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cDNA Cloning and Expression of the Human Homolog of the Sea Urchin *fascin* and *Drosophila singed* Genes Which Encodes an Actin-Bundling Protein

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ABSTRACT

cDNA clones having extensive sequence identity with the sea urchin *fascin* and the *Drosophila singed* gene products were isolated from a human teratocarcinoma cDNA library. The human homolog, termed *hsn*, is a single-copy gene that was localized to human chromosome 7p22 by fluorescence *in situ* hybridization and is predicted to encode a 493-amino-acid product with a molecular mass of approximately 55,000. This protein would be similar in size to the fascin and singed proteins, as well as a previously described 55-kD actin-bundling protein that was purified from HeLa cells. Monoclonal antibodies directed against the 55-kD HeLa protein were reactive against a bacterially expressed hsn fusion protein, indicating that the *hsn* gene probably encodes the 55-kD protein. The *hsn* mRNA was variably expressed in all human tissues analyzed and was highly expressed in actively growing renal carcinoma cell lines and in activated, but not in resting, lymphocytes, suggesting a functional role for hsn in proliferation. The fascin family lacks homology with other characterized actin-binding proteins, and the high degree of evolutionary conservation of these proteins indicates a functional importance of their actin-bundling properties.

INTRODUCTION

ACTIN CROSS-LINKING PROTEINS function to regulate the assembly of actin filaments into bundles or networks. Such microfilament networks define cell shape, support organelles and the translational machinery, are involved in cytokinesis, and provide attachment sites for cell-cell contact and cell-extracellular matrix interactions. Two groups of actin cross-linking proteins that have been described are actin bundling proteins and gelation proteins (Matsudaira, 1991). Fascin was one of the first actin-bundling proteins extensively characterized and can cross-link actin filaments *in vitro* (Bryan and Kane, 1982). Recently, the cDNA for sea urchin *fascin* was identified (Bryan *et al.*, 1993) and shown to be homologous to the *Drosophila singed* (*sn*) gene (Patterson and O'Hare, 1991). In addition, the deduced fascin and sn protein sequences showed homology to peptide sequences obtained from a HeLa cell-derived, 55-kD protein (Bryan *et al.*, 1993) that is also an actin bundling protein (Yamashiro-Matsumura and Matsumura, 1985, 1986). However, similarity to other characterized actin-binding proteins was not observed.

We report here the identification of the human homolog of the sea urchin *fascin* and *Drosophila sn* genes. The human homolog, termed *hsn*, was ubiquitously expressed in human tissues and its pattern of expression in resting and phytohemagglutinin (PHA)-activated lymphocytes indicated that *hsn* expression may be regulated through the cell cycle.

MATERIALS AND METHODS

cDNA cloning

A cDNA library prepared from the Ntera2D1 human teratocarcinoma cell line was provided by Dr. M. Singer

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(Carnegie Institute of Washington). The λ gt11 cDNA library was initially screened by plaque hybridization of 10^6 phage clones using a radiolabeled DNA fragment from a chromosome 3 cosmid contig (Latif *et al.*, 1993a). Subsequent plaque hybridizations were performed using radiolabeled partial cDNA clones that were identified in the initial library screening. Sequencing primers were synthesized on a model 392 DNA synthesizer (Applied Biosystems) and the DNA sequence of inserts was obtained from both strands using a model 373A automated DNA sequencer (Applied Biosystems) using dye terminators. Sequence analysis was performed using the University of Wisconsin GCG package, version 7.1 and the DNADraw program (M. Shapiro, DCRT, NIH). Database searches using FASTA and TFASTA were performed with Genbank release 80.0.

Southern hybridizations

Evolutionary conservation blots containing *Eco* RI- and *Hind* III-digested DNA samples from various species were purchased from BIOS. Eight micrograms of human DNA and proportional equivalents of nonhuman DNA were loaded per lane. The *hsn* probe used was a 1.7-kb *Sma* I fragment containing the entire open reading frame. Hybridizations were performed at 55°C in 10% dextran sulfate, 6× SSC, 1% $NaDodSO_4$, 5× Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA. Blots were washed in 2× SSC containing 0.1% $NaDodSO_4$ at room temperature followed by two 20-min washes in 0.1× SSC containing 0.1% $NaDodSO_4$ at 50°C.

