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Advillin (p92): a new member of the gelsolin/villin family of actin regulatory proteins

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SUMMARY

A new member of the gelsolin/villin family of actin regulatory proteins was initially identified by screening an adult murine brain cDNA library with a probe for bovine adseverin. The predicted amino acid sequence of the 92 kDa murine protein p92 (advillin) is 75% homologous to villin and 65% homologous to gelsolin and adseverin. It shares a six domain structure with other gelsolin family members and has a carboxy-terminal headpiece, similar to, yet distinct from, villin. Northern blot analysis shows a high level of mRNA expression in murine uterus and human intestine. In situ mRNA analysis of adult murine tissues demonstrates that the message is most highly expressed in the endometrium of the uterus, the intestinal lining, and at the surface of the tongue. In murine embryonic development, strong expression of the message is observed by day 14.5 in dorsal root ganglia and trigeminal ganglia. Expression is also noted at day 16.5 in cerebral cortex. We propose that p92 (advillin) has unique functions in the morphogenesis of neuronal cells which form ganglia, and that it may compensate to explain the near normal phenotype observed in villin-deficient mice.

Key words: Cytoskeleton, Actin, Gelsolin, Villin, Advillin, Ganglion

INTRODUCTION

Cell motility is an integral part of numerous physiologic and pathologic processes including development, wound healing, and immune defense. The crawling movement of most mammalian cell types is based upon dynamic changes in the actin cytoskeleton (Stossel, 1993). In order to understand this process, it is necessary to define the proteins and other molecules involved in the regulation of actin polymerization. Gelsolin is a multifunctional actin-binding protein with important effects on actin dynamics, as defined by analysis of tissues and cells from gelsolin-null mice (Azuma et al., 1998; Furukawa et al., 1997; Witke et al., 1995; Yin and Stossel, 1979). Several other proteins from diverse species have been identified which share similarity either in whole or in part with gelsolin, delineating a family of related molecules (Schafer and Cooper, 1995). Members of the family are capable of capping and severing actin filaments in a regulated fashion.

Gelsolin itself consists of six homologous structural domains, as defined by analysis of its primary sequence and crystal structure (Kwiatkowski et al., 1986; Burtnick et al., 1997). The actin-binding activity of this protein is regulated by calcium, pH, and polyphosphoinositides (Yin and Stossel, 1979; Janmey and Stossel, 1987). Gelsolin is expressed in most mammalian tissues, whereas adseverin (also known as scinderin), a family member with similar overall structure, has a more limited tissue distribution. It is found mainly in neural and endocrine tissues (Sakurai et al., 1990), and its function has been studied most extensively in adrenal chromaffin cells. In these cells, there is evidence that adseverin is involved in exocytosis (Zhang et al., 1996); however, the involvement of this protein in other processes is also possible, given its activity and tissue distribution in other mammalian cell types (Tchakarov et al., 1990).

Villin, which shares the six domain structure with gelsolin and adseverin, also contains a carboxy-terminal headpiece domain (Arpin et al., 1988; Bazari et al., 1988; Bretscher and Weber, 1980; Hesterberg and Weber, 1986; Matsudaira et al., 1985). This domain confers actin filament bundling activity to the protein (Arpin et al., 1988). Villin is expressed mainly in differentiated epithelial tissues with a brush border: intestinal villi, proximal renal tubule, oviduct, and seminiferous ducts (Ezzell et al., 1989; Gröne et al., 1986; Horvat et al., 1990). Despite villin's postulated importance in the morphogenesis of microvilli (Franck et al., 1990; Friedrich et al., 1989), targeted disruption of the villin gene produces minimal changes in the structure of microvilli, suggesting that functional redundancy may exist (Pinson et al., 1998). In this regard, several more recently identified proteins contain a domain with similarity to villin headpiece which is likely to provide actin-binding activity. These include the two subunits of dematin (Rana et al., 1993), abLIM (Roof et al., 1997), supervillin (p205) (Pestonjamasp et al., 1997), and a putative villin homologue identified by DNA sequencing along the region of chromosome 3p22-p21.3 (Ishikawa et al., 1997).

Other mammalian members of the gelsolin/villin family include capG, which consists of only the first three homologous repeats of gelsolin and which lacks actin-severing activity

(Southwick and DiNubile, 1986), and flightless-1 which has an amino-terminal leucine-rich region hypothesized to be involved in macromolecular interactions (de Couet et al., 1995).

In order to better define the role of gelsolin and other family members in mammalian cell motility, we have generated transgenic mice deficient in one or more of these proteins. The finding that transgenic mice completely lacking gelsolin have specific defects (Azuma et al., 1998; Furukawa et al., 1997; Witke et al., 1995), yet are viable and can reproduce, led to investigation of whether other family members could compensate for gelsolin's absence. In attempting to clone murine adseverin, so as to be able to examine its expression pattern in mice lacking gelsolin, a cross-reactive signal led to the identification and cloning of a new member of the gelsolin/villin family with significant similarity to villin. The expression patterns of the murine and human isoforms of this protein of 92 kDa predicted molecular mass are described herein.

