

Sequence Similarity of the Amino-terminal Domain of *Drosophila* Beta Spectrin to Alpha Actinin and Dystrophin

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Abstract. We used chicken alpha spectrin as a ligand probe to isolate *Drosophila* beta spectrin cDNA sequences from a lambda gt11 expression library. Analysis of 800 residues of deduced amino acid sequence at the amino-terminal end revealed a strikingly conserved domain of ~230 residues that shows a high degree of sequence similarity to the amino-terminal domains of alpha actinin and dystrophin. This conserved domain constitutes a new diagnostic criterion for spectrin-

related proteins and allows the known properties of one of these proteins to predict functional properties of the others. The conservation of the amino-terminal domain, and other regions in spectrin, alpha actinin, and dystrophin, demonstrates that a common set of domains were linked in different combinations through evolution to generate the distinctive members of the spectrin superfamily.

THE properties of spectrin are well known, especially in the human erythrocyte membrane where this protein cross-links F-actin and associates with several other proteins to form a submembrane meshwork. The distinctive repeating structure of spectrin (Speicher and Marchesi, 1984), once considered diagnostic for that protein (Speicher, 1986), is now recognized as a unifying feature of a group of related proteins that includes alpha actinin (Baron et al., 1987; Wasenius et al., 1987; Noegel et al., 1987; Arimura et al., 1988) and dystrophin, the product of the Duchenne muscular dystrophy gene (Davison and Critchley, 1988; Koenig et al., 1988). Although each repeating segment in spectrin is characterized by a set of consensus residues and a length of ~106 amino acids, the primary sequence is not highly conserved between repeats in a given molecule.

An understanding of the relations between spectrin, alpha actinin, and dystrophin has been limited by the paucity of beta spectrin sequence data. Although full-length alpha spectrin cDNA sequences have been analyzed (Wasenius et al., 1989; Dubreuil et al., 1989), the only available beta spectrin sequence has been a partial peptide and cDNA sequence from the central region and carboxy-terminal end of the human erythroid isoform (Speicher and Marchesi, 1984; Prchal et al., 1987; and Winkelmann et al., 1988). The sequence of the COOH-terminal region of alpha spectrin is similar to the COOH-terminal half of alpha actinin (Wasenius et al., 1989; Dubreuil et al., 1989) and includes two EF-hand (Ca⁺⁺-binding) structures. There is also limited similarity between *Dictyostelium* alpha actinin and dystrophin in the same region (Koenig et al., 1988). In addition, alpha actinin shares a conserved NH₂-terminal domain with dystrophin (Hammonds, 1987; Koenig et al., 1988). This latter observa-

tion is important in view of the fact that alpha actinin is an actin cross-linking protein and the NH₂-terminal domain is thought to contain the site of actin binding activity (Mimura and Asano, 1986; Baron et al., 1987). By analogy to alpha actinin, Wasenius et al. (1989) suggested that the principle actin-binding activity of spectrin resides near the amino terminus of the beta subunit, but no sequences have been reported for spectrin that match the NH₂-terminal domain of alpha actinin.

Drosophila melanogaster spectrin is similar to other known spectrins in structure and composition (Byers et al., 1987; Dubreuil et al., 1987). Using a ligand probe screening strategy, similar to that used by Sikela and Hahn (1987) to clone calmodulin-binding proteins, we now report the identification and characterization of cDNA clones that encode *Drosophila* beta spectrin. We have discovered a striking sequence conservation between the NH₂-terminal domains of beta spectrin, alpha actinin, and dystrophin that must reflect conserved structural and functional properties of these proteins. In addition, we describe a complementary pattern of sequence similarity between alpha spectrin, beta spectrin, and alpha actinin that provides important clues about their evolutionary relations.