mRNA expression

Poly(A)$^+$mRNA from human tissues was purchased from Clontech. Total RNA was prepared according to the method of Chomczynski and Sacchi (1987). RNA was electrophoresed on 1% agarose/0.22 *M* formaldehyde gels, and blotted to nylon membranes (Magnagraph, MSI, Inc). Northern blots were probed with a 1.7-kb *Sma* I fragment containing the entire *hsn* open reading frame. The IL-2 Receptor α chain probe used was obtained from Dr. Warren J. Leonard (NHLBI, NIH). Hybridizations were performed at 42°C in 50% formamide, 10% dextran sulfate, 6× SSC, 1% $NaDodSO_4$, and 100 μg/ml denatured salmon sperm DNA. Blots were washed in 2× SSC containing 0.1% $NaDodSO_4$ at room temperature followed by two 20-min washes in 0.1× SSC containing 0.1% $NaDodSO_4$ at 60°C. Peripheral blood mononuclear cells (PBMC) from normal volunteer donors were isolated on lymphocyte separation medium (LSM, Organon Teknika). After isolation, PBMC were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, glutamine, antibiotics, and 10 μg/ml phytohemagglutin (PHA) (Wellcome Diagnostics) for 72–96 hr. Cells were harvested by centrifugation and washed one time in cold phosphate-buffered saline (PBS) prior to RNA extraction.

Bacterial expression of hsn

A 1.7-kb *Sma* I fragment containing the *hsn* open reading frame and stop codon was subcloned into the *Sma* I site of pGEX-2T (Pharmacia). This resulted in an ~80-kD glutathione-*S*-transferase (GST)-hsn fusion protein whose expression was inducible by isopropyl β-D-thiogalactopyranoside (IPTG). Ligated plasmids were transformed into DH5α (BRL) and orientation was confirmed using *Pst* I sites within the *hsn* insert and the vector. Appropriate plasmids were then transformed into the *Escherichia coli* strains JR600 (a C600 derivative) and BL21(DE3). Similar results were seen in either bacterial strain and multiple bacterial clones containing *hsn* inserts in both orientations were examined with expected results. Bacteria were grown in LB plus 100 μg/ml ampicillin overnight at 30°C with shaking, diluted 1:10 in 2× Tryptone plus ampicillin, grown for an additional hour, and then induced for 3 hr with IPTG (final concentration of 1 m*M*) at 30°C with shaking. A 1.5-ml culture was harvested by centrifugation and lysed with three freeze–thaw cycles in 50 μl of PBS containing 1% TX-100 and protease inhibitors. After lysis, the extracts were spun for 10 min at 14,000 rpm in a microfuge. The supernatant constituted the soluble fraction, and the pellet which was extracted in 50 μl of 2× protein gel sample buffer (Laemmli, 1970) constituted the insoluble fraction. Proteins from both soluble and insoluble fractions were electrophoresed on 8% $NaDodSO_4$-polyacrylamide gels (Laemmli, 1970) and either stained with Coomassie blue or transferred to nitrocellulose membranes (Schleicher & Schuell) for Western blotting analysis (Towbin *et al.*, 1979). Samples analyzed by Western blotting were loaded at a 1:10,000 dilution of the material needed to visualize the fusion protein by Coomassie blue staining. The primary antibodies, raised against a 55-kD protein purified from HeLa cells (Yamashiro-Matsumura and Matsumura, 1985, 1986), were used at a 1:1,000 dilution. The secondary antibody was a goat anti-mouse IgG horseradish peroxidase conjugate (Bio-Rad Laboratories) used at a 1:3,000 dilution. Western blots were detected using chemiluminescence (ECL, Amersham).

RESULTS

An approximate 1-megabase contig was generated in the region of chromosome 3p25 and cDNA clones corresponding to at least seven different genes were cloned (Latif *et al.*, 1993a,b; Kuzmin *et al.*, 1994). A 2.2-kb cDNA corresponding to one of these genes, termed group 6 (Latif *et al.*, 1993b), was identified from a human teratocarcinoma library. However, sequence analysis indicated that the clone was chimeric: poly(A) sequences were identified at both ends of the insert, implying that two cDNA clones were ligated in a head-to-head orientation. One fragment from the clone was clearly from chromosome 3p and corresponded to the group 6 gene (Latif *et al.*, 1993b). The other fragment was used to isolate a cosmid that was localized to human chromosome 7p22 by fluorescence *in situ* hybridization (data not shown). The two fragments were subsequently used to screen additional cDNA libraries, and two independent groups of cDNA clones were identified.

