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Fascin, an echinoid actin-bundling protein, is a homolog of the *Drosophila* singed gene product

(cytoskeleton/microvilli/sea urchin)

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ABSTRACT **A cDNA for fascin, an actin-bundling protein in echinoderms, has been cloned, sequenced, and expressed. The predicted mass of the protein is ≈55 kDa, similar to that observed for fascin purified from sea urchin eggs. Bacterially expressed fascin reacts with antibodies prepared against sea urchin egg fascin. Fascin has a strong sequence similarity to the singed gene (*sn*) product in *Drosophila* and has similarities with a 55-kDa human actin-bundling protein. No extensive similarities were found with other known actin-binding/bundling proteins, indicating that this is a separate gene family.**

Fascin was one of the first molecules identified in cytoplasmic actin gels induced to form in low Ca^{2+} extracts of eggs from the sea urchin *Tripneustes gratilla* by Kane ≈18 years ago (1, 2) and it was the first actin-bundling protein to be characterized extensively (see ref. 3 for review). Fascin crosslinks actin filaments into bundles *in vitro*. Reconstituted actin–fascin bundles show a characteristic 11-nm periodic striping pattern (2, 4) that can also be identified in actin bundles *in situ* (5, 6). Optical diffraction and image reconstruction studies (7–9), coupled with the determination of the actin–fascin stoichiometry in bundles, indicate that there is one fascin molecule per filament crosslink (4). This suggests that each fascin molecule must contain two actin filament binding sites.

Early functional studies demonstrated the importance of fascin in the organization of F-actin into egg microvillar cores, which form shortly after fertilization (10). In phagocytic coelomocytes from echinoderms, fascin has been shown to be involved in the reorganization of actin filaments into bundles that form the cores of filopodia, which develop as part of the host defense mechanism (11, 12). Later studies have localized fascin in the actin bundles of starfish oocyte spikes (13) and starfish sperm acrosomes (14).

Here, we report the sequence of a sea urchin (*Strongylocentrotus purpuratus*) fascin cDNA.¶ The protein sequence shows that the fascins are a separate family of actin-binding proteins, which includes the *Drosophila* singed (*sn*) gene product (15) and a group of ≈55-kDa actin-bundling proteins in mammalian cells (16, 17). These proteins are not normally believed to be localized in classical microvilli and may represent an unusual filament organizing principle. No extensive similarities were found with other known actin-binding/bundling proteins (18).

MATERIALS AND METHODS

Protein Purification. *S. purpuratus* fascin was purified from egg extracts prepared essentially as described by Kane (1, 2) with the following modifications. The isotonic glycerol buffer (0.9 M glycerol/5 mM EDTA/0.1 M Pipes, pH 7.15) used to homogenize the eggs contained 1 mM dithiothreitol, 10 μg of leupeptin per ml, 10 μg of aprotinin per ml, 10 μg of pepstatin A per ml, 1 mM N^{α}-*p*-tosyl-L-arginine methyl ester, 1 mM phenylmethylsulfonyl fluoride, 40 μg of soybean trypsin inhibitor per ml, 10 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone per ml, and 10 mM benzamidine. The extract was centrifuged at 30,000 × *g* for 45 min to remove large particulates. The supernatant was removed and recentrifuged at 100,000 × *g* for 75 min. The supernatant of the second spin was carefully removed to avoid floating lipid contamination and was dialyzed overnight against two changes, 20–40 vol per change, of dialysis buffer (5 mM Pipes/0.1 mM EGTA/0.1 mM ATP, pH 7.0) containing the protease inhibitors aprotinin, leupeptin, pepstatin A, all at 2.5 μg/ml. After dialysis, the egg extract was centrifuged at 30,000 × *g* for 30 min. The supernatant was adjusted to final concentrations of 0.1 M KCl/1 mM EGTA/2 mM ATP/1 mM $MgCl_2$/20 mM Hepes, pH 7.4, plus the protease inhibitors used for the initial homogenization. Phalloidin was added to the extract to a final concentration of 5 μg/ml from a 1-mg/ml stock in methanol, and the extract was incubated for 1 hr at room temperature and then overnight at 4°C. An actin-based precipitate forms that contains many actin-associated proteins including fascin, myosin, and α-actinin along with many unknown proteins. The precipitate was collected by centrifugation at 30,000 × *g* for 30 min, washed with the solution described above, and solubilized by Dounce homogenization in high salt (1 M KCl) in column buffer (10 mM Pipes/0.1 mM EGTA/5 mM 2-mercaptoethanol, pH 7.2, plus protease inhibitors). After 30 min to 1 hr, the solution was centrifuged for 2 hr at 100,000 × *g* to remove F-actin. The supernatant was dialyzed overnight against 0.5 M KCl and then centrifuged for 2 hr at 100,000 × *g* to remove residual F-actin. The supernatant from this centrifugation was dialyzed against two changes (1000 × vol) of column buffer. Before application to a DEAE anion-exchange column (DE52; Whatman), the solution was centrifuged at 30,000 × *g* for 30 min to remove a precipitate that forms during dialysis to low ionic strength. The column was eluted with a gradient of 0–0.5 M NaCl in column buffer. Fascin elutes between 50 and 80 mM NaCl without obvious contamination by other proteins.

