). Vinculin interacts with talin via residues within the N-terminal globular head region (residues 1-258) of vinculin (Jones et al., 1989; Gilmore et al., 1993) whereas the actin-binding site(s) are in the C-terminal region of the molecule (Johnson and Craig, 1994), which can form an extended tail. It will be interesting to establish whether talin enhances the ability of vinculin to bind to actin in a manner similar to its ability to augment the actin-bundling activity of α-actinin (Muguruma et al., 1992). Bearing in mind that vinculin also binds α-actinin (McGregor et al., 1994), and both talin (Horwitz et al., 1986) and α-actinin (Otey et al., 1993) are reported to bind to the cytoplasmic face of integrins, it seems likely that the interactions among these proteins play a pivotal role in the assembly of actin filaments and stress fibres on the cytoplasmic face of focal adhesions.

If talin is a key component of focal adhesions as suggested by the results from a variety of experimental approaches, one might expect that microinjection of sub-domains of the protein would competitively inhibit the interactions between talin and

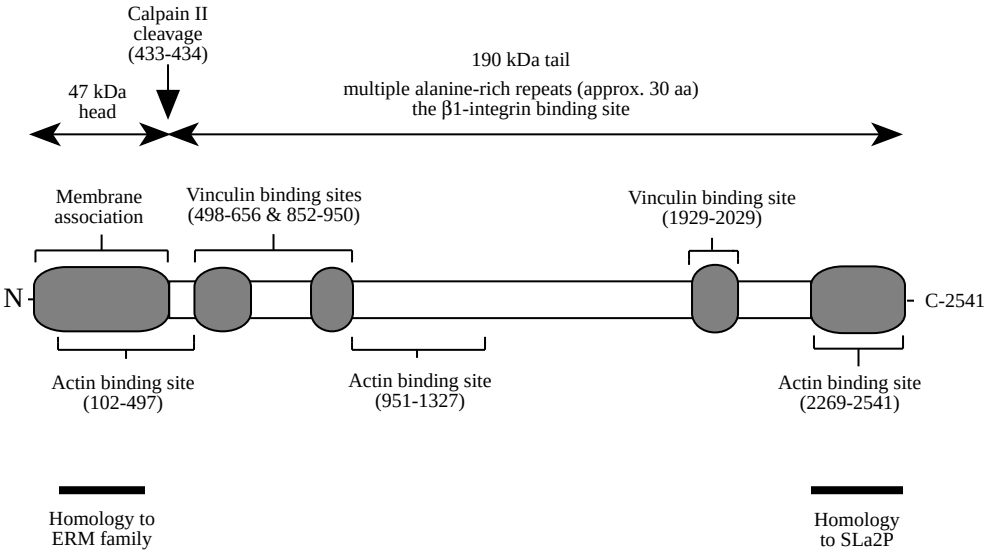

Fig. 10. Domain structure of talin.

its various binding partners, and so disrupt focal adhesions. This prediction was confirmed when we observed that a fusion protein (residues 1,646-2,541) containing both the C-terminal vinculin and actin-binding sites disrupted focal adhesions and actin stress fibres in CEF. However, a smaller C-terminal fusion protein (2,269-2,541) containing the actin-binding site (but not the vinculin-binding site) was ineffective in this regard. One potential explanation for this observation is that the larger fusion protein disrupts focal adhesions by inhibiting the interaction between talin and vinculin. Vinculin is known to be a relatively low abundance protein essential for focal adhesion assembly (Rodriguez-Ferandez et al., 1993), at least in fibroblasts. The concentrations of actin in the cell may be too great for the C-terminal talin polypeptide to compete the interaction between actin and endogenous talin. The fact that a fusion protein (residues 102-497) containing the N-terminal actin-binding site, but no vinculin-binding site, was also able to disrupt focal adhesions could be explained if this region contains an additional binding site for another focal adhesion protein, as discussed earlier.

Whether the actin and vinculin-binding sites in talin are always exposed or are regulated in some way remains to be established. In vinculin, an intramolecular association between the globular N-terminal head and the C-terminal tail has been shown to obscure the actin and talin-binding sites (Johnson and Craig, 1994). The head-tail association is inhibited by acidic phospholipids such as PIP2 exposing the actin-binding site (Weekes et al., 1996). Talin has been observed to exist in both a globular and an extended configuration in vitro, whereas the 190 kDa rod domain is always fully extended (Molony et al., 1987). Interestingly, the 190 kDa talin polypeptide has been reported to bind vinculin more tightly than does intact talin (Burridge and Mangeat, 1984).

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REFERENCES

Albiges-Rizo, C., Frachet, P. and Block, M. R. (1995). Down regulation of talin alters cell adhesion and the processing of the α5β1 integrin. *J. Cell Sci.* **108**, 3317-3329.

Algrain, M., Turunen, O., Vaheri, A., Louvard, D. and Arpin, M. (1993). Ezrin contains cytoskeleton and membrane binding domains accounting for its proposed role as a membrane-cytoskeletal linker. *J. Cell Biol.* **120**, 129-139.