The DNA sequence of the 2,777-nucleotide cDNA clone from human chromosome 7p22 is shown in Fig. 1. The clone contains a 3′ poly(A) sequence and an open reading frame of 493 amino acids that would encode a protein of 54,529 M_r with a predicted isoelectric point of 7.2. The methionine designated as the translation start site is the first AUG in the transcript and has a near-consensus translation initiation sequence (Kozak, 1989). When the sequence was compared with the GenBank/EMBL database the only significant homologies indicated were with the *Drosophila melanogaster singed (sn)* cDNA and the *Strongylocentrotus purpuratus* (sea urchin) *fascin* cDNA. These homologies were at both the nucleotide as well as the protein levels and extended throughout the entire length of the open reading frame (Fig. 2). The human-derived protein, termed *hsn*, showed a 63% similarity and 41% identity to the sn protein and a 60% similarity and 38% identity to the fascin protein (data not shown). These results indicate that *hsn* is the human homolog of the *fascin* and *sn* genes.

Our data from Southern blotting analyses and fluorescence *in situ* hybridization indicate that *hsn* should represent a single-copy gene. The evolutionary relatedness of *hsn* was analyzed, and *hsn* was found to be highly conserved across species, with cross-hybridization at least down to *Caenorhabditis elegans* (Fig. 3). Interestingly, cross-hybridization was not noted in yeast whereas a faint signal was seen in *E. coli*. This result was seen in both *Eco* RI- and *Hind* III-digested DNA samples; however, because the hybridizing band was about 20 kb with both restriction enzymes, the significance of this result is unclear and we cannot immediately rule out the possibility that the *E. coli* hybridization seen was an artifact of nonspecific hybridization.

The *hsn* mRNA was expressed at various levels in all human tissues examined (Fig. 4 and data not shown), with reduced levels in fetal kidney as compared to adult kidney. The *hsn* transcript was additionally expressed in human placenta and lung, and at somewhat lower levels in heart, liver, and skeletal muscle. The *hsn* transcript was also expressed at high levels in all human renal and prostate carcinoma cell lines examined (data not shown). Resting PBMC did not express the *hsn* transcript, as examined by Northern blotting. However, expression was seen within 5–14 hr of PHA stimulation, with maximal expression within 14–24 hr (Fig. 5). In multiple experiments, expression of the IL-2Rα transcript, a gene transcriptionally regulated in lymphocytes (Leonard *et al.*, 1985), preceded *hsn* expression. The *hsn* transcript diminished such that after 72–96 hr of stimulation levels were slightly greater than resting PBMC (Fig. 5 and data not shown). In analogous experiments *hsn* mRNA expression was low in serum-starved normal lung fibroblast or NIH-3T3 cell cultures, but increased after serum stimulation (data not shown). Similarly, confluent renal carcinoma cell cultures showed decreased *hsn* mRNA expression relative to subconfluent cultures (data not shown). Therefore, *hsn* mRNA expression was highest in actively proliferating cells.

It was previously reported that the sea urchin fascin protein was homologous to a 55-kD protein purified from

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gcggagggtgcgtgcgggccgcggcagccgaacaaaggagcaggggcgccgccgcaggga   -51
cccgccacccacctcccggggccgcgcagcggcctctcgtctactgccaccATGACCGCC    -9
                                                      M T A      3

AACGGCACAGCCGAGGCGGTGCAGATCCAGTTCGGCCTCATCAACTGCGGCAACAAGTAC     69
N G T A E A V Q I Q F G L I N C G N K Y                    23

CTGACGGCCGAGGCGTTCGGGTTCAAGGTGAACGCGTCCGCCAGCAGCCTGAAGAAGAAG    129
L T A E A F G F K V N A S A S S L K K K                    43

CAGATCTGGACGCTGGAGCAGCCCCCTGACGAGGCGGGCAGCGCGGCCGTGTGCCTGCGC    189
Q I W T L E Q P P D E A G S A A V C L R                    63

AGCCACCTGGGCCGCTACCTGGCGGCGGACAAGGACGGCAACGTGACCTGCGAGCGCGAG    249
S H L G R Y L A A D K D G N V T C E R E                    83

