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Identification of I-Plastin, a Human Fimbrin Isoform Expressed in Intestine and Kidney

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The complete cDNA sequence of human intestine-specific plastin (I-plastin) was determined from a clone derived by PCR. It consists of a 97-bp 5′ untranslated region, a 1,887-bp coding region, and a 1,655-bp 3′ untranslated region. The coding region predicts a 629-residue polypeptide whose sequence displays 86, 75, and 73% identities with chicken intestine fimbrin, human T-plastin, and human L-plastin, respectively. Recombinant I-plastin cross-linked actin filaments into bundles in the absence but not in the presence of calcium. The I-plastin gene was mapped by PCR to human chromosome 3; the L- and T-plastin genes were previously mapped to chromosomes 13 and X, respectively. I-plastin mRNA was detected in the small intestine, colon, and kidneys; relatively lower levels of expression were detected in the lungs and stomach. In contrast, L-plastin expression was restricted to the spleen and other lymph node-containing organs, while T-plastin was expressed in a variety of organs, including muscle, brain, uterus, and esophagus. In contrast to the situation for the intestine, high levels of L- and T-plastin mRNAs were detected in Caco-2, a human colon-derived cell line. Immunofluorescence microscopy detected I-plastin in the brush border of the small intestine and colon. These results identify I-plastin as the human homolog of chicken intestine fimbrin and as a third plastin isoform in humans.

Fimbrin was originally discovered in chicken intestinal brush border microvilli and identified as a 68-kDa actin cross-linking protein of the core actin bundle (6, 7, 11, 21). Antibodies raised against chicken intestine fimbrin detected fimbrin in actin bundles of cells and tissues of nonintestinal origin. Fimbrin is also found at cell-substratum adhesion sites and at the leading edge of motile cultured cells (7). Clones from cDNA libraries identified two human fimbrin homologs, T-plastin and L-plastin (14, 15). The plastins were first described as transformation-induced proteins identified on two-dimensional gels (12). Later studies showed that L-plastin is a phosphoprotein (2, 12, 19) whose expression is restricted to leukocytes but increases in neoplastic cells (9, 17). L-plastin phosphorylation is stimulated by interleukin-1, interleukin-2, and phorbol myristate acetate (19, 22, 29). In contrast, T-plastin expression was detected in cell lines derived from a wide variety of tissues, including the intestine (9). Homology among fimbrin, T-plastin (72% identity), and L-plastin (69%)

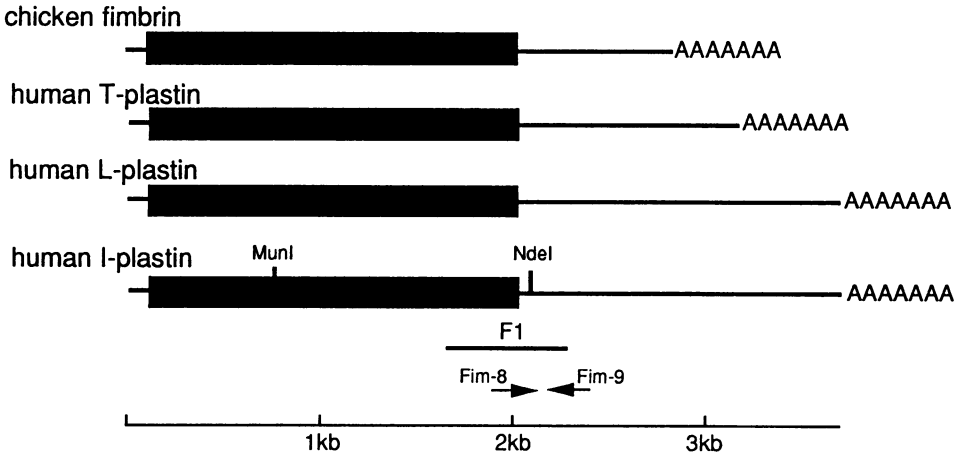

FIG. 1. Organization and comparison of the human plastin and chicken fimbrin cDNAs. The thick bars represent the coding regions, and the lines are either the 5′ UTR or the 3′ UTR. A partial clone isolated from the cDNA library is indicated as F1. *Mun*I and *Nde*I are the two restriction enzyme sites used to ligate the three PCR-generated I-plastin cDNA fragments. Fim-8 and Fim-9 are two PCR primers used in chromosome mapping.

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 M  E  N  S  T  T  T  I  S  R  E  E  L  E  E  L  Q  E  A  F  N  K  I  D  I  D  N  S  G  Y  V  S  32
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 V  N  W  I  N  K  A  L  E  N  D  P  D  C  K  H  L  I  P  M  N  P  N  D  D  S  L  F  K  S  L  A 160
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FIG. 2. Complete I-plastin cDNA nucleotide sequence and deduced amino acid sequence. Nucleotides 1 to 97 form the 5′ UTR, and nucleotides 2081 to 3639 form the 3′ UTR. The remaining sequence encodes a polypeptide of 629 amino acids, which are shown in the single-letter code. The polyadenylation signal sequence AATAAA is underlined. The GenBank accession number is L20826.

identified a cytoskeletal function for these proteins. Studies of the yeast fimbrin homolog, Sac6p (43% identity with chicken fimbrin) (1), have provided the best evidence for a critical function of this actin-bundling protein. In yeast cells, fimbrin is localized to actin cables and cortical actin patches. Deletion of the gene results in growth inhibition and cell cycle arrest at the restrictive temperature.