Materials and Methods

Isolation of Beta Spectrin cDNA Clones

Chicken erythrocyte alpha spectrin was purified by preparative gel electrophoresis from chicken erythrocyte ghosts. This protein was used to probe a cDNA expression library in lambda gt11 (Young and Davis, 1984) prepared from *Drosophila* head poly A⁺ RNA (Itoh et al., 1986). 12 150-mm petri plates were screened at a density of 90,000 plaques per plate using

Escherichia coli Y1090 as host. The filters were blocked in Tween buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween-20) plus 5% newborn calf serum (Hyclone Laboratories, Logan, UT), followed by an overnight incubation at 4°C with ~0.5 µg/ml chicken alpha spectrin in the same buffer. The filters were washed with Tween buffer, blocked as above, then incubated sequentially with a 1:500 dilution of rabbit anti-chicken alpha spectrin (many thanks to Elizabeth Repasky [Roswell Park Memorial Institute, Buffalo, NY] for supplying this antibody) and a 1:250 dilution of affinity-purified goat anti-rabbit antibody conjugated to alkaline phosphatase (Zymed Laboratories, South San Francisco, CA), both in Tween buffer plus 5% newborn calf serum. The presence of alkaline phosphatase-positive plaques was detected by incubation with 60 µg/ml nitro blue tetrazolium and 60 µg/ml 5-bromo-4-chloro-3-indolyl phosphate in 0.15 M Tris-HCl, pH 8.8. Positive plaques were replated at successively lower densities to obtain clonal phage populations.

Phage DNA was prepared as described by Ziai et al. (1988). cDNA inserts from clones of interest were excised from lambda gtl1 with Eco RI and subcloned into the Eco RI site of the Bluescript SK+ vector (Stratagene, La Jolla, CA). The same cDNA library was rescreened with a ³²P-labeled cDNA probe essentially as in Benton and Davis (1977) to isolate 5' sequences.

Hybridization Analysis

To prepare hybridization probes, DNA fragment bands were excised from agarose gels, electroeluted, and either labeled with [³²P]dATP using random primers (Feinberg and Vogelstein, 1983) or nick translated with biotin-7-dATP (Bethesda Research Laboratories, Gaithersburg, MD).

Drosophila head RNA was prepared using hot phenol/SDS and further purified on an oligo (dT)-cellulose column (New England Biolabs, Beverly, MA). Genomic DNA was prepared from a 24-h collection of *Drosophila* embryos.

Poly A⁺ RNA was separated on a 0.5% agarose gel and blotted to Genescreen Plus (Dupont Co., Wilmington, DE) as described by Fournier et al. (1988). Genomic DNA was digested to completion with restriction enzymes as noted and separated on a 0.8% agarose gel for blotting onto Genescreen Plus (Dupont Co.) as suggested by the manufacturer. Hybridization was carried out in 50% formamide, 1% SDS, 1 M NaCl, 10 µg/ml salmon sperm DNA, and 10% dextran sulfate overnight at 42°C. The blots were rinsed in 1× SSC, 1% SDS at 43°C, then the DNA blot was rinsed in 0.5× SSC, 1% SDS at 65°C, and the RNA blot was rinsed in 1× SSC, 1% SDS at 50°C, followed by 0.1× SSC at room temperature for both.

Biotinylated cDNA was hybridized to the polytene chromosomes of *Drosophila* larvae essentially as in Engels et al. (1986). Labeled chromosomes were photographed under phase-contrast optics without counterstaining.

DNA Sequencing and Analysis

The B15 and B32 cDNA inserts were subcloned into Bluescript KS+ and SK+ vectors in both orientations. Each plasmid was digested with Apa I to protect the vector and Cla I to allow digestion into the insert by Exo III exonuclease. Nested deletions were constructed according to the procedure supplied with the Bluescript vectors. T3 and M13 primers were used as appropriate for double stranded DNA sequencing using the dideoxy chain termination procedure with Sequenase enzyme and Sequenase kit reagents as recommended by the supplier (United States Biochemical Corp., Cleveland, OH). All reported sequences were determined on both strands and dITP was used to eliminate compression artifacts where required. Compilation of DNA sequences and their analysis was carried out using the University of Wisconsin Genetics Computer Group (Madison, WI) software package.