GTGCCCGGTCCCGACTGCCGTTTCCTCATCGTGGCGCACGACGACGGTCGCTGGTCGCTG    309
V P G P D C R F L I V A H D D G R W S L                   103

CAGTCCGAGGCGCACCGGCGCTACTTCGGCGGCACCGAGGACCGCCTGTCCTGCTTCGCG    369
Q S E A H R R Y F G G T E D R L S C F A                   123

CAGACGGTGTCCCCCGCCGAGAAGTGGAGCGTGCACATCGCCATGCACCCTCAGGTCAAC    429
Q T V S P A E K W S V H I A M H P Q V N                   143

ATCTACAGTGTCACCCGTAAGCGCTACGCGCACCTGAGCGCGCGGCCGGCCGACGAGATC    489
I Y S V T R K R Y A H L S A R P A D E I                   163

GCCGTGGACCGCGACGTGCCCTGGGGCGTCGACTCGCTCATCACCCTCGCCTTCCAGGAC    549
A V D R D V P W G V D S L I T L A F Q D                   183

CAGCGCTACAGCGTGCAGACCGCCGACCACCGCTTCCTGCGCCACGACGGGCGCCTGGTG    609
Q R Y S V Q T A D H R F L R H D G R L V                   203

GCGCGCCCCGAGCCGGCCACTGGCTACACGCTGGAGTTCCGCTCCGGCAAGGTGGCCTTC    669
A R P E P A T G Y T L E F R S G K V A F                   223

CGCGACTGCGAGGGCCGTTACCTGGCGCCGTCGGGGCCCAGCGGCACGCTCAAGGCGGGC    729
R D C E G R Y L A P S G P S G T L K A G                   243

AAGGCCACCAAGGTGGGCAAGGACGAGCTCTTTGCTCTGGAGCAGAGCTGCGCCCAGGTC    789
K A T K V G K D E L F A L E Q S C A Q V                   263

GTGCTGCAGGCGGCCAACGAGAGGAACGTGTCCACGCGCCAGGGTATGGACCTGTCTGCC    849
V L Q A A N E R N V S T R Q G M D L S A                   283

AATCAGGACGAGGAGACCGACCAGGAGACCTTCCAGCTGGAGATCGACCGCGACACCAAA    909
N Q D E E T D Q E T F Q L E I D R D T K                   303

AAGTGTGCCTTCCGTACCCACACGGGCAAGTACTGGACGCTGACGGCCACCGGGGGCGTG    969
K C A F R T H T G K Y W T L T A T G G V                   323

CAGTCCACCGCCTCCAGCAAGAATGCCAGCTGCTACTTTGACATCGAGTGGCGTGACCGG   1029
Q S T A S S K N A S C Y F D I E W R D R                   342

CGCATCACACTGAGGGCGTCCAATGGCAAGTTTGTGACCTCCAAGAAGAATGGGCAGCTG   1089
R I T L R A S N G K F V T S K K N G Q L                   363

GCCGCCTCGGTGGAGACAGCAGGGGACTCAGAGCTCTTCCTCATGAAGCTCATCAACCGC   1149
A A S V E T A G D S E L F L M K L I N R                   383

CCCATCATCGTGTTCCGCGGGGAGCATGGCTTCATCGGCTGCCGCAAGGTCACGGGCACC   1209
P I I V F R G E H G F I G C R K V T G T                   403

CTGGACGCCAACCGCTCCAGCTATGACGTCTTCCAGCTGGAGTTCAACGATGGCGCCTAC   1269
L D A N R S S Y D V F Q L E F N D G A Y                   423

AACATCAAAGACTCCACAGGCAAATACTGGACGGTGGGCAGTGACTCCGCGGTCACCAGC   1329
N I K D S T G K Y W T V G S D S A V T S                   443

AGCGGCGACACTCCTGTGGACTTCTTCTTCGAGTTCTGCGACTATAACAAGGTGGCCATC   1389
S G D T P V D F F F E F C D Y N K V A I                   463

AAGGTGGGCGGGCGCTACCTGAAGGGCGACCACGCAGGCGTCCTGAAGGCCTCGGCGGAA   1449
K V G G R Y L K G D H A G V L K A S A E                   483