Sequence analysis shows that chicken fimbrin and human plastin are modular proteins consisting of a calmodulin-like calcium-binding domain at the N terminus followed in tandem by a pair of α-actinin-like actin-binding domains. Adjacent actin-binding domains suggest the ability to cross-link actin filaments into bundles (9). The calmodulin-like domain of fimbrin suggests that calcium may regulate actin binding, but biochemical studies of chicken intestine fimbrin have provided conflicting evidence on whether bundling is calcium insensitive or calcium inhibited (6, 11). Recent studies of L-plastin have

TABLE 1. Oligonucleotides used in RT and PCR[a]

Oligonucleotide	Sequence	Location (base)
Fim-1	CAATATGTTTAATGTGGCAG (RT)	2224
Fim-2	GGCAGAAAATCATATGAAAT (downstream)	2009
Fim-3	GAAGGGCCCCAGCATTCTTATTC (upstream)	437
Fim-4	TTTTTCTTATTGATGGCTCT (RT)	654
Fim-5	CTTTCATCAATTGTATCTGG (downstream)	636
Fim-8	ATTTCATATGATTTTCTGCC (upstream)	1990
Fim-9	GTATGAATGCATTTATGATC (downstream)	2388
UAP	GACTCGAGTCGACATCG	
dT-UAP	GACTCGAGTCGACATCGA$(T)_{17}$	

[a] All DNA sequences are from 5′ to 3′. Those used for RT are indicated with RT, and those used for PCR are indicated with either downstream or upstream. Restriction sites used for cloning are underlined. The first nucleotide from the 5′ end of each of the Fim oligonucleotides was used to determine its location in the I-plastin cDNA sequence (Fig. 2).

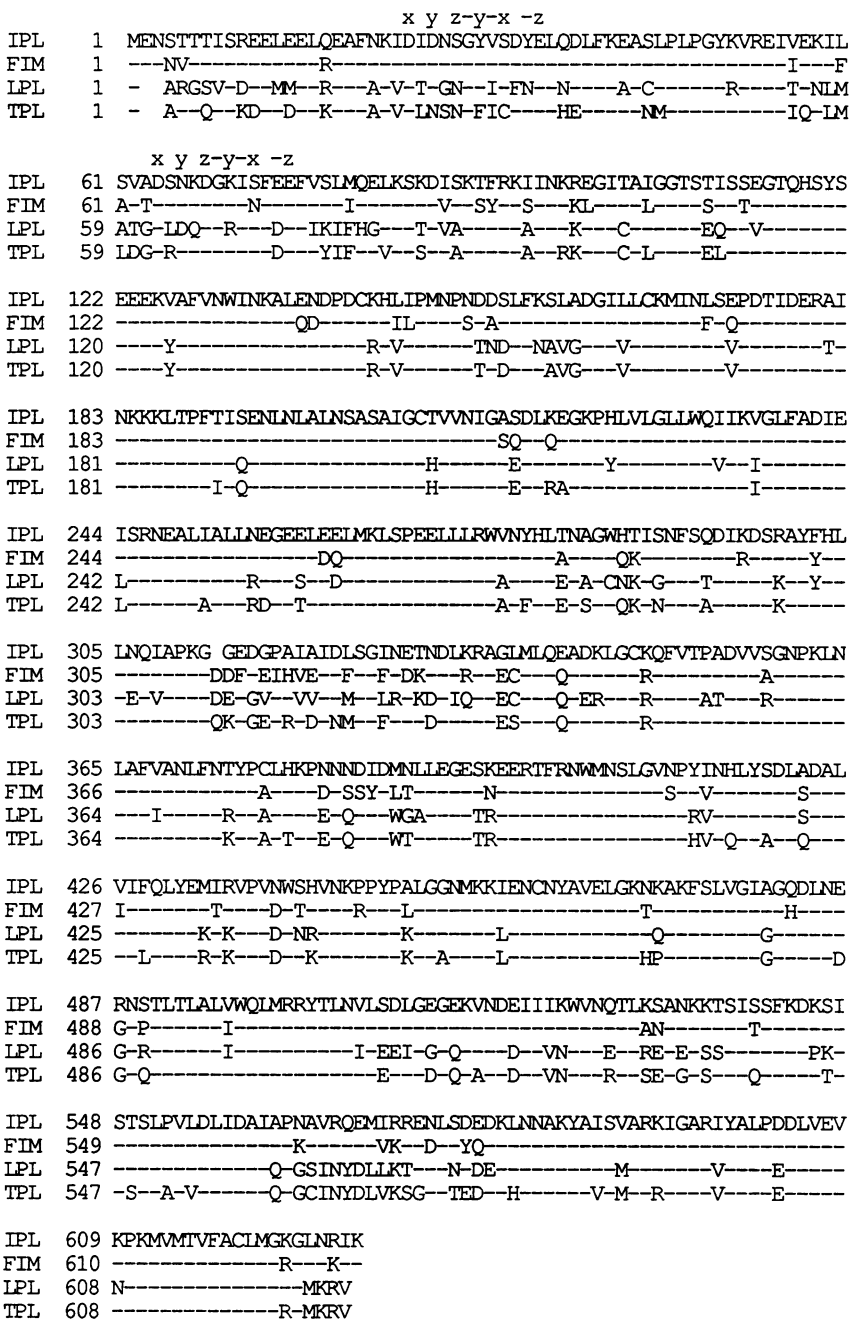

FIG. 3. Comparison of fimbrin and plastin isoform amino acid sequences. Amino acid sequences of human I-plastin (IPL), L-plastin (LPL), and T-plastin (TPL) and chicken fimbrin (FIM) are aligned for maximal homology. Residues identical to those in I-plastin are indicated by dashes. Following the convention for describing EF-hand calcium-binding sites, the positions of the residues that coordinate with calcium are labeled x, y, and z.

