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# Cloning of human erythroid dematin reveals another member of the villin family

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**ABSTRACT** **Dematin is an actin-bundling protein originally identified in the human erythroid membrane skeleton. Its actin-bundling activity is abolished upon phosphorylation by the cAMP-dependent protein kinase and is restored after dephosphorylation. Here we report the complete primary structure of human erythroid dematin, whose sequence includes a homologue of the "headpiece" sequence found at the C terminus of villin. This headpiece is essential for villin function in inducing microvillar development and actin redistribution. The widespread expression of dematin transcripts in human tissues suggests that dematin and its homologues may substitute for villin in villin-negative tissues to regulate actin reorganization by a phosphorylation-regulated mechanism.**

Human erythrocyte dematin consists of two polypeptide chains of 48 and 52 kDa, which have been identified as protein 4.9 on SDS/polyacrylamide gels (1–3). The 48- and 52-kDa subunits are present in a ratio of 3:1 as determined by staining with Coomassie blue (1–3). In solution, dematin exists as a trimer and bundles actin filaments in a phosphorylation-dependent manner (1–3). Dematin is a substrate of multiple protein kinases (4, 5) including protein kinase C, but only phosphorylation by the cAMP-dependent protein kinase abolishes its actin-bundling activity (2, 3). However, the mechanism by which phosphorylation and dephosphorylation regulate dematin's actin-bundling activity is not known.

The core of the human erythroid membrane skeleton consists of spectrin, actin, protein 4.1, and dematin (1). Although many of the functional interactions of spectrin, actin, and protein 4.1 have been characterized in the past, virtually nothing is known about the *in vivo* function of dematin. Since isoforms of dematin exist in many nonerythroid cells (6), it was considered of interest to elucidate dematin's primary structure. Here we report the complete amino acid sequence of human dematin translated from the nucleotide sequence of reticulocyte cDNA.** The availability of dematin's primary structure will permit further experimentation to determine its physiological function in both erythroid and nonerythroid cells.

## MATERIALS AND METHODS

A human reticulocyte cDNA library was constructed in a λZAP II vector (Stratagene). This cDNA library containing $3.6 \times 10^6$ primary plaques (>5% nonrecombinants) was screened with a 155-bp cDNA probe, which was radiolabeled using a random primer kit (Amersham). This cDNA probe was originally isolated from a chicken reticulocyte cDNA library using affinity-purified dematin antibodies. Positive plaques were further purified, and cDNA inserts in pBluescript plasmid were excised from the λZAP II vector. The 3′ rapid amplification of cDNA ends protocol was performed essentially as described (7); 2.0 μg of total reticulocyte RNA was reverse transcribed using the following primer: 5′-GACTCGAGTCGACATCGATTTTTTTTTTTTTTT-3′ (primer 1). The cDNA pool was amplified using an adapter primer [5′-GACTCGAGTCGACATCG-3′ (primer 2)] and an upstream primer [5′-TGTCACCCAAATCCACAT-3′ (primer 3; nucleotides 706–724; Fig. 2)] derived from dematin's sequence. The 5′ end was reverse transcribed using the primer 5′-GGAGGTAAGTGGTTGCTT-3′ (reverse primer 4; nucleotides 465–483; Fig. 2) essentially as described above (7). The cDNA was tailed with poly(dA) and amplified using appropriate concentrations of primers 1 and 2 and a nested dematin primer, 5′-ACTCTCCAGGCTTAGACAG-3′ (reverse primer 5; nucleotides 271–290; Fig. 2). The amplified products were subcloned in a TA Cloning vector system (Invitrogen) and sequenced by the dideoxynucleotide chain-termination method (8). Both strands of the cDNA clones were sequenced. The nucleotide and deduced amino acid sequences were analyzed by using the PC-GENE program from Intelligenetics and the hydrophilicity plot was generated by using the MACVECTOR program from IBI. The mass of dematin was determined by matrix-assisted laser desorption mass spectrometry using a Finnigan MAT lasermat at the Whitehead Institute, Massachusetts Institute of Technology, in the laboratory of P. Matsudaira.