Peptide Sequencing. The N-terminal amino acid sequence was obtained from fascin blotted onto poly(vinylidene difluoride) membranes (19). Internal peptide sequences were obtained by following the procedure described by Aebersold (20). Fascin blotted onto nitrocellulose membranes was digested with trypsin or *Achromobacter* protease I. The peptides were eluted from the membrane and separated by reverse-phase HPLC. Sequences were obtained by gas-phase sequencing using an ABI model 475 or Porton 2090E instru-

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. L12047).

ment equipped with on-line identification of phenylthiohydantoin-derivatized amino acids.

Cloning and Sequencing of *S. purpuratus* Fascin. A cDNA clone, pFAS4, was isolated from a *S. purpuratus* larval stage λ ZAP cDNA library prepared by Stratagene. Approximately 10^6 recombinant phage were screened with a degenerate oligonucleotide, 5′-ATGAA(C/T)(C/T)T(A/C/G/T)AA(A/G)TA(C/T)AA(A/G)TT-3′. Ten candidate clones were isolated and the Bluescript plasmids were excised according to the manufacturer's (Stratagene) instructions and partially sequenced by using T3 and T7 primers. pFAS4 was chosen because the translated peptide matched that obtained by protein sequencing. After restriction mapping, fragments were subcloned into M13 and sequenced by the dideoxynucleotide chain-termination method using either M13 or synthetic oligonucleotide primers and Sequenase version II (United States Biochemical).