MATERIALS AND METHODS

All restriction enzymes, Klenow fragment, and polynucleotide kinase were obtained from New England Biolabs (Beverly, MA). Oligonuclotides were obtained from either Integrated DNA Technologies (Coralville, IA) or Research Genetics (Huntsville, AL). Chemicals of molecular biologic grade were obtained from American Bioanalytical (Natick, MA). Southern blot analysis was performed by capillary transfer onto GeneScreen Plus (Dupont, Boston, MA), followed by radioactive detection using ^{32}P-labeled oligonucleotides or [^{32}P]dGTP-labeled cDNA probes. cDNA and genomic clones were sequenced with either [^{35}S]dATP and Sequenase 2.0 (United States Biochemical, Cleveland, OH) or polynucleotide kinase ^{32}P-labeled oligonucleotides and Cycle Sequencing (Perkin Elmer, Foster City, CA). All animal studies were conducted in accordance with the guidelines for care and use of experimental animals.

cDNA and genomic library screening

An adult mouse brain lambda-ZAP II library (Stratagene, La Jolla, CA) was screened with a 1 kb probe corresponding to the 5′ coding sequence of bovine adseverin kindly provided by Dr Walter Witke. A single weakly hybridizing clone was plaque purified, phagemid rescue was performed, and the DNA sequence of the insert was determined as previously described (Marks and Kwiatkowski, 1996). A 0.4 kb fragment with nucleic acid homology to villin at the derived from this clone was used to rescreen the initial cDNA library and several others, eventually yielding a cDNA clone of length 1.1 kb with nucleic acid homology to segments 3 to 5 of villin. The 1.1 kb partial-length cDNA was used to identify corresponding murine genomic clones, from which selected subclones were prepared and sequenced, providing an additional 0.25 kb of probable cDNA sequence 5′ to that obtained from the previous cDNA clones. This genomic fragment was used to rescreen the adult murine cDNA libraries, but a full length cDNA clone could not be identified.

Reverse transcriptase-polymerase chain reaction (RT-PCR) cloning

To obtain a full length murine cDNA, a strategy utilizing RT-PCR was employed. Total RNA was prepared from the brains of adult Balb/c mice using Trizol reagent (Gibco Life Sciences, Grand Island, NY). Poly(A)$^+$ RNA was then isolated using the Oligotex Poly(A)$^+$ purification kit from Qiagen (Chatsworth, CA). cDNA synthesis and ligation to adaptor primers was performed using the Marathon cDNA amplification kit (Clontech, Palo Alto, CA). Primers were developed for PCR amplification of the internal segment, 5′ and 3′ ends. Overlap was provided to facilitate creation of a full length cDNA from the fragments. Two rounds of PCR were performed to amplify each of these segments using KlenTaq polymerase (Clontech) according to the manufacturer's instructions. AP1 and AP2 primers supplied with the Marathon kit were used in round 1 and round 2, respectively, of the 5′ and 3′ rapid amplification of cDNA ends (RACE) reactions. Other primers were as follows:
internal round 1: 5′-GGGAGACTGCTACATCGTCC-3′;
5′-CCTGCCAGATGTACAAGATGTAATG-3′;
internal round 2: 5′-CCTCTCGACCCGGAGAGTGG-3′;
5′-CACATCGTACGTGTAGAGAACCAG-3′;
5′ RACE round 1: 5′-CAAGCCAGAAGTGGCTGCACAGGAG-3′;
5′ RACE round 2: 5′-GGTGGACGACGGCAAAGGCCAGGTT-3′;
3′ RACE round 15′-GATGATGCCCTGCTTGAAGTAGCCACGG-3′;
3′ RACE round 2 5′-GGAAGGTGTCGGACTCGTGGTACTGG-3′.

After the second round of PCR, products of expected size were band isolated from low melting temperature agarose gels using Geneclean Spin Preps (Bio101, Vista, CA). These were then cloned into pUC18 by blunting/kinasing with the SureClone ligation kit (Pharmacia Biotech, Piscataway, NJ), and sequence analysis was used to identify clones encoding the desired product.

To assemble the full length clone, a double ligation was performed using an *Eco*RI/*Bss*SI fragment containing the 5′ end of the gene, a *Bss*SI/*Bsi*WI fragment containing the middle portion, and *Eco*RI/*Bsi*WI digested plasmid (pUC18) containing the 3′ cDNA. Both strands of the full length clone were sequenced (GenBank/EMBL/DDBJ accession number AF041448).

Database searches revealed several partial length human expressed sequence tag (EST) clones which appeared to encode the human counterpart of this protein. One of these clones was from Soares pregnant uterus and appeared to encode the 3′ end of the gene (GenBank/EMBL/DDBJ accession number AA098895); the other clone was from Soares fetal liver spleen and appeared homologous to a region roughly in the middle of the murine cDNA (GenBank/EMBL/DDBJ accession number AA034150). A full-length human cDNA clone was generated from human uterus Poly(A)$^+$ RNA (Clontech) by RT-PCR in a manner similar to the procedure described above. 5′ RACE was used in order to determine the 5′ sequence of the cDNA. This 5′ sequence was then used in combination with the known 3′ sequence in order to generate primers which amplified the cDNA (with the exception of the 5′ and 3′ most ends) in two rounds of PCR. In order to facilitate cloning, *Bam*HI restrictions sites were engineered at the 5′ and 3′ ends of the product. The gene specific primers for 5′ RACE were:
5′ RACE round 1: 5′-ACGGTCTCCACATTGGTGCTGCTGGGG-3′;
5′ RACE round 2: 5′-GATGAAGCCCAGCGCTTTAGACATGGC-3′.
The primers for amplification of the cDNA were:
5′ round 1: 5′-GATGTTTCAGTCCTGGAAAGACAAGC-3′;
3′ round 1: 5′-GATCTGGGTATTTGGATTTTGCCTATGG-3′;
5′ round 2: 5′-GCGGGATCCCTCTGACCAGTGCCTTCAGGG-3′;
3′ round 2: 5′-CGCGGATCCGCTAGAGGGGCCACAAAACCC-3′.

Sequence corresponding to the full length human cDNA was determined on both strands (GenBank/EMBL/DDBJ accession number AF041449).