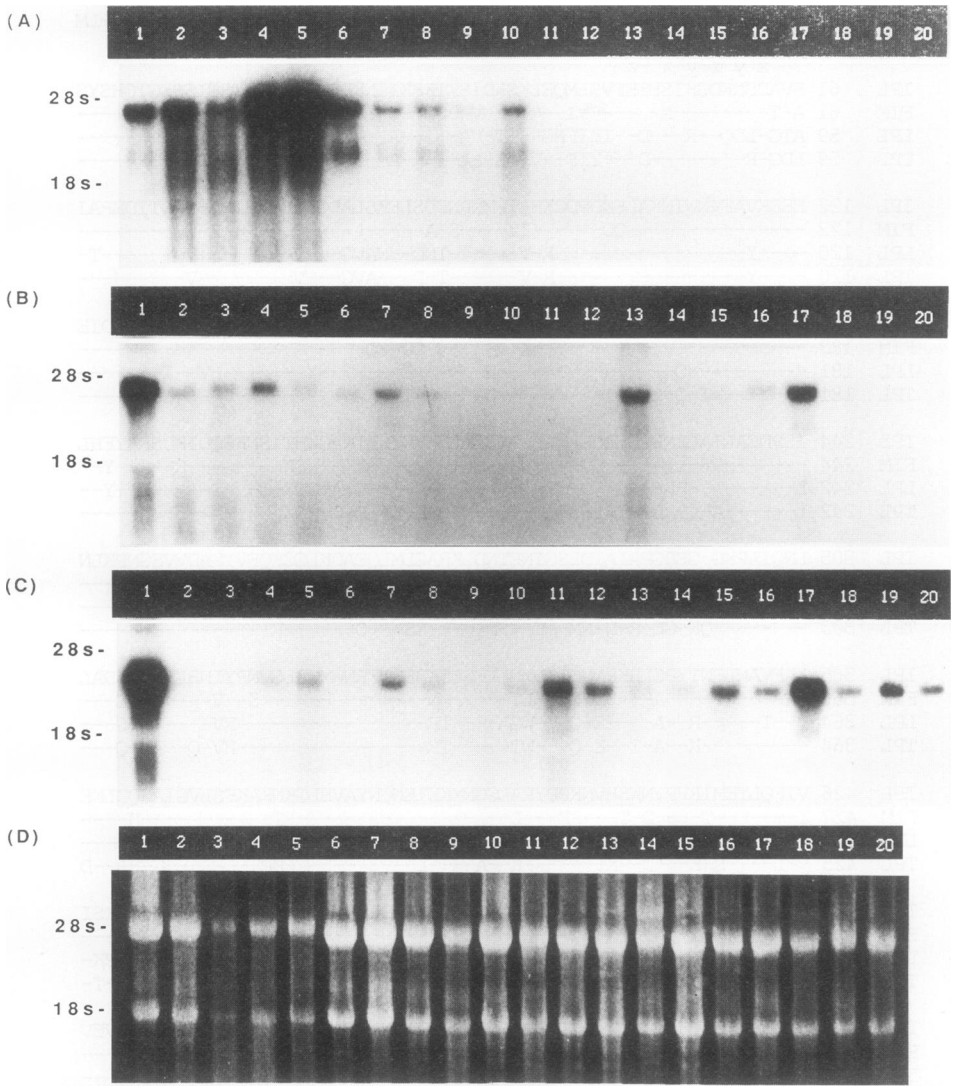

FIG. 4. Tissue distribution of plastin isoforms. Total RNAs prepared from various human and rat tissues were fractionated by gel electrophoresis, blotted onto a nylon membrane, and hybridized to ^{32}P-labeled cDNA probes for I-plastin (A), L-plastin (B), and T-plastin (C). The relative amount of each RNA is displayed in an ethidium bromide-stained gel (D). The 28S and 18S rRNAs are indicated on the left. Human tissues were (lanes) colon adenocarcinoma cell line Caco-2 (1), colonic tumor (2) and normal colon (3) from one patient, and small intestine (4) and colon (5) from another patient. Rat tissues were (lanes) jejunal epithelium (6), colon (7), ileum (8), jejunum (9), duodenum (10), uterus (11), kidney (12), spleen (13), leg muscle (14), liver (15), stomach (16), lung (17), heart (18), esophagus (19), and brain (20).

TABLE 2. Tissue distribution of plastin isoforms

Tissue	Expression[a] of:		
	I-plastin	L-plastin	T-plastin
Human			
Small intestine[b]	++	+	±
Colon[b]	++	±	+
Normal colon[c]	+	+	−
Colonic tumor[c]	+	+	−
Caco-2	+	++	+++
Rat			
Esophagus	−	−	+
Stomach	−	+	+
Duodenum	+	−	±
Jejunum	+	−	−
Jejunum (epithelium)	+	±	−
Ileum	+	+	+
Colon (whole tissue)	+	+	+
Kidneys	+	−	+
Uterus	−	−	+
Spleen	−	+	±
Liver	−	−	+
Lungs	−	+	++
Heart	−	−	+
Brain	−	−	+
Leg muscle	−	−	+

[a] The amount of mRNA was estimated according to the Northern blot hybridization results shown in Fig. 4. +++, ++, and +, detected; ±, marginally detected; −, not detected.
[b] Tissues were from one patient.
[c] Tissues were from another patient.

demonstrated that calcium inhibits actin bundling (23–25). In the homologous region of yeast Sac6p, critical positions are occupied by residues that would prevent calcium binding, suggesting that this protein is not calcium regulated in *Saccharomyces cerevisiae* (1).

Several negative results prompted us to investigate whether an intestine-specific plastin isoform existed. Antibodies specific for T- or L-plastin failed to highlight the brush border microvilli; Northern (RNA) blot analysis of various tissues with probes specific for the 5′ untranslated region (UTR) of T- or L-plastin hybridized to mRNAs from nonintestinal but not intestinal tissues. In this report, we describe the cloning and preliminary characterization of I-plastin, an intestine-specific plastin isoform that is the human homolog of chicken intestine fimbrin. Our results suggest that in humans, plastin is encoded by three distinct genes which display tissue-specific expression.