Asijee, G. M., Sturk, A., Bruin, T., Wilkinson, J. M. and Ten Cate, J. W. (1990). Vinculin is a permanent component of the membrane skeleton, and is incorporated in the (re)organising cytoskeleton upon platelet activation. *Eur J. Biochem.* **189**, 131-136.

Bertagnolli, M. E., Locke, S. J., Hensler, M. E. Bray, P. F. and Beckerle, M. C. (1993). Talin distribution and phosphorylation in thrombin-activated platelets. *J. Cell Sci.* **106**, 1189-1199.

Burridge, K. and Connell, L. (1983). A new protein of adhesion plaques and membrane ruffles. *J. Cell Biol.* **97**, 359-367.

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

Chen, H.-C., Appeddu, P. A., Parsons, J. T., Hildebrand, J. D., Schaller, M. D. and Guan, J.-L. (1995). Interaction of focal adhesion kinase with cytoskeletal protein talin. *J. Biol. Chem.* **270**, 16995-16999.

DePasquale, J. A. and Izzard, C. S. (1991). Accumulation of talin in nodes at the edge of the lamellipodium and separate incorporation into adhesion plaques at focal contacts in fibroblasts. *J. Cell Biol.* **113**, 1351-1359.

Evans, R. R., Robson, R. M. and Stromer, M. H. (1984). Properties of smooth muscle vinculin. *J. Biol. Chem.* **259**, 3916-3924.

Geiger, B., Volk, T. and Volberg, T. (1985). Molecular heterogeneity of adherens junctions. *J. Cell Biol.* **101**, 1523-1531.

Gilmore, A. P., Wood, C., Ohanian, V., Jackson, P., Patel, B., Rees, D. J. G., Hynes, R. O. and Critchley, D. R. (1993). The cytoskeletal protein talin contains at least two distinct vinculin-binding sites. *J. Cell Biol.* **122**, 337-347.

Gilmore, A. P., Ohanian, V., Spurr, N. K. and Critchley, D. R. (1995). Localisation of the human gene encoding the cytoskeletal protein talin to chromosome 9p. *Hum. Genet.* **96**, 221-224.

Hildebrand, J. D., Schaller, M. D. and Parsons, J. T. (1995). Paxillin, a tyrosine phosphorylated focal adhesion-associated protein binds to the carboxy terminal domain of focal adhesion kinase. *Mol. Biol. Cell* **6**, 637-647.

Holtzman, D. A., Yang, S., Drubin, D. G. (1993). Synthetic-lethal interactions identify 2 novel genes, SLA1 and SLA2, that control membrane cytoskeleton assembly in S. cerevisiae. *J. Cell Biol.* **122**, 635-644.

Horwitz, A., Duggan, K., Buck, C., Beckerle, M. C. and Burridge, K. (1986). Interaction of plasma-membrane fibronectin receptor with talin - a transmembrane linkage. *Nature* **320**, 531-533.

Jackson, C. W., Hutson, N. K., Steward, S. A. and Stenberg, P. E. (1992). A unique talin antigenic determinant and anomalous megakaryocyte talin distribution associated with abnormal platelet formation in the Wistar-Furth rat. *Blood* **79**, 1729-1737.

Jackson, C. W., Hutson, N. K., Steward, S. A. and Rees, D. J. G. (1993). Detection of a mutation in the cytoskeletal protein talin in the Wistar-Furth (WF) rat - A rat strain with defective platelet formation and a high tumour incidence. *Blood* **82**, 340A.

Jockusch, B. M., Bubeck, P., Giehl, K., Kroemker, M., Moschner, J., Rothkegel, M., Rudiger, M., Schluter, K., Stanke, G. and Winkler, J. (1995). The molecular architecture of focal adhesions. *Annu. Rev. Cell Dev. Biol.* **11**, 379-416.

Johnson, R. P. and Craig, S. W. (1994). An intramolecular association between the head and tail domains of vinculin modulates talin binding. *J. Biol. Chem.* **269**, 12611-12619.

Jones, P., Jackson, P., Price, G. J., Patel, B., Ohanian, V., Lear, A. L. and Critchley, D. R. (1989). Identification of a talin binding site in the cytoskeletal protein vinculin. *J. Cell Biol.* **109**, 2917-2927.

Kaufmann, S., Piekenbrock, T., Goldmann, W. H., Barmann, M. and Isenberg, G. (1991). Talin binds to actin and promotes filament nucleation. *FEBS Lett.* **284**, 187-191.

Kreitmeier, M., Gerisch, G., Heizer, C. and Muller-Taubenberger, A. (1995). A talin homologue of *Dictyostelium* rapidly assembles at the leading edge of cells in response to chemoattractant. *J. Cell Biol.* **129**, 179-188.

Kupfer, A., Swain, S. L. and Singer, S. J. (1987). The specific direct interaction of helper T-cells and antigen presenting B-cells. II. Reorientation of the microtubule organising centre and reorganisation of the membrane-associated cytoskeleton inside the bound helper T-cells. *J. Exp. Med.* **165**, 1565-1580.

Lee, S.-W., Wulfkuhle, J. D. and Otto, J. J. (1992). Vinculin binding site mapped on talin with an anti-idiotypic antibody. *J. Biol. Chem.* **267**, 16355-16358.