Preparation of Full-length cDNA Coding Region for Expression

A deletion subclone (B15-4(2)) of clone B15 was chosen as a donor for 5' sequences because it lacked in-frame stop codons in the 5' untranslated leader sequence. The B15-4(2) cDNA insert was first removed by cutting at the polylinker sites Kpn I and Eco RI, and transferred to the Bluescript KS II+ vector prepared by cutting with the same enzymes. The 3' Sph I/Eco RI fragment of clone B15-4(2) was then replaced with the 3' 5-kb Sph I/Eco RI fragment of clone B32. The sequence of this hybrid gene (called KBA) predicted an upstream fusion sequence consisting of 20 amino acids from the start codon of the Bluescript lac Z gene to the polylinker Kpn I site, 10 amino acids contributed by 5' untranslated leader sequences followed by the predicted translational start site and remaining sequences of *Drosophila* beta spectrin.

SDS-PAGE, Antibodies, and Immunoblots

Analytical SDS-PAGE, protein blots, and purification of proteins by preparative SDS-PAGE and electroelution were carried out as described by Byers et al. (1987). Polyclonal antiserum A089 was produced as in Byers et al. (1987) using ~25 µg of clone B32 fusion protein for the primary injection and 20 µg for a boost after 1 mo. Reactivity of antiserum A089 to *Drosophila* beta spectrin was detected 1 wk after the boost. Antiserum A089 was affinity purified by absorption to B32 fusion protein on nitrocellulose blot strips as described (Byers et al., 1987).

Protein blot strips were preincubated in Tween buffer plus 5% newborn calf serum, then incubated in the same buffer plus diluted polyclonal antiserum or a 1:1 dilution of affinity-purified antibody for 1 h (polyclonal) or overnight (affinity purified). The strips were washed with Tween buffer, then incubated in Tween buffer plus serum with a 1:1,000 dilution of alkaline phosphatase-conjugated goat anti-rabbit antibody (Zymed Laboratories) for 1 h. After washing with Tween buffer, the blots were developed as described above.

Results

Isolation of cDNA Sequences

Lacking an antibody or nucleotide probe for *Drosophila* beta spectrin, we used vertebrate alpha spectrin as an affinity probe to screen a *Drosophila* cDNA expression library. We have previously shown that vertebrate alpha and beta spectrin subunits can specifically associate with the complementary subunits of *Drosophila* spectrin (Byers et al., 1987). Purified chicken erythrocyte alpha spectrin functions as a sensitive and specific probe for *Drosophila* beta spectrin (Fig. 1 a). An anti-chicken erythrocyte alpha spectrin antibody (kindly provided by Elizabeth Repasky, Roswell Park Memorial Institute) was used to detect the bound alpha spectrin probe because it showed very little cross-reactivity with *Drosophila* proteins at the dilution used. This probe combination detected ~50 strongly positive plaques in a lambda gtl1 expression library (Itoh et al., 1986) out of a screen of ~1 × 10⁶ plaques. An advantage of this screening procedure is that the isolated cDNA clones probably encode interchain binding sites of beta spectrin and will be useful for the study of those sites. A 6-kb cDNA insert (designated as B32) from a group of cross-hybridizing positive clones was chosen for further analysis.

To isolate cDNA extending to the 5' end of the message, we first established the 5' and 3' ends of clone B32 by determining the orientation productive for fusion protein expression. We then reprobated the cDNA library with the Sph I/Eco RI fragment at the 5' end of clone B32 (Fig. 2). One of the positive cDNA clones that extended the map farthest in the 5' direction (designated B15) plus the overlapping B32 cDNA add to a total of 7 kb of contiguous cDNA sequence (Fig. 2).

Demonstration of *Drosophila* Beta Spectrin cDNA Identity

To confirm the identity of the cDNA clones, we raised antibodies against a fusion protein expressed by the B32 cDNA. Clone B32 expressed a fusion protein with an apparent molecular weight of ~210,000 as determined by SDS-PAGE. When injected into a rabbit, that polypeptide induced the production of antibodies (antiserum A089) that reacted strongly with purified *Drosophila* beta spectrin (Dubreuil et al., 1987) and detected a protein of the same mobility on blots of whole *Drosophila* head homogenates (Fig. 1, b and c, lanes 3).