MATERIALS AND METHODS

Screening of the cDNA library and PCR amplification of I-plastin cDNA. Approximately 50,000 λ bacteriophage plaques plated from a human small intestine cDNA library (13; a kind gift from Yvonne Edwards) were screened with a chicken fimbrin cDNA probe (9; clone M38) by previously described procedures (16). One clone (F1) was isolated (Fig. 1); its sequence corresponded to the C-terminal 137-amino-acid sequence of I-plastin and overlapped the 3′ UTR (Fig. 2). Reprobing of the library with this clone yielded no additional plaques. To isolate the complete I-plastin cDNA sequence, reverse transcription (RT)-PCR of human small intestine mRNA (15) was used. The sequences and locations of oligonucleotide primers used in RT-PCR are listed in Table 1. In brief, primer Fim-1 was used for RT, and primers Fim-2 and Fim-3 were used for PCR. The resulting product was cloned into a modified Bluescript plasmid (Stratagene, San Diego, Calif.) containing an *Nde*I restriction site. Two independent clones were analyzed by the dideoxy chain termination method (26). The resulting sequence corresponded to the I-plastin sequence from bases 437 to 2009 (Fig. 2). To isolate the 5′-end cDNA, the anchored PCR technique (15) was used. In brief, the first strand was synthesized by RT with primer Fim-4 and amplified with primers Fim-5 and dT-UAP. The product was cloned, and seven independent clones were analyzed. The resulting sequence corresponded to the I-plastin sequence from bases 1 to 636 (Fig. 2). Similarly, RT with primer dT-UAP and amplification with primers Fim-8 and UAP generated the 3′-end cDNA. One clone of the PCR product was analyzed, and the sequence corresponded to the I-plastin sequence from bases 1990 to 3639 (Fig. 2). With the *Mun*I and *Nde*I restriction enzyme sites (Fig. 1), these three RT-PCR products were assembled into a complete I-plastin cDNA.

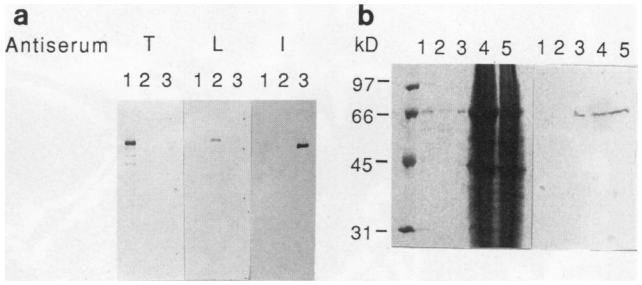

FIG. 5. Immunoblots of plastins from human intestine and colon tissues. (a) Control experiments demonstrating the specificity of isoform-specific peptide antisera for recombinant T-plastin (lane 1), L-plastin (lane 2), and I-plastin (lane 3) stained with anti-T-plastin (panel T), anti-L-plastin (panel L), and anti-I-plastin (panel I) antisera. (b) Immunoblot of human intestine and colon tissues. The left panel is the SDS–15% polyacrylamide gel stained with Coomassie blue, and the right panel is the immunoblot stained with antibody against I-plastin. Lanes 1 to 5, recombinant T-plastin, L-plastin, I-plastin, human small intestine, and human colon, respectively. Molecular mass markers are indicated on the left.

Preparation of RNAs and Northern analysis. Total cellular RNAs were prepared by the guanidinium isothiocyanate method (8) and enriched for poly(A)$^+$ RNAs by oligo(dT)-cellulose chromatography (3). Rat jejunal epithelial cell RNA was a gift from Loren Fong (Research Institute, Palo Alto Medical Foundation). RNAs purified from colon adenocarcinoma cell line Caco-2 (ATCC HTB37), various rat organs, and human intestine biopsy specimens (Deaconess Hospital Core Tissue Bank) were fractionated through formaldehyde-agarose gels, and the blots were probed with ^{32}P-labeled cDNAs.

Expression of I-plastin in bacteria and bundling assay. The full-length coding sequence of the human I-plastin cDNA was cloned into pET-3C (28). The recombinant plasmid was transformed into *Escherichia coli* BL-21 (DE3), and expression was induced with isopropyl-β-D-thiogalactopyranoside (IPTG). I-plastin was purified by a protocol used for the purification of bacterially expressed T- and L-plastins (23).

Purified I-plastin (5 μM) was mixed with rabbit F-actin (10 μM) in the presence or absence of 1 mM $CaCl_2$ and incubated in a buffer containing 10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 1 mM $MgCl_2$, 100 mM NaCl, 0.1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.1 mM ATP, and 0.1 mM dithiothreitol for 2 h at room temperature. One microliter of the reaction mixture was loaded onto a carbon-coated grid and negatively stained