Expression of *S. purpuratus* Fascin in *Escherichia coli*. pFAS-T7 was constructed by engineering an *Nde* I site at the ATG start codon using a PCR primer, 5′-AACATATTTC-CATATGCCTGCATG-3′, with the three mutant nucleotides marked in boldface. This primer was paired with a second primer, 5′-TCGAGCTGCTCTCTTCTTGC-3′, beginning 4 nucleotides downstream of an internal *Bam*HI site at position 211 in the sequence shown in Fig. 1. The PCR product generated by using 1 ng of pFAS4 plasmid DNA and *Taq* polymerase under conditions specified by the manufacturer (Cetus) was digested with *Nde* I and *Bam*HI. The resulting 130-nucleotide fragment was subcloned into pAED4, a T7-based expression vector (a gift from D. S. Doering, Whitehead Institute for Biomedical Research), which had been digested with *Nde* I and *Bam*HI. A transformant was selected on ampicillin and used to prepare plasmid DNA. The construction was verified by plasmid sequencing and then digested with *Eco*RI and blunt ended by using the Klenow fragment of DNA polymerase. This DNA was redigested with *Bam*HI, purified on a low melting point agarose gel, and ligated to the remainder of fascin cDNA prepared as follows: pFAS4 plasmid DNA was digested with *Xho* I, blunt ended with the Klenow fragment of DNA polymerase, and then redigested with *Bam*HI. The resulting 2150-nucleotide fragment was gel purified on low melting point agarose and used as indicated above. The resulting plasmid, pFAS-T7, was transformed into *E. coli* strain BL21(DE3) (21) carrying the T7 RNA polymerase gene under control of the *lac* promoter and selected on ampicillin. Transformants were tested for expression by adding isopropyl β-D-thiogalactopyranoside to 1.0 mM to cells with an approximate optical density of 1.0, growing the cells for 3 hr at 37°C, and then harvesting the cells by centrifugation and solubilizing in SDS sample buffer (22). After SDS/PAGE (22), the gel was semidry electroblotted onto nitrocellulose. The blot was incubated with antibodies to sea urchin fascin, followed by peroxidase-labeled protein A. Chloronaphthol and H_2O_2 were used as substrates for the peroxidase. The antibodies are polyclonal and monospecific and have been extensively characterized as described (11). The antibodies have been used to localize and quantitate echinoid fascin in both sea urchin eggs and coelomocytes (10–12).

RESULTS

Fascin purified from the eggs of *S. purpuratus* was functionally equivalent to that previously characterized from *T.*

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                                                ACTTGAAAGTGGATAAAATCGACT GATACCAAAACAACATTGTTTTACAGAAGT GGTCGTTTGAGGACATCAACATATTTCACA    84

    ATGCCTGCTATGAATTTAAAATACAAATTT GGCCTGGTCAACTCGGCCGGCAGATACCTC ACTGCTGAGAAGTTTGGTGGCAAAGTCAAT GCCTCAGGAGCAACGTTAAAAGCCAGGCAA   204
  1 MetProAlaMetAsnLeuLysTyrLysPhe GlyLeuValAsnSerAlaGlyArgTyrLeu ThrAlaGluLysPheGlyGlyLysValAsn AlaSerGlyAlaThrLeuLysAlaArgGln

    GTATGGATCCTAGAGCAAGAAGAGAGCAGC ACGATCAGCTACTTGAAGGCGCCCTCTGGT AACTTCCTCTCTGCAGATAAAAACGGTAAC GTCTATTGCAGTGTTGAGGACAGGACGGAG   324
 41 ValTrpIleLeuGluGlnGluGluSerSer ThrIleSerTyrLeuLysAlaProSerGly AsnPheLeuSerAlaAspLysAsnGlyAsn ValTyrCysSerValGluAspArgThrGlu

    GACGCGGATACAGGATTCGAGATCGAGTTG CAACCCGATGGTAAATGGGCCCTCAAGAAT GTTTCTCACCAGAGGTACCTAGCTTGCAAT GGTGAGGAGCTGATCTGCAGTGAATCCAGC   444
 81 AspAlaAspThrGlyPheGluIleGluLeu GlnProAspGlyLysTrpAlaLeuLysAsn ValSerHisGlnArgTyrLeuAlaCysAsn GlyGluGluLeuIleCysSerGluSerSer

    ACCAGCAACCCCTCAGCAAACTGGACTGTC CAGCTGGCCATCCATCCACAGGTCTGCATG AAGAACGTCCAGCACCAACGCTACGCACAT CTCAAAACCAGTGAGGAGGGTGAAGACAGC   564
121 ThrSerAsnProSerAlaAsnTrpThrVal GlnLeuAlaIleHisProGlnValCysMet LysAsnValGlnHisGlnArgTyrAlaHis LeuLysThrSerGluGluGlyGluAspSer