The total reticulocyte RNA was isolated by using a method based on selective lysis of leukocytes (9). This RNA was separated on a 1.2% agarose gel containing 2.2 M formaldehyde, transferred to nitrocellulose, and then hybridized with $^{32}$P-labeled cDNA probes. Both the 1.3-kb and 2.0-kb cDNA probes were incubated under the following conditions: 5× standard saline phosphate/EDTA, 10× Denhardt's solution, 50% (vol/vol) formamide, 2% SDS, and salmon sperm DNA (100 μg/ml) at 42°C. Blots were washed under stringent conditions using 0.2× standard saline citrate and 0.1% SDS at 65°C (10) and exposed at −70°C for 48 hr.

Fast protein liquid chromatography-purified dematin (2, 3) was incubated with the appropriate concentrations of chymotrypsin on ice for 90 min. Protease activity was then quenched by the addition of 2.0 mM diisopropyl fluorophosphate. A portion of each sample was tested for actin-bundling activity by phase-contrast microscopy. Actin filaments were added to the remaining sample volumes, which were incubated in the bovine serum albumin-coated ultracentrifuge tubes for 30 min at room temperature. Actin was then sedimented at 35,000 rpm for 1 hr in a type 42.2 Ti rotor.

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. L19713).

Supernatants and pellets were carefully separated and subjected to SDS/PAGE on 14–18% gradient gels.

## RESULTS AND DISCUSSION

As a first step toward elucidating the mechanism by which phosphorylation abolishes dematin's actin-bundling activity, the primary structure of erythroid dematin was determined. A cDNA library was constructed from human reticulocyte mRNA, which was primed with both oligo(dT) and random primers. A 1.3-kb cDNA fragment was isolated by using a combination of antibody and cDNA probes. The complete 3′ end of the cDNA was isolated using the rapid amplification of cDNA ends protocol (7). A 2.0-kb overlapping cDNA fragment was amplified by using the polymerase chain reaction and then subcloned and sequenced.

The organization of the cDNA clones shows an open reading frame beginning at nucleotide 451 and predicting a protein containing 383 amino acids (Figs. 1*A* and 2). The predicted sequence has a calculated molecular mass of 43,121 Da and an isoelectric point of 9.54. To demonstrate that this predicted amino acid sequence encodes an authentic dematin, the sequences of seven peptides derived from highly purified erythroid dematin were compared with the predicted sequence. All of the peptides matched the predicted sequence as underlined in Fig. 2. As further evidence, direct quantification of the molecular mass of the 48-kDa subunit by matrix-assisted laser desorption mass spectrometry (11) yielded a value of 43,265 Da. The discrepancy between the actual and electrophoretically estimated molecular masses is likely to be due to dematin's high proline content (10.7%) and the presence of a negatively charged amino acid cluster, both of which are known to alter protein mobility on SDS gels (12, 13). The molecular mass of the 52-kDa polypeptide of dematin was quantified as 46,175 Da by laser desorption spectrometry. Differential phosphorylation has been ruled out as a mechanism for generating the 52-kDa subunit (data not shown), and at present the origin of the 52-kDa subunit is not known. The close correspondence between the measured and predicted molecular masses of the 48-kDa subunit thus suggests that the open reading frame encodes the 48-kDa subunit of dematin (Fig. 2).

The total 2.7-kb nucleotide sequence of the cDNA clones shown in Fig. 2 is in agreement with the 2.8-kb transcript of dematin in human reticulocytes (see Fig. 4*A*). The evidence for the initiation codon, ATG, comes from a sequence match with an N-terminal peptide derived from purified dematin (Fig. 2). It should be noted that the N termini of both the 48- and 52-kDa polypeptides of dematin are blocked. In addition, agreement between the predicted and measured molecular masses of dematin further supports the validity of the initiation codon. The evidence for the complete 5′ end of the cDNA was obtained using the rapid amplification of cDNA ends protocol (7). Analysis of the coding sequence indicated a highly hydrophilic protein consisting of 122 charged residues (Fig. 1*B*). One especially interesting region in the amino acid sequence of dematin consists of a negatively charged cluster containing seven consecutive glutamic acid residues followed by three aspartic acid residues (Fig. 2; residues 216–225). Although the functional significance of this motif is not known, a sequence homology search using BLAST (14) indicated the presence of this motif in many proteins. A number of these proteins are known to be associated with the cell nucleus. This group of proteins includes human major centromere autoantigen (13), frog nucleoplasmin A (15), chicken progesterone receptor (16), and nucleosome assembly protein (17). The closest sequence similarity to the poly(glutamic acid) motif of dematin was found in a motif present in murine neuronal band 3-like proteins (18). A 73% identity was noted, which included flanking amino acids:

| | |
|---|---|
| GAEEEEEEEDDDSGEEM | Dematin |
| GAEEEEEEEEEEEGESE | Mouse brain AE3 |

The poly(glutamic acid) motif in mouse brain AE3 (band 3-like protein) (18) is located near the N terminus and is not found in mature erythrocyte band 3 (19). Indeed, when an alternatively spliced form of AE3, which was encoded by the N-terminal 600 amino acids, was expressed in COS cells, the expressed protein was almost completely localized in the cell nucleus (R. Kopito and C. Morgans, personal communication). These observations suggest that the negatively charged poly(glutamic acid) motif may constitute a nuclear localization/interaction signal in these proteins. In fact, a punctate pattern of dematin was observed by immunofluorescence around the cell nucleus in human erythroblasts cultured *in vitro* (A.H.C. and M. Hanspal, unpublished data). Interestingly, actin bundles associated with the cell nucleus have been visualized in late erythroblasts undergoing enucleation (20), and whether dematin plays a functional role in such bundling events remains to be elucidated.

The primary structure of dematin includes a PEST sequence (residues 90–103), a motif generally found in signal-transduction proteins that undergo rapid turnover *in vivo* (21). The presence of a PEST sequence in dematin is consistent with our earlier observation that dematin synthesized early (day 5) in avian erythroblasts is rapidly degraded (22). A computer search predicted three potential sites of phosphorylation by cAMP/cGMP kinases (residues 211, 267, and 381) and seven phosphorylation sites each of protein kinase

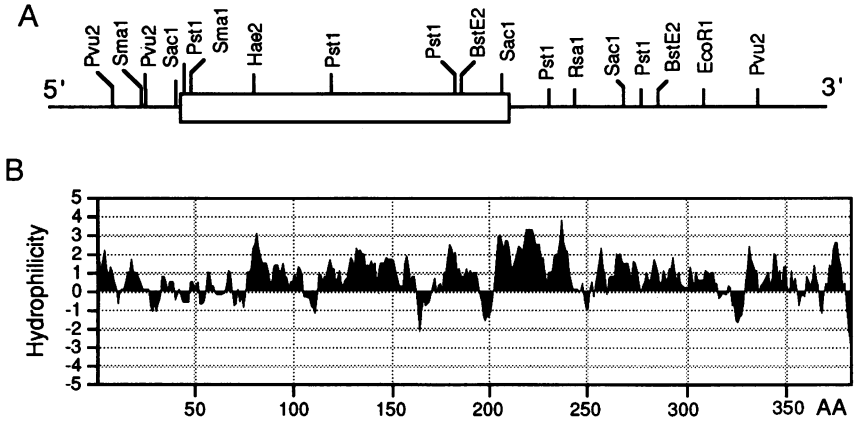


FIG. 1. (*A*) Composite restriction map of two cDNA fragments encoding human reticulocyte dematin. (*B*) Hydrophilicity plot of dematin. AA, amino acids.

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CACGAGAAGACAGGAGGAAGAAAGGGAGAGAGGGCCAGGCAGTCGCACTGTGAACAGAAC   60
AGGAGAAGGCGAAGCGGGGCAAAGTTCCCTGCCCACCGACGCCAGCCTGCTTGGATGACT  120
TGCCTCGTTTCATAATTCACTTACTGTCTGCACCAGCCGGCCTCAGCCTGGCTGGACCCT  180
GCTGCCTGTGTGGCCCGGAGCCAGAGGCCCCCACACTCCCAGCTGCTCTTCTACAGATGC  240
CATCAACGAGCAGGACTCTGGGTGGCTCCACTGTCTAAGCCTGGAGAGTCACCGCCGAGG  300
GATGAGGACGCGCCAGCCCGGGGGAACGCGCCAGCTGCTTTCGCGGCCCCAAGGCGCGAG  360
TGCCCAGCAGCCGCGCCGAGCCTGACACGCTGTCCTCTCCCCTCGCGCACAGGGCTCTGC  420
GAGTGACCCGGCGGGCGAGCTCCGTGCTGCATGGAACGGCTGCAGAAGCAACCACTTACC  480
                    M  E  R  L  Q  K  Q  P  L  T                10