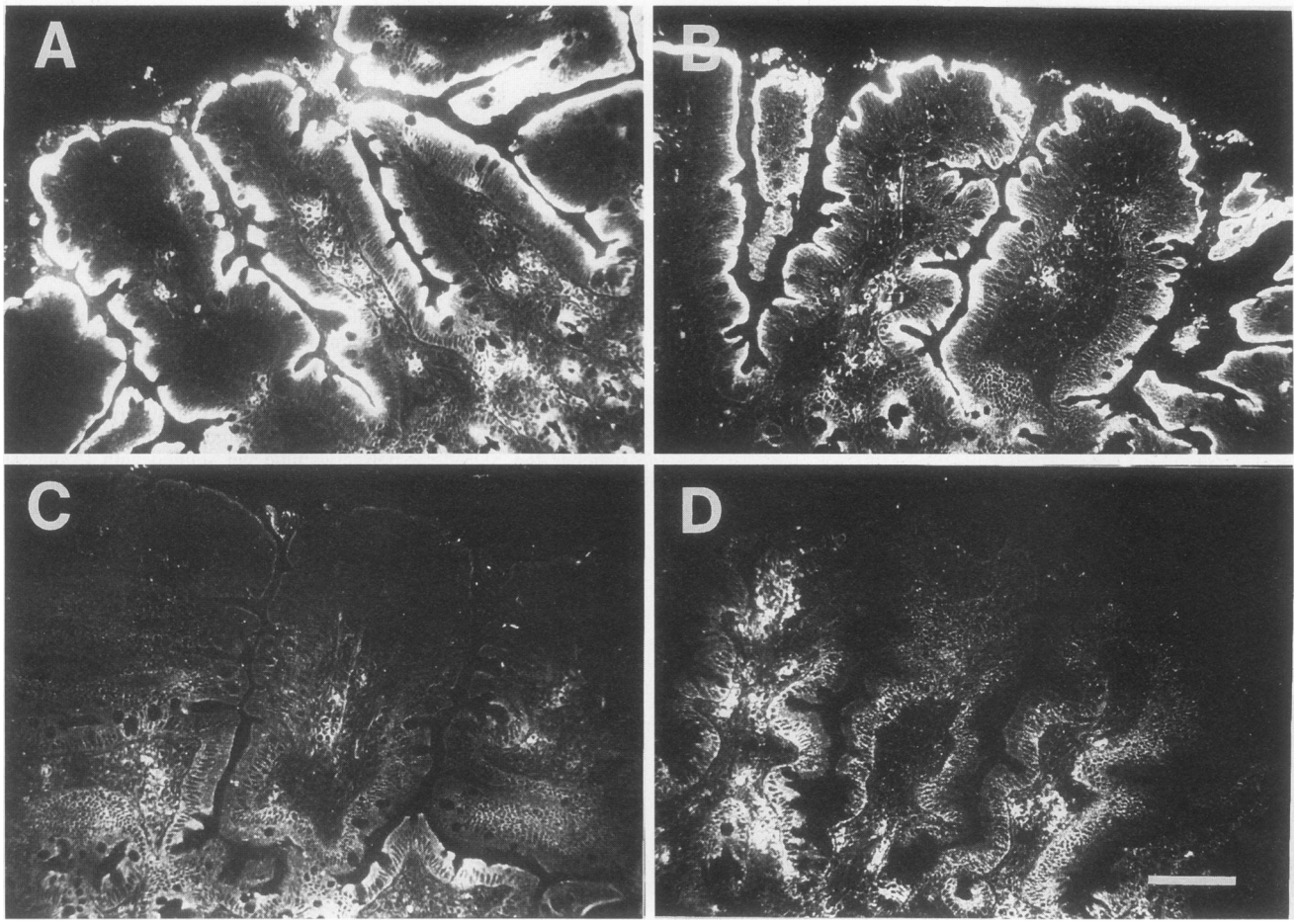

FIG. 6. Localization of I-plastin in the human small intestine. Human small intestine tissue sections were stained with antibodies against human I-plastin (R406.3) (A), chicken fimbrin (R163.3) (B), human L-plastin (R325.4) (C), and human T-plastin (R330.4) (D). Bar, 100 mm.

with 1% uranyl acetate. Bundles were examined in a Philips 410 electron microscope.

Production of plastin isoform-specific antibodies. Antibodies specific for each plastin isoform were elicited by synthetic peptides that corresponded to unique protein sequences at the N terminus. The peptide sequences (antiserum numbers) used were as follows: T-plastin, DEMATTQISKDELDELKEGC (R330.4); I-plastin, ENSTTTISREELEELQEAC (R406.3); and L-plastin, ARGSVSDEEMMELREGC (R325.4). Peptides were cross-linked through the C-terminal cysteine residue to thyroglobulin and then were injected into rabbits by previously described procedures (5). The specificities of the antisera were assessed on immunoblots against a total lysate of *E. coli* expressing different plastin isoforms and against lysates of human colon and small intestine samples (see Results). Immunoblotting was performed by a procedure described by Ezzell et al. (10). The human colon and small intestine samples were obtained from the Deaconess Hospital Core Tissue Bank and prepared accordingly for electrophoresis on a sodium dodecyl sulfate (SDS)–15% polyacrylamide minigel (20). Polyclonal antisera were diluted 1:2,500 and incubated overnight with the blot at 4°C. The positions of the immunoreactive proteins were identified with an alkaline phosphatase-conjugated secondary antibody (ICN Biomedicals Inc.).

Immunofluorescence staining. Human colon and small intestine biopsy samples obtained from the Deaconess Hospital Core Tissue Bank were snap-frozen in liquid N_2 and stored at −80°C. Sections were cut on a cryotome and processed for immunostaining as previously described (10). Antisera were diluted 1:50 in bovine serum albumin-phosphate-buffered saline, and sections were examined with a Bio-Rad MRC600 confocal microscope.

Chromosomal mapping by PCR. The chromosomal location of the I-plastin gene was identified by the PCR-based method previously used to map the human L- and T-plastin genes to chromosomes 13 and X, respectively (17). DNA samples (0.5 μg each) from 18 different human-hamster hybrid cell lines and from control human and hamster cell lines served as templates for PCR amplification with a pair of I-plastin-specific oligonucleotides, Fim-8 and Fim-9 (Table 1 and Fig. 1).

RESULTS

Sequence comparison of fimbrin and plastin isoforms. The organization of the human I-plastin cDNA shown in Fig. 1 was compared with that of the cDNAs of human L-plastin, human T-plastin, and chicken intestine fimbrin. The cDNA contained coding regions of 1,887, 1,881, 1,881, and 1,890 nucleotides (human I-, L-, and T-plastins and chicken fimbrin, respectively), a 5′ UTR of approximately 100 nucleotides, and a 3′ UTR [excluding the poly(A) tail] of 1,655, 1,671, 1,112, and 793