ACCGTGGACCCCGCCTCGCTCTGGGAGTACtagggccggcccgtccttccccgccctgc    1509
T V D P A S L W E Y *                                     493

ccacatggcggctcctgccaacctccctgctaacccctttctccgccaggtgggctccag    1569
ggcgggaggcaagccccttgcctttcaaactggaaaccccagagaaaacggtgccccca    1629
cctgtcgccccatatggactccccactctcccctccgcccgggttccctactccctcggg    1689
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ctgtcagtggccctccctggtgcactgtccccgaaaccccctgcttgggaagggaagctgt    1989
cgggagggctaggactgacccttgtggtgttttttttgggtggtggctggaaacagccccct  2049
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acagggtctgcccgctgcacgttctgccaaggtggtggtggcgggcgggtagggtgtgg     2169
gggccgtcttcctcctgtctctttccttcacccctagcctgactggaagcagaaaatgac    2229
caaatcagtatttttttttaatgaaatattattgctggaggcgtcccaggcaagcctggct    2289
gtagtagcgagtgatctggcgggggggcgtctcagcaccctccccaggggtgcatctcag    2349
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tccccaacatgcatctcactctgggtgtcttggtcttttattttttgtaagtgtcatttg    2589
tataactctaaacgcccatgatagtagcttcaaactggaaatagcgaaataaaataactc    2649
agtctgcaaaaaaaaaa                                               2656
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FIG. 1. Nucleotide and deduced amino acid sequence of the *hsn* cDNA (GenBank/EMBL accession number U03057). The deduced amino acid sequence is shown below the nucleotide sequence using the single-letter code. The first AUG of the cDNA is numbered +1 for the sequence. The polyadenylation signal in the 3′ untranslated region is underlined.