TCCCCCGGGAGCGTGAGCCCCTCCCGAGATTCCAGTGTGCCTGGCTCTCCCTCCAGCATC  540
 S  P  G  S  V  S  P  S  R  D  S  S  V  P  G  S  P  S  S  I   30

GTGGCCAAGATGGACAATCAGGTGCTGGGCTACAAGGACCTGGCTGCCATCCCCAAGGAC  600
 V  A  K  M  D  N  Q  V  L  G  Y  K  D  I  A  A  I  P  K  D   50

AAGGCCATCCTGGACATCGAGCGGCCCGACCTCATGATCTACGAGCCTCACTTCACTTAT  660
 K  A  I  L  D  I  E  R  P  D  L  M  I  Y  E  P  H  F  T  Y   70

TCCCTCCTGGAACACGTGGAGCTGCCTCGCCAGCGCGAGCGCTCGCTGTCACCCAAATCC  720
 S  L  L  E  H  V  E  L  P  R  Q  R  E  R  S  L  S  P  K  S   90

ACATCCCCCCCACCATCCCCAGAGGTGTGGGCGGACAGCCGGTCGCCTGGAATCATCTCT  780
 T  S  P  P  P  S  P  E  V  W  A  D  S  R  S  P  G  I  I  S  110

CAGGCCTCGGCCCCCAGAACCACTGGAACCCCCCGGACCAGCCTGCCCCATTTCCACCAC  840
 Q  A  S  A  P  R  T  T  G  T  P  R  T  S  L  P  H  F  H  H  130

CCTGAGACCTCCCGCCCAGATTCCAACATCTACAAGAAGCCTCCCATCTATAAGCAGAGA  900
 P  E  T  S  R  P  D  S  N  I  Y  K  K  P  P  I  Y  K  Q  R  150

GAGTCCGTGGGAGGCAGCCCTCAGACCAAGCACCTCATCGAGGATCTCATCATCGAGTCA  960
 E  S  V  G  G  S  P  Q  T  K  H  L  I  E  D  L  I  I  E  S  170

TCCAAGTTTCCTGCAGCCCAGCCCCCAGACCCCAACCAGCCAGCCAAAATCGAAACCGAC 1020
 S  K  F  P  A  A  Q  P  P  D  P  N  Q  P  A  K  I  E  T  D  190

TACTGGCCATGCCCCCCGTCTCTGGCTGTTGTGGAGACAGAATGGAGGAAGCGGAAGGCG 1080
 Y  W  P  (C)  P  P  S  L  A  V  V  E  T  E  W  R  K  R  K  A  210

TCTCGGAGGGGAGCAGAGGAAGAGGAGGAGGAGGAAGATGACGACTCTGGAGAGGAGATG 1140
 S  R  R  G  A  E  E  E  E  E  E  E  D  D  D  S  G  E  E  M  230

AAGGCTCTCAGGGAGCGTCAGAGAGAGGAACTCAGTAAGGTTACTTCCAACTTGGGAAAG 1200
 K  A  L  R  E  R  Q  R  E  E  L  S  K  V  T  S  N  L  G  K  250

ATGATCTTGAAAGAAGAGATGGAAAAGTCATTGCCGATCCGAAGGAAAACCCGCTCTCTG 1260
 M  I  L  K  E  E  M  E  K  S  L  P  I  R  R  K  T  R  S  L  270

CCTGACCGGACACCCTTCCATACCTCCTTGCACCAGGGAACGTCTAAATCTTCCTCTCTC 1320
 P  D  R  T  P  F  H  T  S  L  H  Q  G  T  S  K  S  S  S  L  290