Northern blot analysis

Total RNA was isolated from the organs of adult Balb/c mice, the RNA blotted onto GeneScreen Plus, and hybridization was performed as previously described (Marks and Kwiatkowski, 1996). Approximately 10 μg of RNA as determined by A_{260} was loaded into each lane, and equivalence of loading was demonstrated by ethidium bromide staining of ribosomal subunits. The probe, which was labeled with ^{32}P using Klenow fragment, consisted of a 349 bp *Pst*I/*Xba*I fragment from the 3′-untranslated region (UTR) of the murine p92 cDNA. Determination of the murine and human expression patterns were also made using Clontech multiple tissue northern blots using ExpressHyb solution according to the manufacturer's instructions. In the latter case, the probe was generated from a 210 bp *Bam*HI/*Stu*I fragment corresponding to the 3′-UTR of the human cDNA. These probes had no significant

nucleic acid homology with murine or human villin, and a search of the GenBank sequence database produced no corresponding murine or human cDNA clones. All northern blots were probed in a similar manner to the above with a human beta-actin probe (Clontech) in order to estimate the amount of RNA which had been loaded into each lane.

In situ mRNA analysis

A 575 bp *Pst*I fragment corresponding to coding sequence and a *Pst*I/*Xba*I fragment corresponding to the 3′-UTR of the murine cDNA were cloned into pGEM-3Z and used for in situ analysis of the distribution of RNA in fetal and adult tissues. These two probes gave identical results. Embryos were obtained from Balb/c mice, dissected from surrounding tissue, and placed into OCT (TBS, Durham, NC) for cryosectioning into slices of 10 to 16 μm thickness. Gestational age was determined by the stage of digit formation. Adult tissues were obtained from 3 to 6 month old Balb/c females and treated similarly.

[^{35}S]dUTP labeled sense and antisense probes were prepared from linearized plasmid DNA using an in vitro transcription system with T3, T7, and SP6 RNA polymerases (Promega, Madison, WI). After phenol-chloroform extraction these probes were purified using G-50 Sephadex Quick Spin columns (Boehringer Mannheim, Indianapolis, IN). Their specific activity was adjusted to 4×10^4 cpm/μl. In situ hybridization was performed as described by Zheng et al. (1994). Sections on slides were prehybridized for 1 to 3 hours and then hybridized with the probes overnight. After washing, the slides were dehydrated and autoradiographs were exposed for 1 to 3 days. Nuclear emulsion autoradiography was then performed using NTB-2 emulsion diluted 1:1 (Kodak, Rochester, NY) for 2 to 4 weeks exposure at 4°C. After exposure, these slides were developed in Kodak D-19, fixed, and counterstained with hematoxylin and eosin (H & E). Slides were examined with conventional light microscopy and dark field microscopy using an upright microscope. Experiments were all repeated at least twice using both antisense and sense probes of similar specific activity.

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mur p92   1 .MSLSSAFRA VSN..DPRII TWRIEKMELA LVPLSAHGNF YEGDCYIVLS TRRVGSLLSQ NIHFWIGKDS SQDEQSCAAI YTTQLDDYLG GSPVQHREVQ
hum p92   1 .MPLTSAFRA VDN..DPGII VWRIEKMELA LVPVSAHGNF YEGDCYVILS TRRVASLLSQ DIHFWIGKDS SQDEQSCAAI YTTQLDDYLG GSPVQHREVQ
hum vil   1 MTKLSAQVKG SLNITTPGLQ IWRIEAMQMV PVPSSTFGSF FDGDCYIILA IHKTASSLSY DIHYWIGQDS SLDEQGAAAI YTTQMDDFLK GRAVQHREVQ

mur p92  98 YHESDTFRGY FKQGIIYKKG GVASGMKHVE TNTYDVKRLL HVKGKRNIQA TEVEMSWDSF NRGDVFLLDL GMVIIQWNGP ESNSGERLKA MLLAKDIRDR
hum p92  98 YHESDTFRGY FKQGIIYKQG GVASGMKHVE TNTYDVKRLL HVKGKRNIRA TEVEMSWDSF NRGDVFLLDL GKVIIQWNGP ESNSGERLKA MLLAKDIRDR
hum vil 101 GNESEAFRGY FKQGLVIRKG GVASGMKHVE TNSYDVQRLL HVKGKRNVVA GEVEMSWKSF NRGDVFLLDL GKLIIQWNGP ESTRMERLRG MTLAKEIRDQ

mur p92 198 ERGGRAEIGV IEGDKEAASP GLMTVLQDTL GRRSMIKPAV SDEIMDQQQK SSIMLYHVSD TAGQLSVTEV ATRPLVQDLL NHDDCYILDQ SGTKIYVWKG
hum p92 198 ERGGRAEIGV IEGDKEAASP ELMKVLQDTL GRRSIIKPTV PDEIIDQKQK STIMLYHISD SAGQLAVTEV ATRPLVQDLL NHDDCYILDQ SGTKIYVWKG
hum vil 201 ERGGRTYVGV VDGENELASP KLMEVMNHVL GKRRELKAAV PDTVVEPALK AALKLYHVSD SEGNLVVREV ATRPLTQDLL SHEDCYILDQ GGLKIYVWKG

mur vil 298 KGATKVEKQA AMSKALDFIK MKGYPSSTNV ETVNDGAESA MFKQLFQKWS VKDQTTGLGK IFSTGKIAKI FQDKFDVSLL HTKPEVAAQE RMVDDGKGQV
hum p92 298 KGATKAEKQA AMSKALGFIK MKSYPSSTNV ETVNDGAESA MFKQLFQKWS VKDQTMGLGK TFSIGKIAKV FQDKFDVTLL HTKPEVAAQE RMVDDGNGKV
hum vil 301 KKANEQEKKG AMSHALNFIK AKQYPPSTQV EVQNDGAESA VFQQLFQKWT ASNRTSGLGK THTVGSVAKV EQVKFDATSM HVKPQVAAQQ KMVDDGSGEV