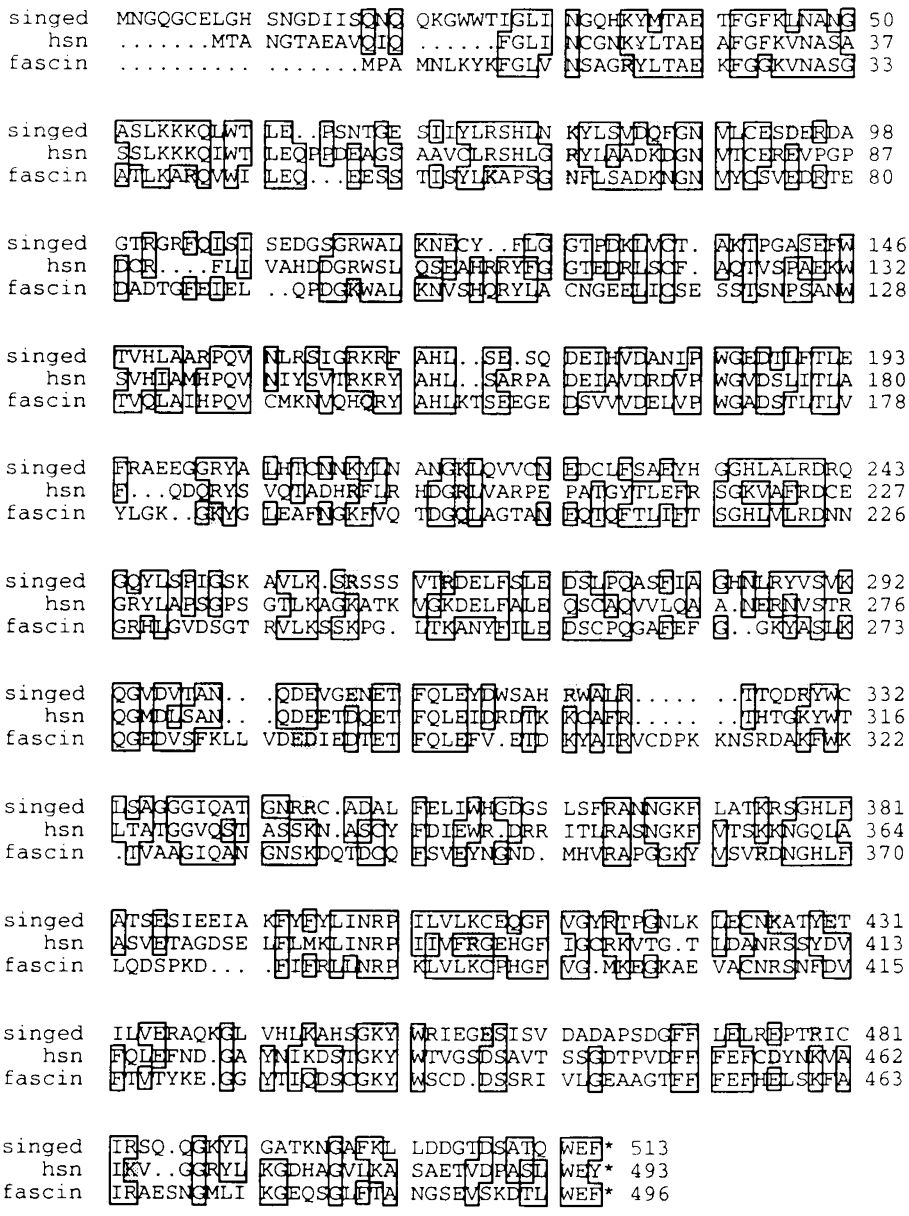

FIG. 2. Comparison of the deduced amino acid sequences of human *hsn*, sea urchin *fascin*, and *Drosophila singed*. Alignment was performed with the Pileup program from the GCG software package. Dots indicate gaps made in the sequences for alignment. Amino acids in at least two of the sequences are boxed, and amino acid substitutions that conserve charge are shaded.

HeLa cells (Bryan *et al.*, 1993). This homology was based on the similarity of the fascin amino acid sequence with peptides obtained by microsequencing of the 55-kD protein and cross-reactivity of fascin with anti-55-kD antibodies. We note that one of the 55-kD protein-derived peptide sequences presented by Bryan *et al.* (1993) contained two differences from the deduced hsn protein sequence: VGKDGLFALEQSSAQ for the 55-kD protein and VGKDELFALEQSCAQ for hsn. The first amino acid difference (G *vs.* E) was found to be an error in the peptide sequencing and has been corrected (F. Matsumura, unpublished data). The second difference may also be due to an error in peptide sequencing. To demonstrate that *hsn* encodes the 55-kD protein, the *hsn* cDNA was subcloned into a bacterial expression vector, and the 80-kD recombinant fusion protein was tested for reactivity with monoclonal antibodies made against the 55-kD protein (Yamashiro-Matsumura and Matsumura, 1985, 1986). The hsn fusion protein was identified in the insoluble bacterial fraction after IPTG induction (Fig. 6) but not in the soluble bacterial fraction

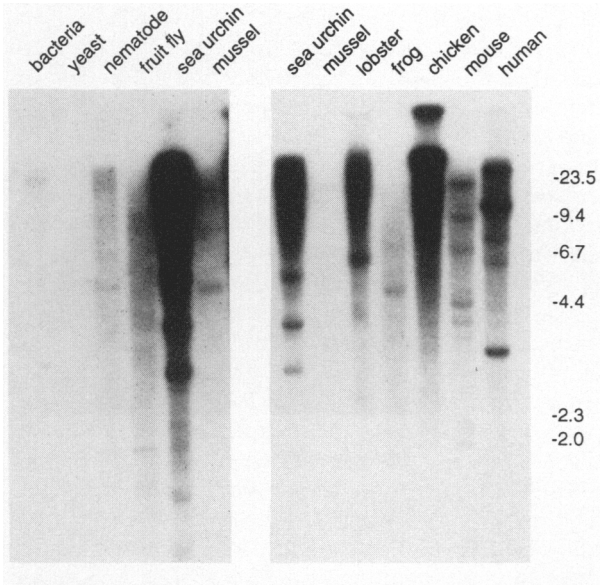

FIG. 3. The *hsn* gene is highly conserved across species. Evolutionary relatedness blots containing *Eco* RI-digested DNA from the indicated species were hybridized with a 1.7-kb *Sma* I fragment containing the *hsn* open reading frame. The right side is an overnight exposure, whereas the left side shows the same blot after a 4-day exposure. Molecular size standards indicated at the right were from a λ DNA *Hin*d III digest.