Antiserum A089, purified by affinity to the B32 polypeptide, cross-reacted specifically with a variety of vertebrate

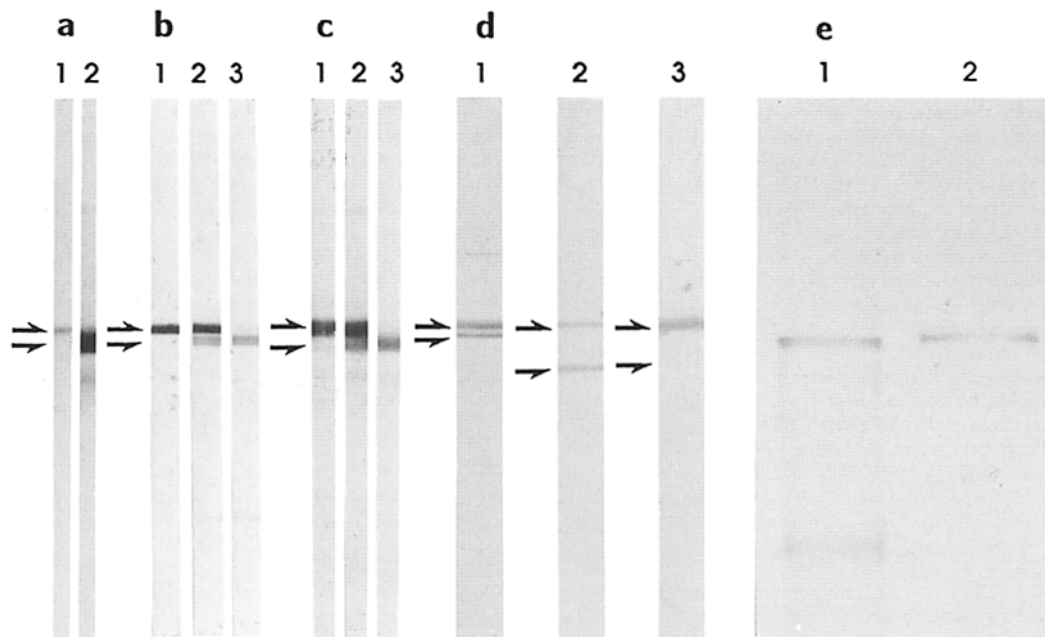


Figure 1. Protein blot analyses. (a) Protein blots of *Drosophila* head lysate probed with (lane 1) 1:500 anti-chicken alpha spectrin; or (lane 2) 0.4 $\mu\text{g/ml}$ purified chicken alpha spectrin followed by 1:500 anti-chicken alpha spectrin. (b and c) Antiserum A089 reacted specifically with *Drosophila* beta spectrin. Because *Drosophila* alpha and beta spectrins are difficult to resolve in SDS-PAGE, we stained adjacent blot strips of (b) *Drosophila* head lysate and (c) partially purified *Drosophila* spectrin (Dubreuil et al., 1987) with (lanes 1) 1:10,000 antiserum 905 (anti-*Drosophila* alpha spectrin; Byers et al., 1987), (lanes 2) 1:10,000 antiserum 905 plus 1:4,000 antiserum A089, and (lanes 3) 1:4,000 antiserum A089. Preimmune sera showed no reaction at the same dilutions. Beta spectrin often appeared as a tight doublet in *Drosophila* head blots. (d) Vertebrate spectrin-containing protein blots reacted with affinity purified antiserum A089: (lane 1) bovine brain membranes; (lane 2) human erythrocyte ghosts; and (lane 3) chicken erythrocyte ghosts. (e) Comparison of apparent molecular weights for (lane 1) *Drosophila* head homogenate and (lane 2) SDS-PAGE purified KBA fusion protein; both blotted to nitrocellulose and stained with antiserum A089. Arrowheads mark the positions of alpha (upper) and beta (lower) spectrin chains for each set of blot strips.

spectrins (Fig. 1 d). Cross-reaction with vertebrate alpha spectrin bands indicates that there are shared determinants for antiserum A089 between *Drosophila* beta and vertebrate alpha spectrins despite a specificity for the beta subunit in *Drosophila*. In fact, there was very little reaction with chicken erythrocyte beta spectrin. But, because beta spectrins often do not transfer as efficiently as the alpha subunits, the staining of the alpha band with antiserum A089 (Fig. 1 d) must not be interpreted quantitatively.