    GTGGTTGTAGACGAATTGGTTCCCTGGGGA GCTGATTCCACACTCACTCTTGTCTACCTG GGCAAAGGAAAGTACGGCCTTGAGGCCTTC AACGGAAAGTTTGTCCAAACCGACGGACAG   684
161 ValValValAspGluLeuValProTrpGly AlaAspSerThrLeuThrLeuValTyrLeu GlyLysGlyLysTyrGlyLeuGluAlaPhe AsnGlyLysPheValGlnThrAspGlyGln

    CTTGCTGGCACAGCCAACGAACAGACGCAG TTCACACTCATCTTCACATCCGGTCACCTG GTACTAAGGGACAACAATGGACGTCACTTA GGAGTGGACAGTGGAACCAGGGTCTTGAAG   804
201 LeuAlaGlyThrAlaAsnGluGlnThrGln PheThrLeuIlePheThrSerGlyHisLeu ValLeuArgAspAsnAsnGlyArgHisLeu GlyValAspSerGlyThrArgValLeuLys

    TCCTCCAAGCCTGGACTGACGAAAGCCAAT TACTTCATCCTAGAGGATAGCTGTCCACAA GGTGCCTTCGAATTTGGTGGCAAATATGCA TCGTTAAAGCAAGGCGAAGATGTTTCATTC   924
241 SerSerLysProGlyLeuThrLysAlaAsn TyrPheIleLeuGluAspSerCysProGln GlyAlaPheGluPheGlyGlyLysTyrAla SerLeuLysGlnGlyGluAspValSerPhe

    AAGCTTCTTGTTGACGAAGATATCGAAGAC ACAGAGACCTTCCAGTTGGAGTTCGTTGAA ACCGACAAGTATGCCATCAGGGTATGTGAC CCCAAGAAGAACTCCAGAGATGCTAAGTTC  1044
281 LysLeuLeuValAspGluAspIleGluAsp ThrGluThrPheGlnLeuGluPheValGlu ThrAspLysTyrAlaIleArgValCysAsp ProLysLysAsnSerArgAspAlaLysPhe

    TGGAAGACCGTCGCTGCTGGTATCCAGGCT AACGGCAACTCAAAGGACCAGACGGACTGT CAATTCTCTGTCGAATACAACGGCAACGAC ATGCATGTGCGTGCTCCAGGAGGCAAGTAT  1164
321 TrpLysThrValAlaAlaGlyIleGlnAla AsnGlyAsnSerLysAspGlnThrAspCys GlnPheSerValGluTyrAsnGlyAsnAsp MetHisValArgAlaProGlyGlyLysTyr

    GTTAGTGTCCGTGACAACGGCCATCTCTTC CTTCAGGATTCACCCAAAGACTTCATCTTC CGTCTGCTCAACCGACCCAAGCTGGTGCTC AAGTGCCCTCATGGATTCGTGGGCATGAAG  1284
361 ValSerValArgAspAsnGlyHisLeuPhe LeuGlnAspSerProLysAspPheIlePhe ArgLeuLeuAsnArgProLysLeuValLeu LysCysProHisGlyPheValGlyMetLys

    GAGGGCAAGGCTGAGGTCGCCTGCAACCGA TCAAACTTTGATGTCTTCACTGTCACCTAC AAGGAAGGCGGATACACTATCCAAGACTCC TGTGGCAAGTACTGGTCTTGTGATGACAGT  1404
401 GluGlyLysAlaGluValAlaCysAsnArg SerAsnPheAspValPheThrValThrTyr LysGluGlyGlyTyrThrIleGlnAspSer CysGlyLysTyrTrpSerCysAspAspSer

    AGCCGCATCGTTCTTGGAGAGGCAGCAGGT ACTTTCTTCTTCGAGTTCCATGAGCTCTCC AAGTTTGCTATCCGAGCAGAAAGCAACGGC ATGTTGATCAAGGGCGAGCAGAGTGGCTTG  1524
441 SerArgIleValLeuGlyGluAlaAlaGly ThrPhePhePheGluPheHisGluLeuSer LysPheAlaIleArgAlaGluSerAsnGly MetLeuIleLysGlyGluGlnSerGlyLeu