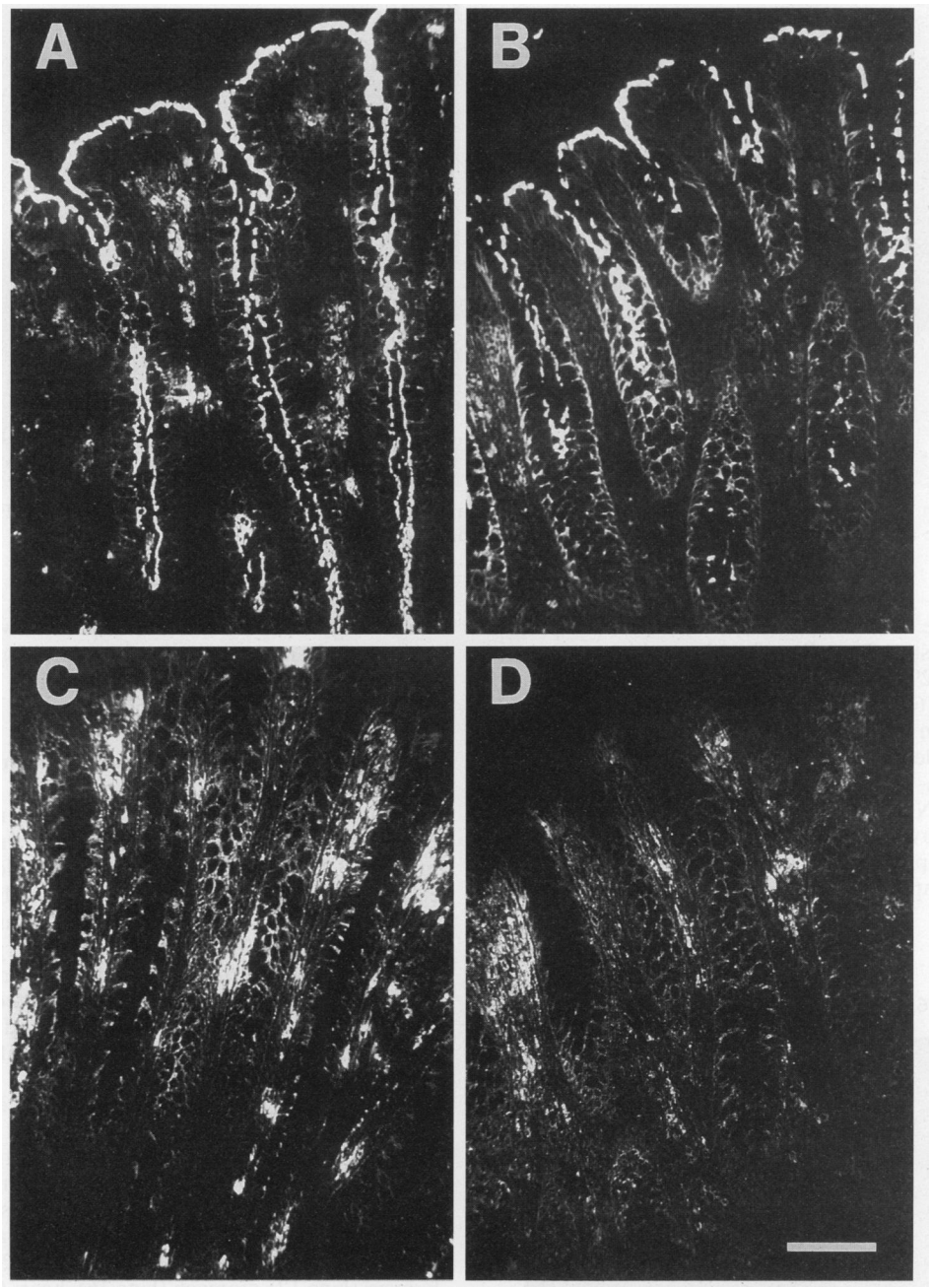

FIG. 7. Localization of I-plastin in the human colon. Human colon tissue sections were stained with antibodies against human I-plastin (R406.3) (A), chicken fimbrin (R163.3) (B), human L-plastin (R325.4) (C), and human T-plastin (R330.4) (D). Bar, 100 mm.

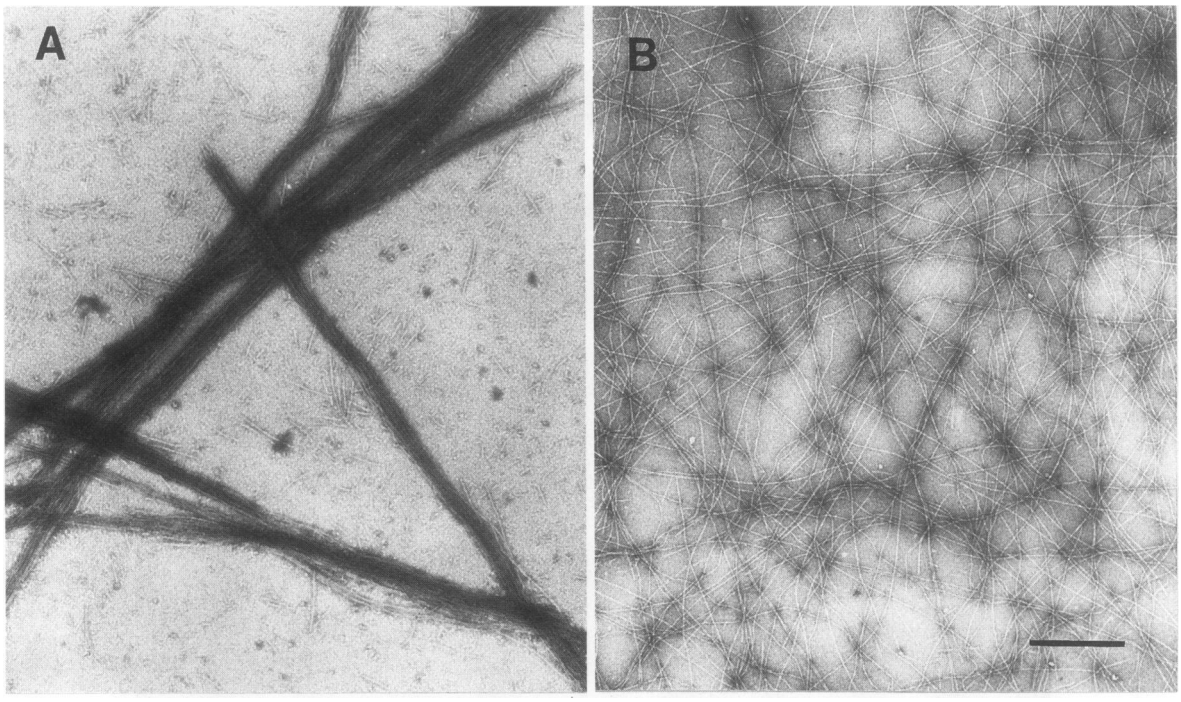

FIG. 8. Formation of actin bundles by recombinant I-plastin. Shown are electron micrographs of negatively stained F-actin incubated with I-plastin either in the absence of calcium (A) or in the presence of calcium (B). Bar, 0.4 mm.