CCCCGCTATGGCAGGACCACCCTGAGCCGGCTACAGTCCACGGAGTTCAGCCCATCAGGG 1380
 P  R  Y  G  R  T  T  L  S  R  L  Q  S  T  E  F  S  P  [S  G  310

AGTGAGACTGGAAGCCCAGGCCTGCAGATCTATCCCTATGAAGTGCTAGTGGTGACCAAC 1440
 S  E  T  G  S  P  G  L  Q  I  Y  P  Y  E  V  L  V  V  T  N  330

AAGGGGCGAACCAAGCTGCCACCGGGGGTGGATCGGATGCGGCTTGAGAGGCATCTGTCT 1500
 K  G  R  T  K  L  P  P  G  V  D  R  M  R  L  E  R  H  L  S  350

GCCGAGGACTTCTCAAGGGTATCTGCCATGTCCCCTGAAGAGTTTGGCAAGCTGGCTCTG 1560
 A  E  D  F  S  R  V  S  A  M  S  P  E  E  F  G  K  L  A  L  370

TGGAAGCGGAATGAGCTCAAGAAGAAGGCCTCTCTCTTCTGATGGCCCCCACCTGCTCCG 1620
 W  K  R  N  E  L  K  K  K  A  S  L  F]  *                   383

GGACGGCCCCCTTACCCCTGCTGCTTCAGGGTTTTTCCCCGGCGGGTTGGGAGGGGCAGG 1680
AGGTGGGGTGGAAATAGGGTGGGCTCCTTTCCTCAGGTAGAGTGGGGGGCCAAAACCTCT 1740
GCAGTCCCCGGCAGTGAGCTATGGACTTTCTTCCCCCTCACGAGGCTGGGGGCCTCCTGC 1800
TCTCGTCCCTGGCCCTCCCTGTACAGGGCAAAGCCAGTCTGGGCTCTGGCACACAGAGTT 1860
CATGTTTGCCGCCCTCTCCCTGCCCCTCACCCCAGAGGTGAGAGGAATGAGGGGCATTGG 1920
TGGTTAGGCCGGTTGGCTGTCTTGAACAGCTGGAGGGAAGATGCAGGGGTGGGAAGCGGC 1980
CAGGCAGAAAGAGCTCCAGGCTCTTGTGTCGCCCACCCAGCCCTCCCATACTCACTCCTG 2040
ACAGCTTTCCTGCACTGCAGCTTCCTGCTCCTCTGACTCTAGTGGGAACAGGCCCCAGCT 2100
CAGCCTCCGCGAGGGAGGTCACCCCTCCACTTCAGCTTGCCCTGACCTCCGCTCGCAAAC 2160
CCCGAGCTTCCAAGCCTTTTGCTCCAGCCCTGCGGCTTCCCCAGAAGCCTGGGCTTAGGG 2220
TGGAGATGCCGCCTACCCGATCCTGGCCCTCCACCTGCCTCCAGGCCACGAAATGGGAAT 2280
TCCAGCACTAAGCCAGGCACCGGGCAGAAGCTGGGCCTTCCGCCTCCCTTGGATGGGGTC 2340
AAGAGGCCAGGCCTGGCACATTTTGGAGTGTCCTGGCTACCAGCTCTCACACCTACACCC 2400
ACGCACCCCCTCACACACTATGCTCTCTCAAGAATGTAATTTATTGGGGCCCCCCCAGCT 2460
GCTTTCCTCACCTGCCCCTGCCCTACCTTACACCCCCAGCTTGACTTCTTTCCAGTCCAC 2520
GTGGATATAATGATATCTATATTTTTGCCCAGGTCTGGGTATTGCTCCTGCCCAGACCCT 2580
GACATCCCTTTCCACTGTGTGTGTGACCATGCTGGGGGAGGGGGACTCTGCTTGAATTA 2640
AAAGGTTGCTTTGGGTCCCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA          2690
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FIG. 2. Nucleotide and amino acid sequence of human reticulocyte dematin. The underlined amino acid residues were confirmed by sequence analysis of dematin peptides obtained from poly(vinylidene difluoride) blots. The last underlined amino acid sequence was obtained from a dematin peptide sedimented with actin filaments as shown in Fig. 5*B*. The dotted line highlights the headpiece of dematin. The single cysteine-194 is circled.