    TTTACCGCCAATGGTTCCGAGGTCTCAAAG GACACACTGTGGGAATTCTAAACAAATTGG GCTTGAAAGAAGCCAAATCCAAATCAGAAG TAGAGTAGCTGACAAGCCAGCCACTCTATC  1644
481 PheThrAlaAsnGlySerGluValSerLys AspThrLeuTrpGluPhe

    TATTATATCAATTGCAAATATTGTACATTT TTTTAATACAAAAATATTTTCAAAGGTGCA TAATAATTATTTCATACTCTGGTGGGATCT TTAGGATCATATTTTCTCATTGCCTTGGCA  1764
    TACTGACTTCCATTATCCCTCATTTTTTAA AAGGTAAATTGATCACTTAATATCAACTGA AAACGAAATGGAAGTAGGTCTCTGGAAATT TAGAAGAATAGATGACTATCCAGTATATTC  1884
    AAATATTTGTTGAACCTGTCTCAAAAAAAC CCCATAAAAAACCCTCTGTTTTGTGCTGTC TCATAGCCATAAATAGAGATCAATTCTGGT GGTATATGCTACTTAAAATCAGGCTTGAAA  2004
    TAGAATAAATGGAATGGAATGGATTTTCAA AAAGATTTGGAATTTTAAATTTGCAGCCAG TGTCCGTGACAACTCTTGCATACCAGAAGC ACTAAACAGTCTCTGCCGCACCGCTCGCCC  2124
    AGGTGTATTGTTGTGACTGTACTTTTGAAC AATAAAACAGATCTTTTCGTCAAGTTTGGA TATAAAAGTGGATTGAAATGCACGAATAGA TTCGACTTGTATAGGGCATGGTGGACATTG  2244
    ATTTTACAGATACTTTCAATATACCGGTAA AAATCAATCATATAGAAAATGAAAACAGGG TGTAATATCTCAAATAAAAAAAAAAAAAAA                                  2334
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FIG. 1. Nucleotide sequence and translation of *S. purpuratus* fascin. The coding region is arranged in triplets beginning with an ATG codon at nucleotide 84 and ending with a stop codon at nucleotide 1573. Amino acid sequences obtained by peptide sequencing are underlined. A potential polyadenylylation sequence (AATAAA) is similarly marked.

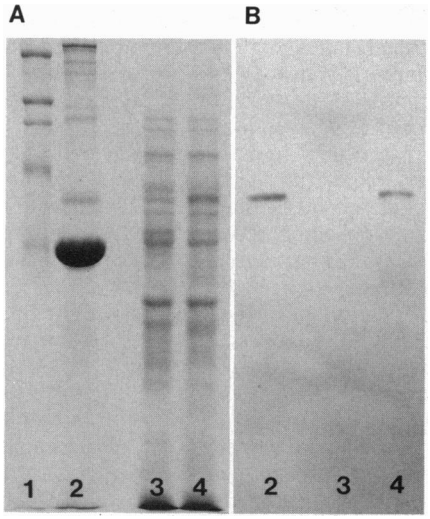

FIG. 2. (*A*) Coomassie blue-stained SDS/polyacrylamide gel showing the isopropyl β-D-thiogalactopyranoside-induced expression of *S. purpuratus* fascin in *E. coli* BL21(DE3) cells transformed with a T7 expression vector, pFAS-T7, containing fascin cDNA. A band migrating at ≈55 kDa is observed in the induced cells. (*B*) A companion immunoblot incubated with polyclonal antibodies produced against fascin isolated from *T. gratilla*. The 55-kDa protein crossreacts with the anti-fascin antibodies. Lanes: 1, molecular size markers (200, 116, 94, 66, and 42 kDa); 2, actin and actin-associated proteins from *S. purpuratus* eggs; 3, uninduced bacteria; 4, induced bacteria.