nucleotides. Figure 2 shows the cDNA and deduced protein sequences of I-plastin. The aligned amino acid sequences of human I-plastin (629 residues), chicken fimbrin (630 residues), human L-plastin (627 residues), and human T-plastin (627 residues) are shown in Fig. 3. Human I-plastin demonstrated a higher degree of identity with chicken intestine fimbrin (86%) than with human L- and T-plastins (73 and 75%, respectively). In particular, the N-terminal domain (residues 1 to 114) was much better conserved (84%) between chicken intestine fimbrin and human I-plastin than between any pair of the three human isoforms (approximately 60% identity). The most diverged region among the four sequences was located between residues 313 and 338 of I-plastin; approximately 60% of the 26 residues were nonidentical between any two isoforms, and residue 313 of chicken fimbrin was not present in human I-plastin.

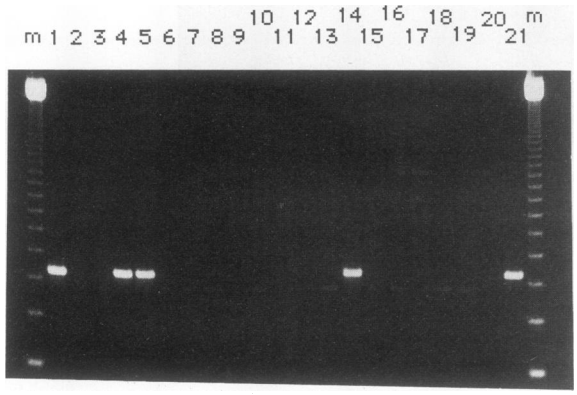

FIG. 9. Chromosomal mapping of the human I-plastin gene by PCR. Cell lines used in this mapping were (lanes) a human positive control (1), 867 (2), 854 (3), 423 (4), 860 (5), 803 (6), 909 (7), 1006 (8), 811 (9), 967 (10), 734 (11), 968 (12), 683 (13), 507 (14), 750 (15), 1099 (16), 324 (17), 940 (18), and 983 (19) and a hamster negative control (20). The RT-PCR product of the human small intestine is shown in lane 21. Size markers of the 123-bp ladder are shown in lanes m.

Differential expression of plastin isoforms. Because L- and T-plastins demonstrated cell-type-specific expression (9, 14), we analyzed human intestine and different rat organs for I-, L-, and T-plastin expression. Figure 4A shows that I-plastin mRNA (3.7 kb) was detected in human colon adenocarcinoma cell line Caco-2 (lane 1) and human tissue biopsies of a colonic tumor (lane 2), normal colon (lane 3), small intestine (lane 4), and colon (lane 5). A survey of rat organs revealed that I-plastin was primarily expressed in the intestine (lanes 6 to 10). A lower level of expression was detected in the kidneys (lane 12). I-plastin expression was detected in the stomach and lungs (lanes 16 and 17, respectively), only after a long exposure of the X-ray autoradiograph.

L-plastin (Fig. 4B) was expressed in the spleen and lungs, two major leukocyte-containing organs, as well as Caco-2 (lanes 13, 17, and 1, respectively). L-plastin mRNA was also detected in human intestine and rat esophagus and intestine.

As shown in Fig. 4C, T-plastin expression was highest in Caco-2 (lane 1), followed by the lungs (lane 17), esophagus (lane 19), uterus (lane 11), brain (lane 20), liver (lane 15), kidneys (lane 12), stomach (lane 16), heart (lane 18), and colon (lane 7). Low levels of T-plastin expression were detected in the spleen (lane 13), skeletal muscle (lane 14), and small intestine (lanes 8 to 10). Table 2 summarizes the different locations of the three plastin isoforms.

Localization of I-plastin in the human intestine. Antisera raised against an N-terminal peptide from human T-, L-, and I-plastins reacted with the cognate recombinant protein but not with the other two (Fig. 5a). In addition, the R406.3 antiserum reacted with a 68-kDa band from SDS-solubilized

TABLE 3. Chromosomal location of the human I-plastin gene in human-hamster hybrid cell lines

Cell line	Plastin	Involvement of human chromosome[a]:																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Human	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
867	−	(+)	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	−
854	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
423	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
860	+	−	−	(+)	−	+	+	−	−	−	(+)	−	−	−	−	−	−	−	−	(+)	−	+	−	−	−
803	−	−	−	−	+	+	−	−	+	−	−	(+)	−	−	−	−	−	−	−	−	−	−	+	+	−
909	−	−	−	+	−	D	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
1006	−	−	−	−	(+)	+	−	+	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	−
811	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−
967	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
734	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
968	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−
967	−	−	−	−	−	+	−	−	−	−	−	+	(+)	−	+	−	−	−	−	+	−	+	+	−	−
507	+	−	−	+	−	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	(+)	−	(+)	−	+
750	−	−	−	−	−	D	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−
967	−	+	−	−	−	D	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	+	−	−
324	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
940	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
983	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Hamster	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] +, −, or (+), presence, absence, or presence of part (from 15 to 60%) of the indicated human chromosome, respectively; D, deletion at p15.1-p15.2 on human chromosome 5.

human small intestine and colon (Fig. 5b). In immunofluorescence studies, R406.3 specifically stained the brush border of human small intestinal (Fig. 6) and colonic (Fig. 7) epithelial cells. An identical pattern of localization was seen with an antiserum (R163.3) raised against intact chicken intestine fimbrin (Fig. 6 and 7). In contrast, peptide antisera specific for T- and L-plastins localized to the lamina propria and not the epithelium (Fig. 6 and 7). Control experiments revealed some autofluorescence from the lamina propria; preimmune sera showed only weak background staining (data not shown).