To demonstrate the coding potential of the cDNA sequences, a hybrid cDNA combining B15 and B32 sequences was constructed for fusion protein expression (see Materials and Methods). This cDNA construct (called KBA) expressed a fusion protein that was recognized by antiserum A089 (Fig. 1 e) and was comparable in size to *Drosophila* beta spectrin. This argues that the 7 kb of cDNA presented in Fig. 2 contains the full beta spectrin coding region.

Taken together, these data establish that we have isolated beta spectrin cDNA sequences. First, the cDNA sequences express polypeptides that bind specifically to alpha spectrin. Second, fusion proteins expressed by these sequences elicit antibodies that react specifically with *Drosophila* beta spectrin. Third, a cDNA construct that is full length for the predicted coding sequence expresses a polypeptide comparable in mobility to *Drosophila* beta spectrin. Finally, as shown below, the cDNA clones show a high degree of sequence identity to human erythroid beta spectrin.

Hybridization Analysis of the *Drosophila* Beta Spectrin Gene

A restriction fragment from the B32 cDNA was used as a hybridization probe to determine the size of the beta spectrin mRNA. A single band of ~ 8.0 kb was detected on Northern blots of poly A⁺ RNA isolated from *Drosophila* heads (Fig. 3 a). A single band of similar molecular weight was detected in total RNA blots of *Drosophila* S3 cells (Schneider and Blumenthal, 1978), bodies without heads, and embryos (data not shown). Since the composite cDNA length of 7 kb (Fig. 2) is sufficient to code for a protein with the apparent molecular weight of beta spectrin, we suspect that either the message size of 8 kb is an overestimate or that there are untrans-

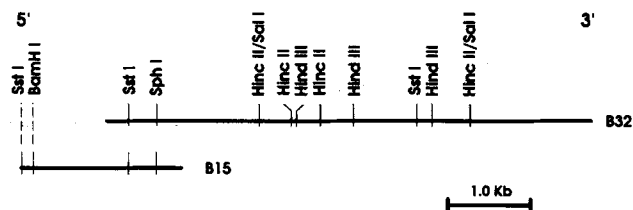


Figure 2. Restriction map of cDNA inserts B32 and B15. The positions of restriction enzyme sites are shown. Both clones were flanked by Eco RI linker sites as isolated from the library.

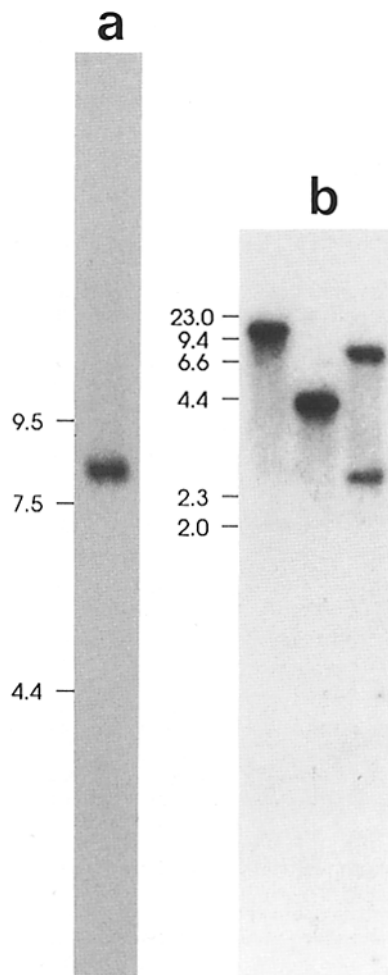


Figure 3. Hybridization analysis reveals an mRNA of ~ 8 kb and suggests that the gene is a single copy. (a) Autoradiogram showing hybridization of the ^{32}P -labeled 3' Eco RI/Hind III fragment (~ 2 kb) of B32 cDNA to a Northern blot of 3 μg poly A⁺ RNA isolated from *Drosophila* heads. (b) Southern blot of 1 μg each of (left lane) Bam HI-digested; (middle lane) Hind III-digested; and (right lane) Sal I-digested genomic DNA from 24-h embryos probed with the same labeled cDNA fragment as in a. The positions of RNA size markers (Bethesda Research Laboratories) or Hind III-digested lambda DNA fragments are indicated in kilobases.

lated regions of RNA not represented in the cDNA that has been cloned.