gratilla with an estimated molecular mass of 55–58 kDa and the ability to bundle F-actin. Peptide sequencing yielded an N-terminal peptide sequence and two blocks of internal sequence. This was used to design and synthesize degenerate oligonucleotides, which were used to screen a cDNA library. The sequence of a positive clone, 2334 bp long, is shown in Fig. 1. The open reading frame encodes a protein of $M_r \approx$ 54,952, in agreement with earlier mass estimates for fascin (2, 3). The calculated isoelectric point is ≈5.5; secondary structure algorithms and dot plots indicate no particular structural features or repeated regions. The estimated α-helix and β-sheet contents are both ≈40%. The estimated extinction coefficient, ϵ^{M}_{280nm}, is 62,390.

Expression of the cDNA in *E. coli* using a T7 promoter generated a 55-kDa protein that reacted with antibodies prepared against egg fascin from *T. gratilla* (Fig. 2). Additional studies have shown that the expressed material is largely insoluble using this expression vector under a variety of different growth conditions and temperatures.

A data base search for genes and proteins having similarities to the fascin sequence produced only two known actin-binding proteins, ABP280 (23) and hisactophilin (24). These similarities are weak and, in the case of ABP280, outside the actin-binding domain. No similarity was found to other known actin-binding proteins or to three known actin-bundling proteins: fimbrin (25), villin (26, 27), and a 30-kDa protein from *Dictyostelium* (28). The search did identify a significant similarity to the singed gene product from *Drosophila* (15) as shown in Fig. 3. The predicted size of the singed gene product, $M_r \approx$ 57,341, is similar to that of sea urchin fascin. The estimated pI values differ, ≈7.0 for singed versus ≈5.5 for fascin. The sequences have ≈35% absolute identity at the amino acid level (174 of 496 residues) with far greater similarity if conservative substitutions are considered.

Peptide sequence information for a third actin-bundling protein shows that this gene family is present in vertebrates. Fumio Matsumura (personal communication) has generously provided peptide sequence information for the 55-kDa actin-bundling protein isolated from HeLa cell extracts (16, 17) (Fig. 3); these sequences show identity with echinoid fascin and the predicted *Drosophila* singed gene product. This protein is localized to microspikes and stress fibers of vertebrate cells in tissue culture (17).

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fascin   1 ---------- -------MPA MNLKYKFGLV NSAGRYLTAE KFGGKVNASG ATLKARQVWI LEQEESS-TI SYLKAPSGNF LSADKNGNVY CSVEDRTEDA   82
singed   1 MNGQGCELGH SNGDIISQNQ QKGWWTIGLI NGQHKYMTAE TFGFKLNANG ASLKKKQLWT LEPSNTGESI IYLRSHLNKY LSVDQFGNVL CESDERDAGT  100

fascin  83 DTGFEIELQP D--GKWALKN VSHQRYLACN GEELICSESS TSNPSANWTV QLAIHPQVCM KNVQHQRYAH LKTSEEGEDS VVVDELVPWG ADSTLTLVYL  180
singed 101 RGRFQISISE DGSGRWALKN ECY--FLGGT PDKLVCT-AK TPGASEFWTV HLAARPQVNL RSIGRKRFAH L--SESQDE- IHVDANIPWG EDTLFTLEFR  194

fascin 181 GK--GKYGLE AFNGKFVQTD GQLAGTANEQ TQFTLIFTSG HLVLRDNNGR HLGVDSGTRV LKSSKPGLTK ANYFILEDSC PQGAFEFG-- GKYASLKQGE  276
singed 195 AEEGGRYALH TCNNKYLNAN GKLQVVCNED CLFSAEYHGG HLALRDRQGQ YLSPIGSKAV LKSRSSSVTR DELFSLEDSL PQASFIAGHN LRYVSVKQGV  294
55 kDa     ---------- ---------- ---------- ---------- ---------- ---------- -------VGK DGLFALEQSS AQ-------- -------QGM