C (residues 87, 120, 133, 170, 211, 284, and 329) and of casein kinase II (residues 71, 134, 226, 269, 309, 350, and 361) (Fig. 2). It is interesting to note that there is only one cysteine residue (cysteine-194) in the predicted sequence of dematin. This was confirmed by direct quantification of cysteic acid, which yielded 1.2 residues of cysteine per dematin molecule (Fig. 2). The significance of a single cysteine may become apparent when elucidating sites of trimer formation, since individual monomers in a dematin trimer are known to be crosslinked by sulfydryl groups (1). However, the resolution of this issue may require an analysis of the 52-kDa polypeptide of dematin, which may participate in the trimer formation. Using both the BLAST (14) and FASTA (23) programs, dematin revealed significant sequence homology only with villin. The C-terminal 66 amino acids of dematin showed 48.5% identity with the C terminus of human villin (24) and 47.0% identity with the C terminus of chicken villin (25) (Fig. 3). Less significant identity extended further upstream including 9 additional amino acids (Fig. 2).

Villin is an actin-binding protein associated with the brush border of certain gastrointestinal and urogenital epithelia (26). *In vitro* villin crosslinks actin filaments into bundles at low calcium concentrations and caps and severs actin filaments at high (micromolar or above) calcium concentrations (27, 28). The C-terminal 75 amino acids of villin define a unique region referred to as the "headpiece," which contains an actin-binding site and is essential for villin's function *in vivo* (29). The discovery of a similar headpiece in dematin marks the first instance, to our knowledge, that this sequence has been found in a protein other than villin. Villin has been extensively investigated as a potential marker for brush border differentiation and to establish the cellular origin of certain epithelial carcinomas (26). When the tissue specificity of dematin was compared with that of villin by Northern blot analysis, dematin transcripts were detected in heart, brain, lung, skeletal muscle, and kidney (Fig. 4). Placenta, liver, and pancreas yielded relatively weak signals (Fig. 4). At this stage, we have not yet examined the expression of dematin in the intestine where the expression of villin is abundant. Previously, immunoreactive forms of dematin have been observed in various nonerythroid tissues (6).

Villin is a monomeric protein that, by binding actin filaments to its headpiece and to a second site within its core domain, produces bundles (30). In contrast, dematin exists in solution as a trimer (1), and whether it contains a second actin-binding site in its undefined domain remains to be investigated (Fig. 3). To directly demonstrate that the head-

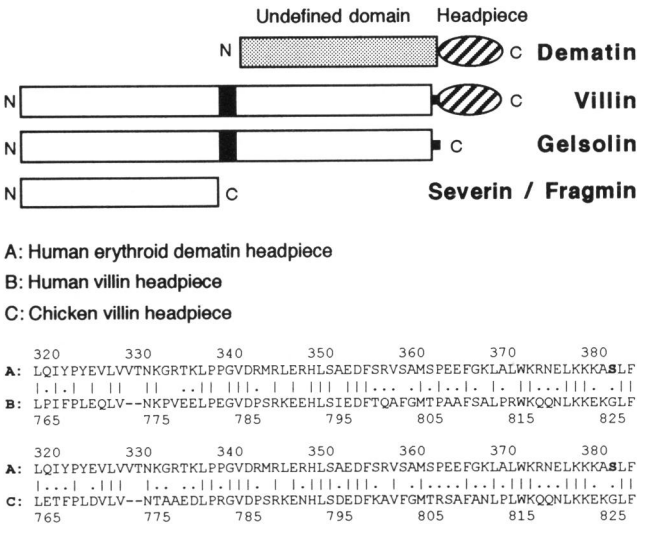


FIG. 3. Domain organization of dematin and its comparison with villin headpiece. Schematic alignment of the C-terminal headpiece of dematin. Sequence alignment of the dematin and villin headpieces was carried out using FASTA. Serine-381 (shown in boldface type) in the dematin headpiece is a consensus phosphorylation site for cAMP-dependent protein kinase. Note that the N-terminal core of villin, which is homologous to gelsolin, severin, and fragmin, does not show any similarity to the undefined domain of dematin.