mur p92 398 EVWRIENLEL VPVEYQWHGF FYGGDCYLVL YTYDVNGKPH YILYIWQGRH ASRDELAASA YRAVEVDQQF DRAPVQVRVS MGKEPRHFMA IFKGKLVIYE
hum p92 398 EVWRIENLEL VPVEYQWYGF FYGGDCYLVL YTYEVNGKPH HILYIWQDRH ASQDELAASA YQAVEVDRQF DGAAVQVRVR MGTEPRHFMA IFKGKLVIFE
hum vil 401 QVWRIENLEL VPVDSKWLGH FYGGDCYLLL YTYLIGEKQH YLLYVWQGSQ ASQDEITASA YQAVILDQKY NGEPVQIRVP MGKEPPHLMS IFKGRMVVYQ

mur p92 498 GGTSRKGNEE PDPPVRLFQI HGNDKSNTKA VEVSASASSL ISNDVFLLRT QAEHYLWYGK GSSGDERAMA KELVDLLCDG NADTVAEGQE PPEFWDLLGG
hum p92 498 GGTSRKGNAE PDPPVRLFQI HGNDKSNTKA VEVPAFASSL NSNDVFLLRT QAEHYLWYGK GSSGDERAMA KELASLLCDG SENTVAEGQE PAEFWDLLGG
hum vil 501 GGTSRTNNLE TGPSTRLFQV QGTGANNTKA FEVPARANFL NSNDVFVLKT QSCCYLWCGK GCSGDEREMA KMVADTISRT EKQVVVEGQE PANFWMALGG

mur p92 598 KTAYANDKRL QQETLDVQVR LFECSNKTGR FLVTEVTDFT QEDLSPGDVM LLDTWDQVFL WIGAEANATE KKGALSTAQE YLVTHPSGRD PDTPILIIKQ
hum p92 598 KTPYANDKRL QQEILDVQSR LFECSNKTGQ FVVTEITDFT QDDLNPTDVM LLDTWDQVFL WIGAEANATE KESALATAQQ YLHTHPSGRD PDTPILIIKQ
hum vil 601 KAPYANTKRL QEENLVITPR LFECSNKTGR FLATEIPDFN QDDLEEDDVF LLDVWDQVFF WIGKHANEEE KKAAATTAQE YLKTHPSGRD PETPIIVVKQ

mur p92 698 GFEPPTFTGW FLAWDPHIWS EGKSYEQLKN ELGDATAIVR ITADMKNATL YL.....NPS DGEPKYYPVE VLLKGQNQEL PEDVDPAKKE NYLSEQDFVS
hum p92 698 GFEPPIFTGW FLAWDPNIWS AGKTYEQLKE ELGDAAAIMR ITADMKNATL SL.....NSN DSEPKYYPIA VLLKNQNQEL PEDVNPAKKE NYLSEQDFVS
hum vil 701 GHEPPTFTGW FLAWDPFKWS NTKSYEDLKA ESGNLRDWSQ ITAEVTSPKV DVFNANSNLS SGPLPIFPLE QLVNKPVEEL PEGVDPSRKE EHLSIEDFTQ

mur p92 793 VFGITRGQFT ALPGWKRLQL KRERGLF* 819
hum p92 793 VFGITRGQFA ALPGWKQLQM KKEKGLF* 819
hum vil 801 AFGMTPAAFS ALPRWKQQNL KKEKGLF* 827
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Fig. 1. Amino acid sequence comparison of murine p92, human p92, and human villin. The single letter code for amino acids is used. Lines between the sequences denote identity. Amino acids indicated in bold correspond to those in villin which have been demonstrated to interact with actin (Doering and Matsudaira, 1996; DiMarkus et al., 1997); underlined residues correspond to those which have been reported to bind polyphosphoinositides in gelsolin/villin family members (Nakamura et al., 1994). The murine and human p92 cDNAs are 2,973 and 2,718 nucleotides, respectively. The predicted protein product of both cDNAs is 819 amino acids and has a molecular mass of approximately 92 kDa. These sequence data are available from GenBank/EMBL/DDBJ under accession numbers AF041448 and AF041449.

RESULTS

Cloning of murine and human p92

While screening an adult mouse brain cDNA library for the gelsolin family member adseverin, a single weakly cross-hybridizing signal was detected. Isolation and analysis of this clone revealed that it had a novel sequence with strongest amino acid identity to villin and adseverin. Initial attempts to clone the full length cDNA involved an iterative process of screening murine cDNA libraries from brain and adrenal gland, among others, as well as screening of a murine genomic library. This strategy yielded partial length sequences for the putative cDNA when compared to its closest homologue, murine villin. Because of the difficulty in obtaining a full length clone by cDNA library screening, RT-PCR was utilized. Poly(A)$^+$ RNA prepared from murine brain was used in the synthesis of an adaptor-ligated cDNA library. PCR on this material using nested internal primers, as well as nested primers for 5′ and 3′ RACE yielded cDNA clones providing the entire sequence of this novel gene.

The murine cDNA consists of 2,973 nucleotides encoding a putative protein of 819 amino acids of molecular mass 92 kDa (Fig. 1), here denoted p92. When the amino acid sequence of p92 is compared to murine gelsolin, adseverin, and villin, there is 46%, 47%, and 59% identity, respectively (Table 1). Although the similarity of p92 is weaker to villin headpiece than to the rest of the molecule, amino-terminal residues demonstrated by multidimensional nuclear magnetic resonance spectroscopy to interact with actin and residues which have been determined by cysteine mutagenesis to be essential for villin headpiece binding to actin are highly conserved (Doering and Matsudaira, 1996; Markus et al., 1997).

After the murine cDNA was cloned, database comparison revealed several partial length human expressed sequence tag (EST) clones which appeared to represent the human homologue of this gene. These clones had been prepared from a variety of different libraries: pregnant uterus, fetal liver-spleen, neuroepithelium, and several different tumor types. Using sequence obtained from EST clones of fetal liver-spleen and pregnant uterus libraries, cDNA clones containing the full length human sequence were obtained by RT-PCR using poly(A)$^+$ uterus RNA and 5′ RACE. The human protein is encoded by a cDNA of 2,718 nucleotides and shares 89% amino acid identity with its murine counterpart (Fig. 1).