fascin 277 DVSFKLLVDE DIEDTETFQL EFVETDKYAI RVCDPKKNSR DAKFWKTVAA GIQANGNSKD QTDCQFSVEY NGN-DMHVRA PGGKYVSVRD NGHLFLQDSP  375
singed 295 DVTAN---QD EVGENETFQL EY---DWSAH RW--ALRTTQ DRYWCLSAGG GIQATGN-RR CADALFELIW HGDGSLSFRA NNGKFLATKR SGHLFATSES  385
55 kDa     DL

fascin 376 KD----FIFR LLNRPKLVLK CPHGFVGMKE -GKAEVACNR SNFDVFTV-T YKEGGYTIQD SCGKYWSCDD SSRIVLGEAA G-TFFFEFHE LSKFAIRAES  468
singed 386 IEEIAKFYFY LINRPILVLK CEQGFVGYRT PGNLKLECNK ATYETILVER AQKGLVHLKA HSGKYWRIEG ESISVDADAP SDGFFLELRE PTRICIRSQ-  484
55 kDa     --------MK LINRPIIVFR GEHG

fascin 469 NGMLIKGEQS GLFTANGSEV SKDTLWEF  496
singed 485 QGKYLGATKN GAFKLLDDGT DSATQWEF  512
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FIG. 3. Protein sequence alignments of *S. purpuratus* fascin with the protein encoded by the *Drosophila* singed gene (Protein Identification Resource no. S15691) and three peptide fragments from a 55-kDa HeLa cell actin-bundling protein kindly supplied by Fumio Matsumura (Rutgers University). Sequences were aligned by using the PIMA (pattern-induced multiple alignment) program of Smith and Smith (29). Vertical bar indicates identity in all aligned sequences; colon indicates a match between two family members. Numbering is given for both fascin and singed sequences.

DISCUSSION

We have cloned and sequenced a cDNA encoding a 496-amino acid protein with the expected molecular mass and properties of sea urchin fascin. The predicted sequence agrees with three peptide fragments obtained by peptide sequencing and the protein expressed in *E. coli* crossreacts with anti-fascin antibodies. To date, the insolubility of the expressed protein has prevented a direct functional test of actin-bundling activity. The fascin sequence is unrelated to two major families of actin-bundling proteins that include (*i*) fimbrin, α-actinin, and the 30-kDa protein from *Dictyostelium* and (*ii*) the family that includes ABP50/EF-1a from *Dictyostelium* (18). This plus the observation that echinoid fascin, the *Drosophila* singed gene product, and a 55-kDa HeLa cell protein that bundles F-actin share similar peptide sequences strongly suggests that these proteins are members of a unique family of actin-crosslinking proteins. Recent work on the sequence of mouse fascin confirms the homology with the mammalian 55-kDa protein and indicates an overall identity of ≈35% between echinoid and mouse fascins. We propose that the family name be "fascin" because all the proteins, including the *Drosophila* singed gene product, as outlined below, tightly bundle actin into linear arrays or fascicles.