Southern blots of restriction-digested, genomic DNA were probed with beta spectrin cDNA to examine the possibility of additional cross-hybridizing genes (Fig. 3 b). Single bands were observed in Bam HI, Hind III, and Eco RI digests (data not shown for Eco RI) of genomic DNA. Two bands were detected in Sal I digests, because the probe fragment contained sequences on either side of a Sal I site (Fig. 2). The ~ 2.6 -kb Sal I band is probably the same as the Sal I fragment of the same size predicted by the cDNA restriction map, indicating that this Sal I fragment lacks introns. These results also argue that this beta spectrin gene is single copy in the *Drosophila* genome, but do not rule out the possibility of related genes that do not cross-hybridize.

The B32 cDNA insert was used for in situ hybridization to polytene chromosomes. Only one site of hybridization was

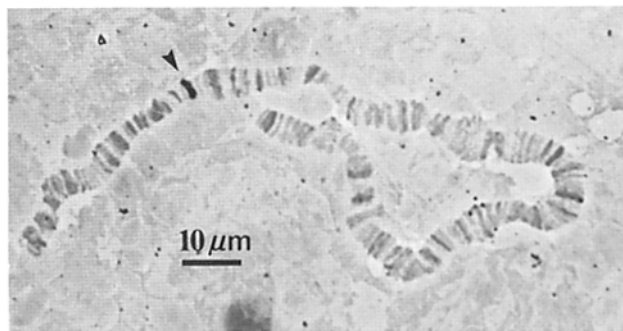


Figure 4. In situ hybridization to polytene chromosomes. The B32 cDNA insert was labeled with biotin-dATP by nick translation and was used for in situ hybridization to polytene chromosomes. Only one site of hybridization was detected in many chromosome preparations, on the X chromosome in the region of bands 16C1-4 (arrowhead). An X chromosome that had broken free of the centromere is presented here for clarity.

detected per nucleus. This locus is on the X chromosome near bands 16C1-4 (Fig. 4).

Sequence Analysis and Comparisons

The nucleotide and predicted amino acid sequence for the B15 cDNA and the 5' portion of B32 are shown in Fig. 5. There was a 941-bp overlap between the B15 and B32 cDNA sequences. The first 90 bp contain more than one stop codon in all three frames. The first possible translational start site in the cDNA sequence occurs at nucleotide 129 and begins a long open reading frame. The sequence preceding this AUG (AGCC) is not a strong match to the *Drosophila* consensus translational start site derived by Cavener (1987), but it meets the criteria of a strong preference for purine at the -3 position and a general preference for C or A at the following positions: -4 , -2 , and -1 .

The beta spectrin identity of the B15 and B32 cDNA sequences is confirmed by comparison with vertebrate beta spectrin sequence. The deduced amino acid sequence of *Drosophila* beta spectrin (residues 45–800) shows 62% identity to the deduced sequence of human erythrocyte beta spectrin (kind thanks to J. Winkelmann, J. Chang, and B. Forget for providing this sequence before publication). The deduced human erythrocyte beta spectrin sequence matches exactly with partial peptide sequence reported by Speicher and Marchesi (1984). The similarity between *Drosophila* and human sequences extended throughout the available sequence.

As discussed below, our data suggest that the NH₂-terminal region of beta spectrin can be divided into segments as indicated in Fig. 5 and as shown schematically in Fig. 6 a. The segment nomenclature is consistent with that proposed by Speicher (1986) and used by Wasenius et al., (1989) and Dubreuil et al., (manuscript submitted for publication) for alpha spectrin. For purposes of comparison with alpha actinin and dystrophin, we will refer to the corresponding regions of those proteins using the same nomenclature.