Calcium-regulated actin cross-linking activity. The presence of I-plastin in the brush border and the high degree of sequence similarity indicate that I-plastin is the human homolog of chicken intestine fimbrin. Because fimbrin is an actin-bundling protein, we tested the actin-binding activity of I-plastin. Electron microscopy (Fig. 8) of negatively stained samples showed that bundles of actin filaments were seen in the absence of calcium (Fig. 8A); in the presence of calcium, individual filaments but not filament bundles were observed (Fig. 8B).

Chromosomal location of the I-plastin gene. To confirm that I-plastin is encoded by a unique gene and is not a spliced isoform of human L- or T-plastin, we mapped its chromosomal location by PCR. Using a pair of I-plastin-specific primers (Fim-8 and Fim-9; Fig. 1) whose corresponding locations in the T- and L-plastin genes are within an isoform-specific exon (17), we generated a 399-bp PCR product from human genomic DNA and from a reverse-transcribed product of human small intestine RNA (Fig. 9, lanes 1 and 21, respectively), confirming that the two primers were located within an exon. Identically sized PCR products were produced (Fig. 9, lanes 4, 5, and 14) from genomic DNAs of three human-hamster hybrid cell lines which all contain human chromosome 3 (Table 3). In contrast, a 399-bp PCR product was not generated in human-hamster hybrid cell lines lacking human chromosome 3. These results clearly demonstrated that the I-plastin gene is located on human chromosome 3.

DISCUSSION

I-plastin is the human homolog of chicken intestine fimbrin. This conclusion is based on the sequence homology, actin-bundling activity, and localization to the brush border. We previously thought that T-plastin represented the intestine homolog primarily because T-plastin expression was detected in intestinal cell lines. However, our results now show that intestinal cell lines display a pattern of plastin expression different from that of isolated intestinal epithelial cells (Table 2). For example, Caco-2, a cell line derived from a human adenocarcinoma, expresses high levels of all three plastin isoforms, including L-plastin, an isoform exclusively expressed in leucocyte-derived cells. L-plastin mRNA is not detected in isolated intestinal epithelial cells. Presumably, the L-plastin mRNA in intestine biopsy samples (Fig. 4) arose from leukocytes which populate the lymph nodes in the submucosa of the intestine. This interpretation is supported by immunofluorescence studies of mouse intestine (result not shown), which revealed L-plastin in single cells infiltrating the epithelium but not in epithelial cells. In contrast to the limited expression of I- and L-plastins, T-plastin is a more generally expressed isoform because of its presence in a wide variety of organs.

The tissue-specific pattern of I-plastin expression reported in this study is strikingly similar to the pattern of villin expression. Both I-plastin and villin are expressed by enterocytes of the intestinal epithelium and are localized by immunofluorescence microscopy to the brush border microvilli. In the kidneys, in which I-plastin mRNA can be detected, villin protein is in the brush border of the proximal tubule epithelium; on the basis of the functional similarity between intestinal and kidney epithelia, we conclude that I-plastin is also localized to the brush border of proximal tubule cells. This conclusion is supported by preliminary studies on I-plastin expression in the visceral yolk sac of the mouse embryo. Our previous studies localized villin and fimbrin to the brush border microvilli of this absorptive epithelium (10). Our preliminary immunofluorescence studies with I-plastin-specific antibodies

confirmed that I-plastin can be detected in the embryonic visceral endoderm (data not shown). The correlation between villin and I-plastin localization to the brush border of a variety of absorptive tissues suggests that the brush border cytoskeleton has evolved to perform a specialized function. Consistent with this suggestion is the observation that the brush border also contains a unique nonmuscle actin isoform (27). In addition, the common pattern of expression of villin and I-plastin may result from coordinate regulation of their genes.

Our observation that the plastin sequence is more highly conserved among intestinal isoforms in chickens and humans than among plastin isoforms in humans suggests that the plastins are functionally different. The difference in sequence is accompanied by a difference in the localization of an isoform within a cell. For example, I-plastin is localized to the actin bundle within brush border microvilli. In contrast, L-plastin is not localized to actin stress fibers in leukocytes but to "podosomes" or regions of adhesion to the substratum in which the ends of stress fibers are associated with the ventral surface of cells (7, 18). It will be interesting to examine cells which express several plastin isoforms. Discrete localization of the isoforms to different cytoplasmic structures would be further evidence for functional specialization.

Limited proteolytic digestion of chicken fimbrin generates two peptide fragments, a 114-residue headpiece peptide and a 516-residue core (9). The headpiece contains two putative calcium-binding sites, while the core contains two duplicated actin-binding domains. Despite its actin cross-linking function, I-plastin is most diverged in sequence from the other plastin isoforms in the headpiece segment. This fact suggests the possibility that the isoforms differ in calcium binding or calcium regulation. Amino acid sequence differences in similar calcium-binding domains of troponin C differentiate cardiac troponin C from skeletal muscle troponin C with regard to their muscle length-dependent calcium sensitivity (4). We speculate that amino acid sequence differences in calcium-binding domains may also differentiate one plastin isoform from another on the basis of their responsiveness to different concentrations of calcium ions. This response may in turn regulate association and dissociation between plastin and actin filaments. These hypotheses could be tested by analyzing the localization of different chimeras constructed between plastin isoforms and transfected into cultured cell lines.

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