Tissue expression pattern of p92

Northern blot analysis was performed on murine and human tissue panels. Because the coding region of p92 shares a high degree of identity with villin, cDNA probes generated from this region were found to be cross-reactive in northern blot experiments even under high stringency hybridization conditions (data not shown). Therefore probes lacking significant homology at the nucleic acid level were generated from the 3′ untranslated regions of the cDNAs.

Northern blot analysis of total and poly(A)$^+$ RNA prepared from various murine tissues revealed expression in uterus and testes, and weaker expression in brain (Fig. 2a,b). Expression was also seen in total RNA prepared from a murine uterus cell line, MBAC57.Ut (Fig. 2a). Examination of a human tissue panel showed that p92 was most highly expressed in small intestine and the colonic lining, and a weak signal was also detected in thymus, prostate, testes, and uterus (Fig. 2c). The difference in expression pattern between the murine and human tissue patterns may reflect interspecies differences in the expression of p92. In the case of the colon, however, the difference in expression pattern may reflect a difference in the portion of the large intestine which was utilized to prepare the RNA: the total thickness of colon, including the muscle

Table 1. Homology of advillin with other members of the gelsolin family

Homology	Gelsolin (%)	Adseverin (%)	Villin (%)	Headpiece of villin (%)
Nucleic acid identitiy	61	60	65	44
Amino acid homology	65	65	75	60
Amino acid identity	46	47	59	40

Murine advillin was compared with GenBank sequences for the murine forms of gelsolin (GenBank accession number J04953), adseverin (GenBank accession number U04354), and villin (GenBank accession number M98454). For comparison, murine gelsolin and adseverin share 64% identity at the nucleic acid level, with 75% homology and 58% identity in amino acid sequence.

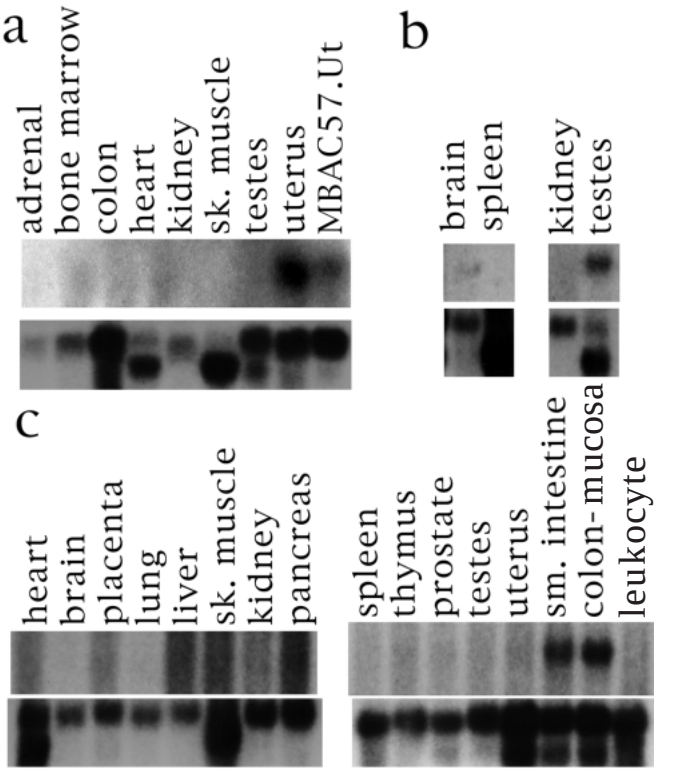

Fig. 2. (a) Expression pattern of p92 in murine tissues using total RNA. (b) Expression pattern of p92 in murine tissues using poly(A)$^+$ RNA. (c) Expression pattern of p92 in human tissues using poly(A)$^+$ RNA. The murine message is about 3.0 kb, and the human 2.8 kb, as determined by size markers. The top panel of each portion of the figure demonstrates the p92 signal, and the lower panel shows the beta-actin signal, which is an approximation of the total amount of RNA loaded into each lane. The beta-actin probe reacts with a 2.0 kb message, as well as a 1.6-1.8 kb message, particularly in heart and skeletal muscle. Differences between the expression of p92 in murine and human intestine may reflect the fact that total colon, rather than colonic lining, was used to prepare the murine RNA.