The singed (*sn*) gene of *Drosophila melanogaster* has been shown to have a role in the development of both somatic and germ cells. The *sn* gene (1-21.0) is located on the X chromosome at band 7D1-2 (30) and has been isolated by chromosomal jumping and characterized extensively (15). The most extreme alleles are female sterile with short, deformed, and gnarled bristles (both macrochaetae and microchaetae); less extreme forms are fertile with wavy bristles (31). Several alleles, including sn^3, are known that have an extreme bristle phenotype but are fertile; these are believed to be transcriptional regulatory mutants (15). Relatively little work has been published on bristle ultrastructure. Bristle development involves four cells: a trichogen, which elongates the bristle; a tormogen, which forms the socket for the bristle; a sensory cell; and a neurilemma cell (32). An early electron microscopic examination of developing bristles shows a bundle of microtubules in the trichogen in the center of the bristle shaft (33). In addition, there are multiple (>6) fiber bundles, immediately under the plasma membrane, arranged around the microtubules and extending the length of the bristle shaft. These fiber bundles are roughly circular and although the micrographs were taken and published before the realization that actin filaments were widely distributed, their morphology is consistent with them being bundles of actin filaments. In addition, we have used rhodamine phalloidin to verify that the fiber bundles contain filamentous actin (J.W. and J.O., unpublished data). These bundles occupy ≈20% of the cross-sectional area of a bristle shaft. The available micrographs are not of the highest resolution, but a periodic striping of ≈11 nm is discernible in the bristle fiber bundles (see inset in figure 13 in ref. 33). This periodicity is the same as that seen in the actin bundles in sea urchin egg microvilli (7) and coelomocyte filopodia (12). In the bristles of sn^3 flies, the fiber bundles are highly disorganized and reduced to twisted bands of filaments. Overton (33) has estimated that these disorganized bundles occupy ≈5% of the bristle cross-sectional area. The results indicate that the *sn* gene product is the insect homologue of echinoderm fascin and suggest that it functions to organize actin filaments in the developing bristle, presumably to serve a structural role during cuticle deposition. The absence of insect fascin, or its mutation, results in the failure to form fiber bundles.

The role of insect fascin in *Drosophila* oocyte development is unclear. To our knowledge, there are no microvilli present in *Drosophila* oocytes. Fibrous bundles, sometimes referred to as composed of microfilaments and/or microtubules, have been described but their function is uncertain (see, for example, figure 16 in ref. 34). Recent work on the chickadee gene, which encodes profilin, a low molecular weight actin-binding protein, suggests a role for the *sn* gene product in nurse cell function. Chickadee mutant cell egg chambers exhibit incomplete transfer of nurse cell cytoplasm to the developing oocyte and the nurse cells lack the actin filaments that form a cytoplasmic network (36). A similar phenotype, failure of cytoplasm transfer, has been described for the singed mutant sn^{36a} by Gutzeit and Straub (37), suggesting that fascin is involved in the organization of this network. This suggestion is reinforced by the development of microfilament bundles, radiating from nurse cell plasma membranes and terminating at or near the nuclear envelope (38), that could function to hold the nucleus in place while the surrounding cytoplasm is transferred to the oocyte. Fascin might function to stabilize these bundles or participate in the formation of the ring canals between nurse cells and the oocyte as recently described for the kelch gene product (35). Fibrous elements (20-nm rods) have been described in ring canals, but their origin and composition are unknown (discussed in ref. 34).

The sequence data and functional evidence strongly support the idea that the 55-kDa protein described by Yamashiro-Matsumura and Matsumura (16) is the mammalian homologue of fascin. This conclusion is further supported by the recent cloning of both human and mouse fascins. The physical characteristics of this protein are similar to fascin and the singed gene product, but our efforts to demonstrate antigenic crossreactivity have been unsuccessful. We note also that the bundles generated by the HeLa cell protein show neither the degree of order nor the characteristic striped periodicity of actin–fascin bundles indicating the evolutionary divergence of the family. The function of fascin in mammalian cells is poorly understood, but the functional studies in sea urchins suggest that it may play a role in the organization of a class of actin bundles and microvilli or cellular projections that differ from those defined by villin and fimbrin.

The authors dedicate this paper to the memory of Robert E. Kane (1931–1993), who discovered and characterized sea urchin fascin. Technical support from Gabriela Gonzalez, Haydee Herrera-Sosa, and Robyn Lee is gratefully acknowledged. We thank Dr. Costas Flytzanis for providing the cDNA library. This work was supported by National Institutes of Health Grants GM26093 to J.B. and CA44704 to P.M. and by National Science Foundation Grant MCB-9012165 to J.O.

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