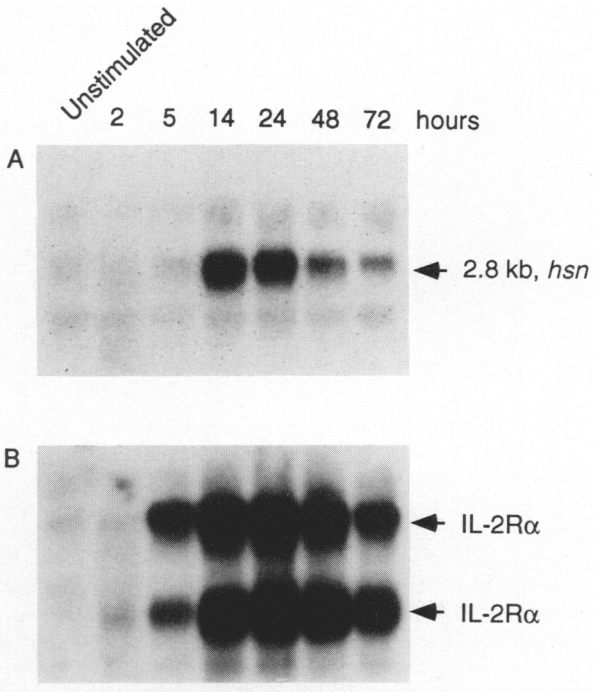

FIG. 5. Expression of the *hsn* gene in PHA-activated lymphocytes. Peripheral blood mononuclear cells were stimulated with PHA, and RNA was extracted at the indicated times. Ten micrograms of total RNA was loaded per lane and the blot was hybridized with a 1.7-kb *Sma* I fragment containing the *hsn* open reading frame. The blot was then stripped and hybridized with an IL-2Rα probe.

(data not shown). In addition, the hsn fusion protein showed immuno-cross-reactivity with two independent anti-55-kD monoclonal antibody (Fig. 6 and data not shown). Therefore, the *hsn* gene likely encodes the previously characterized HeLa protein.

DISCUSSION

The work presented here describes the identification and characterization of *hsn*, a gene encoding a human actin-bundling protein with strong homology to the sea urchin fascin and *Drosophila sn* gene products. On the basis of peptide sequence identity and immuno-cross-reactivity *hsn* likely encodes the 55-kD actin-bundling protein previously characterized in HeLa cells (Yamashiro-Matsumura and Matsumura, 1985, 1986). Because of the homologies between the sea urchin and human products, hsn and the 55-kD protein should be referred to as human fascin. The *hsn* transcript was highly expressed in actively proliferating cells and was induced in lymphocytes after mitogenic activation. Maximal *hsn* expression was seen between 14 and 24 hr after PHA-stimulation of lymphocytes. Previous work using PHA-stimulated PBMC showed that DNA synthesis initiated between 48 and 72 hr after stimulation (Cantrell and Smith, 1983; Robb, 1984). Therefore, maximal *hsn* expression in the work presented here probably occurred prior to entry into S phase. These results indicate that *hsn* expression may be cell cycle regulated, and that the hsn protein may function as an actin-bundling protein during cell division. The *hsn*, fascin, and *sn* genes have no homology to other described actin-binding proteins and, therefore, the fascin family apparently defines a structurally distinct actin-bundling protein. There are no obvious functional domains or motifs in the deduced sequences that would indicate sequences important for actin interactions.

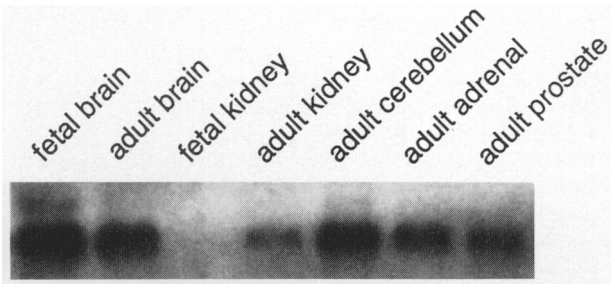

FIG. 4. The *hsn* transcript is expressed in most human tissues. Two micrograms of poly(A)⁺RNA was loaded per lane and hybridized weith a 1.7-kb *Sma* I fragment containing the *hsn* open reading frame. An overnight exposure is shown. A signal was seen in the fetal kidney RNA lane after 4 days.