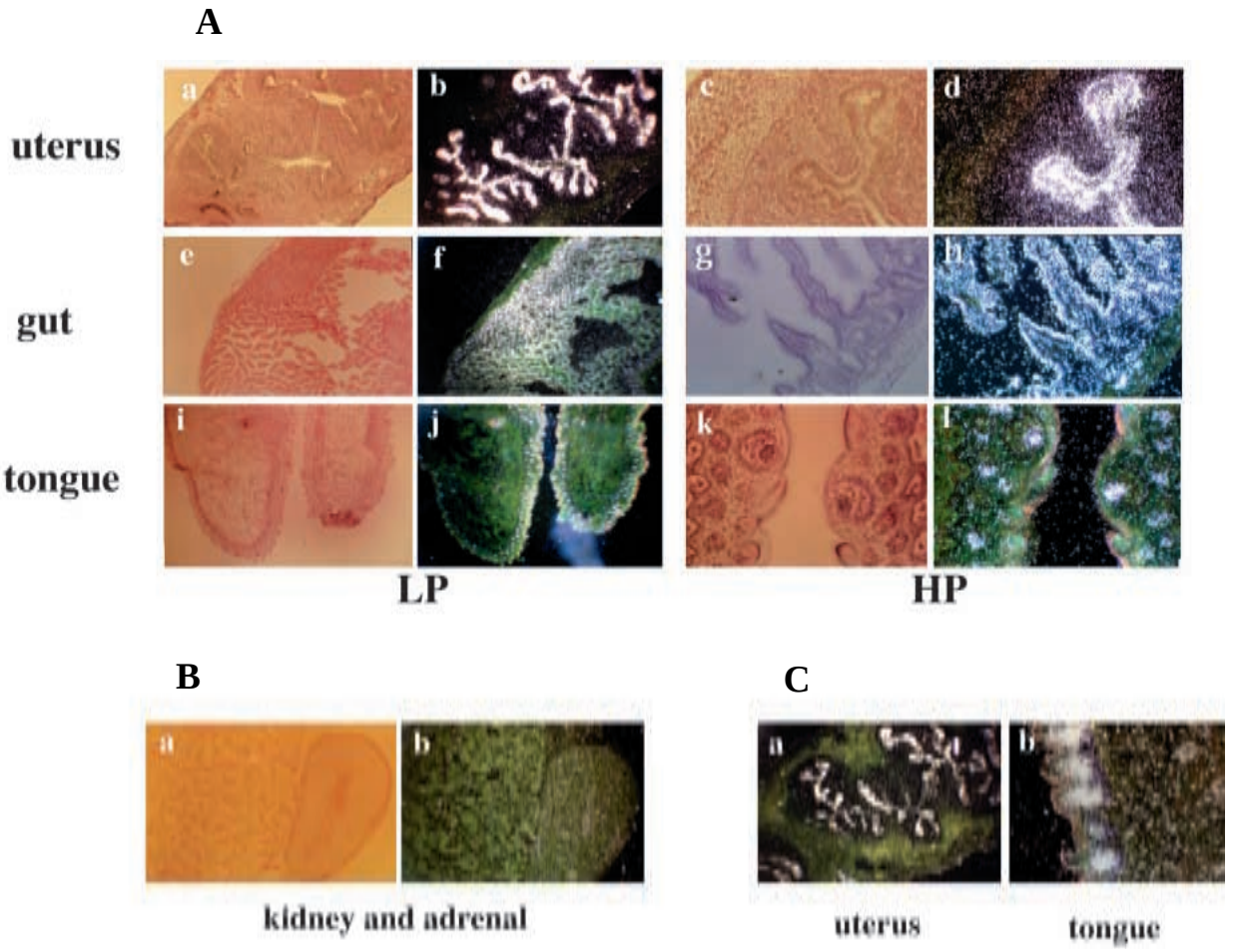

Fig. 3. In situ mRNA analysis of p92 in adult murine tissues. (A) advillin mRNA expression in uterus (a-d), gut (e-h) and tongue (i-l) using an antisense riboprobe for the coding region. Hematoxylin and eosin (H & E) counterstained sections are shown to the left of a darkfield image at low (×40) and high (×200) power magnification, respectively. (B) advillin mRNA expression in kidney and adrenal at low power magnification using the same probe (a) H & E, (b) darkfield. (C) similar expression pattern in tissues probed with an antisense riboprobe from the 3′-UTR (a) uterus at low power and (b) tongue at high power magnification. Staining is most apparent in the surface lining cells of the uterus and in the intestinal villi. In the tongue, staining appears to be concentrated around papillated areas. Tissues such as adult brain, liver, and, and kidney did not show significant expression of p92.

layer, was used to prepare the murine total RNA, whereas the human poly(A)$^+$ RNA was prepared from the lining layer which had been dissected off of the muscle layer.

In situ mRNA analysis

In order to better define the tissue expression of murine p92, in situ analysis of mRNA was performed. Examination of adult tissues revealed that message for p92 was expressed in uterus at the epithelial surface (endometrium), as well as in intestinal villi (Fig. 3A). Although the distribution of p92 message in intestine was similar to that of villin as determined by immunohistochemistry (Bazari et al., 1988; Ezzell et al., 1989), its presence in endometrium was distinctly different (Horvat et al., 1990). Additionally, p92 message was detected on the surface of the tongue in structures which appeared to represent taste buds. Other tissues, such as kidney and adrenal (Fig. 3B) reveal little, if any, p92 message. The specificity of the hybridization signal for p92 message was confirmed through the use of a probe derived from the 3′-UTR of murine p92 (Fig. 3C). Sense controls on all of these tissues were negative.

When embryos were examined for expression of p92, strikingly positive signals were detected in dorsal root and trigeminal ganglia by embryonic day 14.5 and continued through at least day 16.5 (Fig. 4). A strong signal in trigeminal ganglia was also seen in the adult animal. Ossification in the adult, however, made in situ analysis of dorsal root ganglia expression impossible. In addition to the ganglionic distribution, a weaker signal was detected in the subcortical region of E16.5 brain (Fig. 4j-m). As above, sense controls were non-reactive. As an additional control for the specific expression of p92, antisense riboprobes to other members of the gelsolin family (gelsolin, adseverin, and capG) have been used with this in situ method to stain similar embryonic sections, and have revealed distinctly different reactivity (M. Arai and D. J. Kwiatkowski, unpublished).

DISCUSSION

p92 is a new member of the gelsolin/villin family of proteins, most homologous to villin. It shares the six domain structure

of a number of other mammalian gelsolin/villin family members, as well as the carboxyl-terminal headpiece domain of villin (Fig. 5). Because p92 has highest identity in amino acid sequence to *ad*severin and *villin*, we propose that the protein be called advillin.