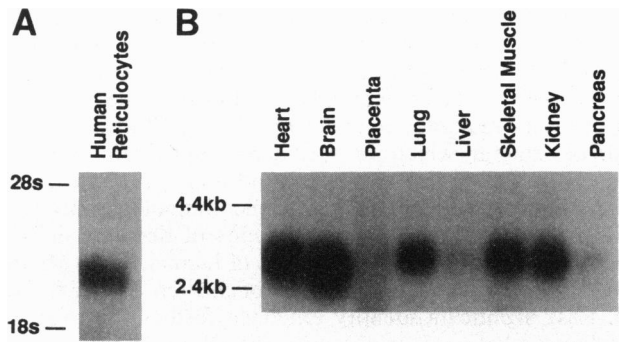


FIG. 4. Northern blot analysis. (*A*) Total RNA from human reticulocytes. (*B*) Northern blot containing 20 μg of total RNA from human tissues was obtained from Clontech. The blots were hybridized with $^{32}$P-labeled cDNA probes of dematin.

piece of dematin binds to actin filaments, purified dematin was subjected to limited proteolysis with increasing concentrations of chymotrypsin (Fig. 5). After protease inactivation, each dematin sample was tested for (*i*) extent of proteolysis, (*ii*) actin-bundling activity, and (*iii*) sedimentation of dematin fragments with actin filaments. As shown in Fig. 5, increasing the proteolysis of dematin was paralleled by the loss of dematin's actin-bundling activity and resulted in the production of a 13-kDa fragment that sedimented with actin filaments (Fig. 5*B*, lane 5, arrowhead). The photograph shown in Fig. 5*C* represents a typical field with no bundles observed in the digested dematin sample in lane 5. Direct microsequencing of the sedimented 13-kDa fragment confirmed its derivation from the headpiece of dematin (Fig. 2, last underlined peptide). These results suggest that the dematin headpiece alone, like the headpiece of villin, can bind but cannot bundle actin filaments (29). An unusual feature of the headpiece of dematin is the presence of a serine residue in place of glycine-381 in villin (Fig. 3). This serine is a consensus phosphorylation site for the cAMP-dependent protein kinase. If serine-381 is indeed phosphorylated by protein kinase A, it may account for the fact that the actin-bundling activity of dematin (2) but not of villin (29) is subject to regulation by phosphorylation.

Experimental evidence thus far supports three potential physiological activities of dematin. (*i*) In mature erythrocytes where actin bundles appear to be absent, dematin's actin-binding activity rather than its bundling property may be functionally more relevant. We propose that dematin, like protein 4.1 (31), links spectrin–actin complexes to the plasma membrane. This proposed mechanism would provide a necessary redundancy in the membrane linkage that is critically important for membrane mechanical properties and erythrocyte shape (32). Preliminary evidence that lends support to this hypothesis includes the following: dematin has been localized to spectrin–actin junctions (33), dematin binds to an unidentified protein in membrane vesicles devoid of spectrin and actin (34), and dematin specifically associates with human erythroid β-spectrin in blot overlay assays (A.H.C., unpublished data). (*ii*) Dematin's localization in erythroblasts (data not shown) suggests a potential role for dematin during erythroid development. The availability of the primary structure of dematin may permit the application of antisense technology to determine whether dematin participates in the formation of actin bundles that exist in erythroblasts undergoing enucleation (20). (*iii*) It has been suggested that RET52,