The HeLa cell 55-kD protein was extensively characterized. Increased amounts of the 55-kD protein were found in retrovirally transformed cells, and the protein localizes to both stress fibers and microspikes (Yamashiro-Matsu-

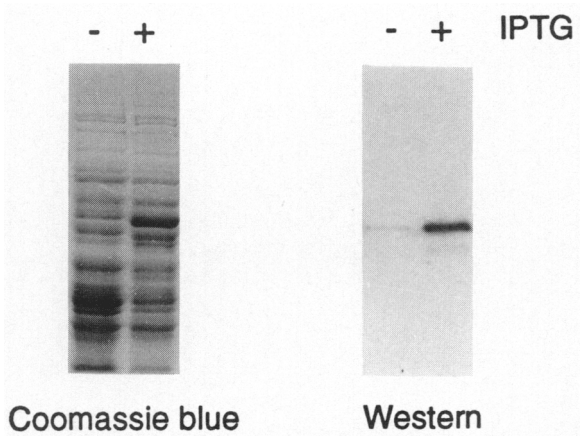

FIG. 6. Bacterial expression of the *hsn* gene product as a fusion protein and reactivity with anti-55-kD monoclonal antibodies. Bacterial cultures were induced with IPTG (+) or not induced (−) and cells were lysed in PBS + 1% TX-100. Insoluble bacterial fractions were run on $NaDodSO_4$-polyacrylamide gels and either stained with Coomassie blue or analyzed by Western immunoblotting using the 55K-2 monoclonal antibody (Yamashiro-Matsumura and Matsumura, 1985, 1986). Similar results were seen with the 55K-14 monoclonal antibody (Yamashiro-Matsumura and Matsumura, 1985, 1986) (data not shown). The ~80-kD IPTG-induced band corresponds to the expected size of the ~55-kD hsn peptide fused to the ~25-kD GST peptide.

mura and Matsumura, 1985, 1986). Increased protein levels seen after transformation are consistent with the mRNA expression studies presented here. The actin-bundling activity in the microspikes was not regulated by divalent cations, unlike another actin-bundling protein, fimbrin (Yamashiro-Matsumura and Matsumura, 1985). Some actin-binding proteins may have "dual" functions with actin-binding as part of the protein's function. The actin-bundling protein, ABP-50, is unrelated to other actin-binding proteins and was shown to be the same as elongation factor 1a (Yang *et al.*, 1990). Another actin-binding protein, profilin, may play a role in signal transduction through interaction with membrane phospholipid (Lassing and Lindberg, 1985) and regulation of phospholipase C-γ1 activity (Goldschmidt-Clermont *et al.*, 1991). Therefore, actin-binding proteins may serve to tether biosynthetic processes physically, such as protein synthesis or molecules involved in signal transduction to the cytoskeleton. The localization of fascin to microspikes may put it in a position to participate in such signal transduction processes.

Understanding of the function of the fascin actin-bundling proteins may be obtained in part from studies on naturally occurring *Drosophila sn* mutants. Many *sn* mutants exhibit two phenotypes: gnarled bristle development and female sterility. However, it was clear from the cloning of the *sn* locus that these two seemingly disparate phenotypes are due to mutations in the same gene (Patterson and O'Hare, 1991). The common feature shared between these two phenotypes involves the association of the sn protein with actin fibers. The hair bristle is made up of four cells. The bristle shaft develops as a cytoplasmic extension of one of these cells, the trichogen (Poodry, 1980). Microtubles run longitudinally along the length of the trichogen shaft (Poodry, 1980) and actin-containing fiber bundles lying just inside the plasma membrane are oriented in the same longitudinal fashion (Bryan *et al.*, 1993). This longitudinal array gives the bristle its shape, and in *sn* mutants a decreased number of microfibril bundles are noted by electron microscopy; as a result, the bristle shaft is short and curved (Poodry, 1980). Similarly, in oogenesis the developing oocyte is surrounded by 15 nurse cells through intercellular bridges (Mahowald and Kambysellis, 1980). The nurse cell cytoplasmic contents flow into the oocyte along actin filaments (Mahowald and Kambysellis, 1980), and at least one *sn* allele associated with female sterility has been implicated in affecting the microfilament structure required for nurse cell cytoplasmic flow (Gutzeit and Straub, 1989). These effects on actin bundles are presumably due to an inability of mutant sn proteins to effectively form cross-linked fibers. Future studies incorporating naturally occurring *sn* mutations and established fascin biochemical properties may help shed light on the function of these proteins.

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