Villin has been thought to play an important role in

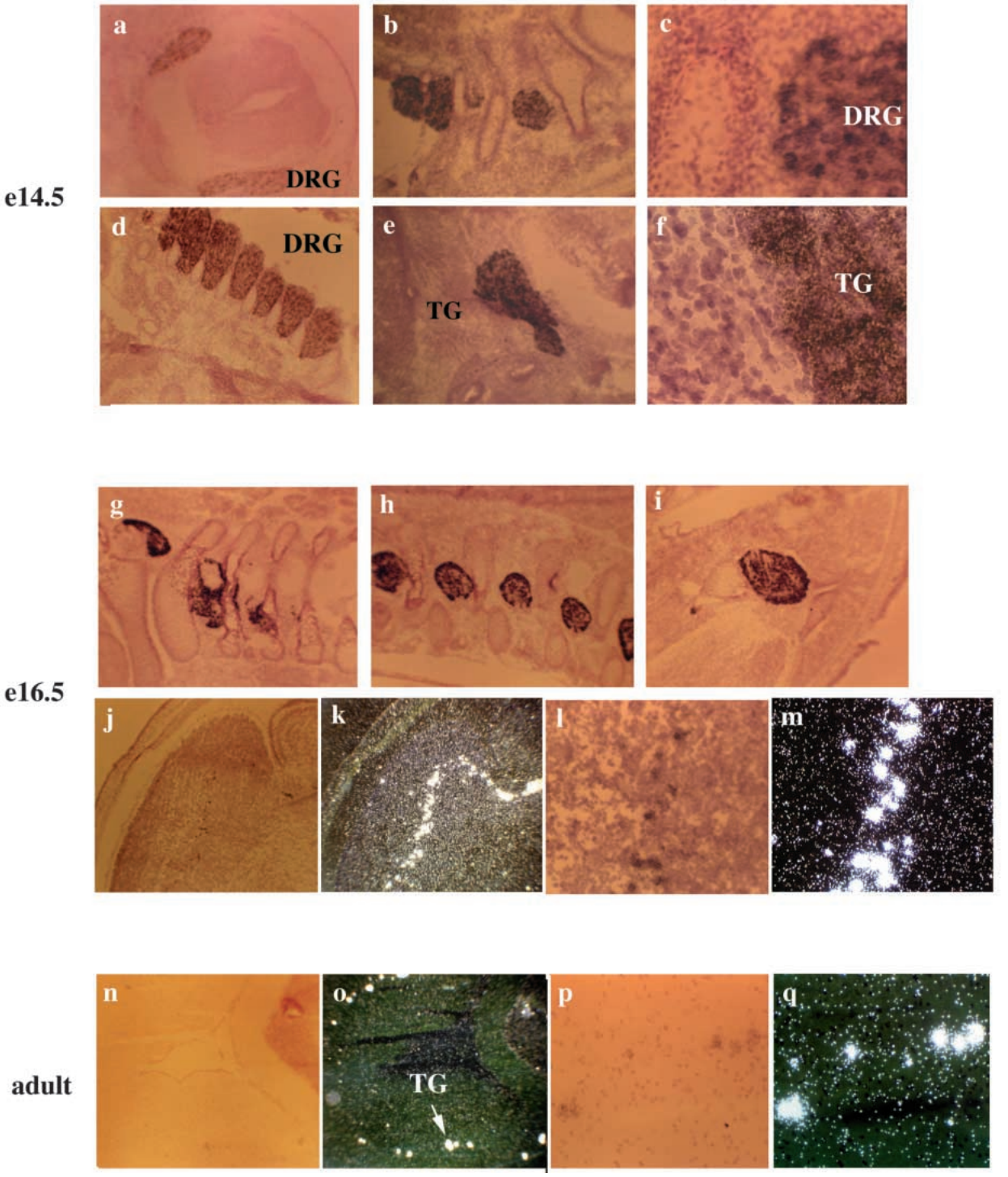

Fig. 4. In situ mRNA analysis of p92 during murine development and in adult neural tissues. Embryonic stages E14.5, E16.5, and adult sections are shown. (a-i) Brightfield images reflecting a high level of expression. e14.5: (a) transverse section through dorsal root ganglia. (b,c) Sagittal sections through dorsal root ganglia (DRG) in the mid-spinal region at low power and high power magnification (d) sagittal section at a higher spinal level viewed at low power. (e,f) Sagittal sections through trigeminal ganglion at low and high power magnification. e16.5: (g,h,i) transverse sections through dorsal root ganglia at low power magnification: from cranial to caudad (g) upper, (h) middle, and (i) lower dorsal root ganglia. (j,k) low and (l,m) high power H & E and corresponding darkfield images of cerebral cortex. Adult: (n,o) low and (p,q) high power H & E and corresponding darkfield images of trigeminal ganglia.

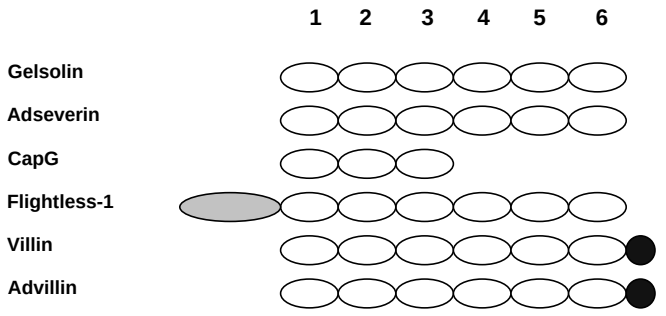

Fig. 5. Mammalian gelsolin/villin family members, including p92. The structure of the prototypical family member gelsolin can be divided into six domains. Domains 1 to 3 are homologous to domains 4 to 6. The shaded oval of flightless-1 represents the leucine rich region, and the black circles of villin and p92 represent the headpiece domains.

conjunction with other cytoskeletal proteins in the morphogenesis of microvilli by crosslinking actin filaments into the uniformly polarized bundles observed by ultrastructural analysis (Louvard, 1989; Mooseker, 1985). Experimental evidence supporting this view includes experiments using transient transfection or microinjection of villin into cells not normally expressing this protein (Franck et al., 1990; Friederich et al., 1989, 1992). In both of these cases, structures resembling microvilli appear, suggesting that villin is capable of facilitating the organized actin structure necessary for the morphogenesis of microvilli. More recently, however, the analysis of villin-null mice has revealed minimal changes in the ultrastructure of microvilli (Pinson et al., 1998). This suggests that other proteins may also function in the generation of these complex and essential structures.