Marfatia, S. M., Lue, R. A., Branton, D. and Chisti, A. H. (1994). In vitro binding studies suggest a membrane-associated complex between erythroid p55, protein 4.1 and glycophorin C. *J. Biol. Chem.* **269**, 8631-8634.

McGregor, A., Blanchard, A. D., Rowe, A. J. and Critchley, D. R. (1994). Identification of the vinculin-binding site in the cytoskeletal protein α-actinin. *Biochem. J.* **301**, 225-233.

McLachlan, A. D., Stewart, M., Hynes, R. O. and Rees, D. J. G. (1994). Analysis of repeated motifs in the talin rod. *J. Mol. Biol.* **235**, 1278-1290.

Menkel, A. R., Kroemaker, M., Bubeck, P., Ronsiek, M., Nikolai, G. and Jockusch, B. M. (1994). Characterisation of an F-actin binding domain in the cytoskeletal protein vinculin. *J. Cell Biol.* **126**, 1231-1240.

Molony, L., McCaslin, D., Abernethy, J., Paschal., B. and Burridge, K. (1987). Properties of talin from chicken gizzard smooth muscle. *J. Biol. Chem.* **262**, 7790-7795

Moulder, G. L., Huang, M.-M., Waterston, R. H. and Barstead, R. J. (1996). Talin requires β-integrin but not vinculin for its assembly into focal adhesion-like structures in the nematode *Caenorhabditis elegans*. *Mol. Biol. Cell*. **7**, 1181-1193.

Muguruma, M., Matsumura, S. and Fukazawa, T. (1990). Direct interactions between talin and actin. *Biochem. Biophys. Res. Commun.* **171**, 1217-1223.

Muguruma, M., Matsumura, S. and Fukazawa, T. (1992). Augmentation of α-actinin-induced gelation of actin by talin. *J. Biol. Chem.* **267**, 5621-5624.

Niggli, V., Kaufmann, S., Goldmann, W. H., Weber, T. and Isenberg, G. (1994). Identification of functional domains in the cytoskeletal protein talin. *Eur. J. Biochem.* **224**, 951-957.

Nuckolls, G. H., Turner, C. E. and Burridge, K. (1990). Functional-studies of the domains of talin. *J. Cell Biol.* **110**, 1635-1644.

Nuckolls, G. H., Romer, L. H., Burridge, K. (1992). Microinjection of antibodies against talin inhibits the spreading and migration of fibroblasts. *J. Cell Sci.* **102**, 753-762.

Otey, C. A., Vasquez, G. B., Burridge, K. and Erickson, B. W. (1993). Mapping of the α-actinin binding site within the β1-integrin cytoplasmic domain. *J. Biol. Chem.* **268**, 21193-21197.

Pestina, T. L., Jackson, C. W. and Stenberg, P. E. (1995). Abnormal subcellular distribution of myosin and talin in Wistar Furth rat platelets. *Blood* **85**, 2436-2446.

Pestonjamasp, K., Amieva, M. R. Strassel, C. P., Nauseef, W. M., Furthmayr, H. and Luna, E. J. (1995). Moesin, ezrin and p205 are actin-binding proteins associated with neutrophil plasma membranes. *Mol. Biol. Cell* **6**, 247-259.

Rees, D., Ades, S. E., Singer, S. J. and Hynes, R. O. (1990). Sequence and domain-structure of talin. *Nature* **347**, 685-689.

Rodriguez Fernandez, J. L. R., Geiger, B., Salomon, D., Ben Ze'ev, A. (1993). Suppression of vinculin expression by antisense transfection confers changes in cell morphology, motility, and anchorage-dependent growth of 3T3-cells. *J. Cell Biol.* **122**, 1285-1294.

Sanger, F., Nickel, S. and Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proc. Nat. Acad. Sci. USA* **74**, 5463-5466.

Smith, D. B. and Johnson, K. S. (1988). Single step purification of polypeptides expressed in E. coli as fusions with glutathione-S-transferase. *Gene* **67**, 31-40.

Takeuchi, K., Kawashima, A., Nagafuchi, A. and Tsukita, S. (1994). Structural diversity of band 4.1 superfamily members. *J. Cell Sci.* **107**, 1921-1928.

Turunen, O., Wahlstrom, T. and Vaheri, A. (1994). Ezrin has a COOH-terminal actin-binding site that is conserved in the ezrin protein family. *J. Cell Biol.* **126**, 1445-1453.

Weekes, J., Barry, S. T. and Critchley, D. R. (1996). Acidic phospholipids inhibit the intramolecular association between the N- and C-terminal regions of vinculin exposing actin-binding and protein kinase C phosphorylation sites. *Biochem J.* **314**, 827-832.

Winder, S. J., Hemmings, L., Maciver, S. K., Bolton, S. J., Tinsley, J. M., Davies, K. E., Critchley, D. R. and Kendrick-Jones, J. (1995). Utrophin actin binding domain: analysis of actin binding and cellular targeting. *J. Cell Sci.* **108**, 63-71.

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