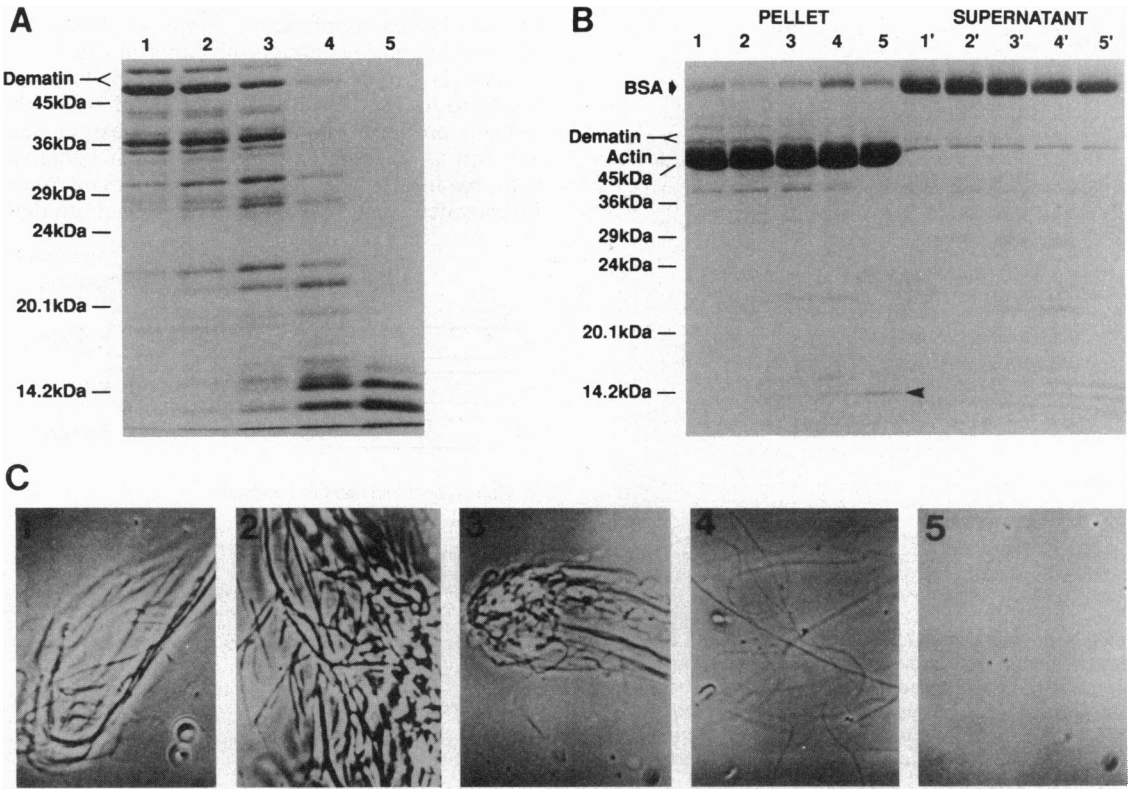


FIG. 5. Limited proteolysis of dematin and sedimentation of headpiece with actin filaments. (*A*) Proteolysis of purified dematin. Dematin (3.0 μg) was subjected to proteolysis with increasing concentration of chymotrypsin: 0.38 ng (lane 1), 0.75 ng (lane 2), 1.5 ng (lane 3), 3.0 ng (lane 4), and 6.0 ng (lane 5). (*B*) Sedimentation of dematin peptides with actin filaments. Lanes 1–5 show pelleted actin filaments and dematin peptides. Lanes 1′–5′ show corresponding supernatants. The N terminus of the sedimented peptide in lane 5 (arrowhead) was directly sequenced after transfer to a poly(vinylidene difluoride) membrane. The N-terminal sequence of the sedimented peptide is shown as the last underlined peptide in Fig. 2. Note that the centrifugal force was sufficient to sediment actin bundles as well as actin filaments. BSA, bovine serum albumin. (*C*) The sample in each lane was assayed for the actin-bundling activity by phase-contrast microscopy.

an immunoreactive homolog of erythroid dematin found in mouse retina (35), may play a role in the regulation of the disk membrane assembly and synapse formation within photoreceptors. The primary sequence will allow identification of nonerythroid forms of dematin, such as in the brain, where dematin has been localized in a subset of neurons (6).

In summary, the molecular cloning of dematin describes a member of a family of proteins whose actin-bundling function may be important in a wide variety of nonerythroid cells. Moreover, the primary structure of dematin will allow elucidation of the mechanism by which phosphorylation abolishes its actin-bundling activity. These observations are likely to reveal a novel pathway that regulates cytoskeletal–membrane interactions and thus cellular shape in many eukaryotic cells.

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