In this regard, a number of proteins have now been cloned which share a villin-like carboxy-terminal headpiece (Fig. 6). This is the region of the villin molecule which is required for its ability to bundle actin filaments (Arpin et al., 1988; Doering and Matsudaira, 1996). These proteins containing headpiece domains have been cloned from diverse sources, and show varying degrees of similarity to the villin molecule outside of the headpiece domain. Dematin was first identified in the erythrocyte membrane skeleton, alhough it has subsequently been found in numerous other tissues as well (Rana et al., 1993). Cloning of dematin has revealed that its two subunits differ by a 22 amino acid insertion within the headpiece domain (Azim et al., 1995). It can bind to and bundle actin filaments (Azim et al., 1995), although it does not contain the six domain repeat structure typical of gelsolin/villin family members. Similarly, abLIM contains a zinc finger motif which is not found in other gelsolin/villin family members, but has an actin-binding villin-like carboxy-terminus (Roof et al., 1997). It is also expressed in a variety of tissues (Roof et al., 1997).

Proteins have also been identified which do share additional regions of similarity to gelsolin outside of the villin-like carboxy-terminal headpiece domain. Supervillin (p205) was isolated from bovine neutrophil plasma membranes (Pestonjamasp et al., 1997). It has strong similarity to gelsolin domains 2 and 5 and contains a villin-like headpiece. Based on immunostaining, a role for this molecule in regulating actin filament assembly at adherens junctions is postulated. Another novel villin-like protein was identified by DNA sequencing along the region of chromosome 3p22-p21.3 (GenBank/EMBL/DDBJ accession number D88154; Ishikawa et al., 1997). In addition to a homologous carboxy-terminal headpiece, this molecule shares 68% homology in amino acid sequence to villin domains 2 through 5, but does not appear to contain domains 1 or 6. In contrast, advillin (p92) which also contains a carboxy-terminal headpiece domain, shares homology in amino acid sequence to villin along its entire length, including domains 1 through 6.

All of the above homologues of villin headpiece are identical at the last 6 of 7 amino acids defined as critical for actin binding by in vitro mutagenesis (Fig 6; Doering and Matsudaira, 1996): Lys38, Glu39, Lys65, Lys70, Lys71, Leu75, and Phe76 (numbering from the beginning of the villin headpiece domain). Of note, human advillin (p92) is the only family member identical at all 7 of these residues. The strong similarity of advillin (p92) to villin (75% at the amino acid level), and its expression in intestinal microvilli may help to explain why villin-deficient mice have minimal deficits in the morphogenesis of these structures (Pinson et al., 1998).

Although advillin (p92) is expressed along with villin in intestine, its presence in uterus, in structures resembling taste buds on the surface of the tongue, and in dorsal root and trigeminal ganglia at high expression level, is different from that reported for villin (Ezzell et al., 1989; Horvat et al., 1990). It is interesting to speculate on the origin of the strong signal in dorsal root and trigeminal ganglia, as well as on its presence in taste buds and embryonic brain. Filipodia of neurons have been observed to contain tight bundles of microfilaments at their cores, which are presumably important for neurite extension (Bridgeman and Dailey, 1989). Perhaps advillin (p92) is involved in generating these actin bundles and in developing the cytoskeletal architecture necessary for this process.

Advillin is similar to several other members of the gelsolin/villin family in that it is evolutionarily conserved, and its expression is restricted to certain embryonic and adult

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            1          11         21         31         41         51         61         71
villin  752 VFNANSNLSS..GPLPIFPLEQLVNKPVEELPEGVDPSRKEEHLSIEDFTQAFGMTPAAFSALPRWKQQNLKKEKGLF
p92     742 MKNATLSLNSNDSEPKYYPIAVLLKNQNQELPEDVNPAKKENYLSEQDFVSVFGITRGQFAALPGWKQLQMKKEKGLF
dematin 306 FSPSGSETGSPGLQIYPYEVLVVTNKGRTKLPPGVDRMRLERHLSAEDFSRVSAMSPEEFGKLALWKRNELKKKASLF
abLIM   774                          NRGRNKILREVDRTRLERHLAPEVFREIFGMSIQEFDRLPLWRRNDMKKKAKLF
D88154  633                          HQAVEDLPEGVDPARREFYLSDSDFQDIFGKSKEEFYSMATWRQRQEKKQLGFF
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Fig. 6. Comparison of the carboxy-terminal domain of villin subfamily members. Human sequences for villin, p92, dematin-1/2, abLIM, and villin-like protein (GenBank/EMBL/DDBJ accession number D88154) are shown. The numbering corresponds to that of the villin headpiece domain. Villin and p92 are identical at the 7 residues (bold) critical for actin binding as demostrated by Doering and Matsuidara (1996). The other subfamily members differ at the first of these residues, but are identical at the remaining 6.

differentiated tissues. These proteins are likely to serve important functions in regulating the actin organization and motility of each tissue where they are expressed. The viability of gelsolin- and capG- and villin-null animals suggest that compensatory mechanisms permit viability of these mice (Pinson et al., 1998; Witke et al., 1995; W. Witke and D. J. Kwiatkowski, unpublished observation), but detailed analysis of gelsolin-null tissues has yielded a harvest of data implicating its critical functions (Azuma et al., 1998; Furukawa et al., 1997). Similar analyses of advillin-null mice and their ganglia will likely be required to delineate the in vivo function of this protein.

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