Fig. 6 a provides a schematic representation of the protein domains, and summarizes the regions of sequence similarity that will be analyzed here. Pairwise comparisons (Fig. 6 b) show that beta spectrin and alpha actinin share sequence similarity through the amino-terminal half of alpha actinin, while alpha spectrin and alpha actinin are similar through the

M T T D I S I V 8

GAGAAGTGGCGAGCTCCCGTGAACGAAACGACAAAACCAACATAGAACTAAGACGTATAAACTAGCAGCTATTAACATACTAAAGCAGAAACCACTGTGAAACCGTAGATCCCCAGCCATGACGACGGACATTCGATG 150

|< SEGMENT 1

R W D P S Q G P G N E Y I D E Y E Y D G G N S S S R L F E R S R I K A L A E E R E S V Q K K T F T K 58
 TTCGCTGGGATCCAGCCAGGCTCCGCAACGAGTACATCGATGAGTACGAGTACGATGGCGGCAACTCCAGTTCCCGACTGTCGAGCGGTCCAGATCAAGGCTCTGGCCGAGGAGCGTGAAGTGTGCAAAAAGAACGATCCACCA 300

W V N S H L C R V N C R I A D L Y V D M R D G K H L I K L L E V L S G E R L P K P T K G K M R I H C 108
 AGTGGGTCAATTCCGATCTGTGCCGGTCAACTGCCGATGCCGATCTCTATGTGGATATGCGGGATGGCAAGCAGCTGATCAAACTGCTGGAGGTCCTCTCCGGCGAGCAGCTGCCAAGCCCAAGGCGAAGATGCGTATCCACT 450

L E N V D K A L Q F L R E Q R V H L E N I G S H D I V D G N A S L N L G L I W T I I L R F Q I Q D I 158
 GCGTGGAGAACGTGGCAAGGCTCTCGAGTTCCTGCGCAAGCAGCGCTTTCATCTGGAGAAATATGGCTCCATGACATAGTGCATGGCAATGCTTCCCTCAATTTGGGCTGATTTGGACCATCTCCGCTCCAGATCCAGGACA 600

T I E E V D N K E T K S A K D A L L L W C Q M K T A G Y H N V N V R N F T T S W R D G L A F N A I I 208
 TCACCATCGAGGAGGTGGCAACAGGACCAAGTCCGCAAGGACGCCCTGCTGCTGCTGTCGAGTGAAGACTGCCGCTATCACAATGTGAAGCTGCGCAACTCACCACCTGCTGGCGGATGGCTGGCTTTAATGCCATCA 750

H K H R P D L V Q F E K L S K T N A I H N L N N A F D V A E D K L G L A K L L D A E D V F V E H P D 258
 TCCAAAACACCGTCCGATTGGTTCAGTTCGAGAACTCTCGAAGCAAGCCATCCACAACCTAAACAATGCTTCGAGCTGCCGAGGACAACTGGGCTGCCAAGCTCTGATGCCGAGGATGTTGCTCGAGCATCCGG 900

>|< SEGMENT 2

E K S I I T Y V V T Y Y H Y F S K L K Q E T V Q G K R I G K V V G I A M E N D K M V H D Y E N F T S 308
 ACGAGAAGTCCATCATCACTACCTGCTGCTACTACTACTTAGCAAACTCAAGCAGGAGCGGTGAGGGCAAGCGTATCGTAAGTGGTCCGATGGCAGGAGCAAAAATGCTCCAGCATACGAGAACTCCACAA 1050

D L L K W I E T T I Q S L G E R E F E N S L A G V Q G Q L A Q F S N Y R T I E K P P K F V E K G N L 358
 GCGATCTGCTCAAGTGGATCGAAACGACCATCCAGTCCGCTGGCGGAGCGGAGTTCGAAAACCTGCTGGCGGCTCCAAAGGCGAGTGGCCAGTCTCCAACCTACCGCACCATCGAGAAGCCGCCAAGTTTGTGAAAAGGGCAAC 1200

>|< SEGMENT 3

E V L L F T L Q S K M R A N N Q K P Y T P K E G K M I S D I N K A W E R L E K A E H E R E L A L R E 408
 TCGAGGTGCTCCTTTCCACCTCGAGTCCAGATGCGGGCCAAACCAAGGACCCCTACACCCCAAGAGGGCAAGATGATTCGGACATCAACAGGCTGGGAGCGTCTGGAGAAGCCGAGCAGCAAGCGAAATGGCCCTGCGCG 1350

E L I R Q E K L E Q L A A R F D R K A S H R E T W L S E N Q R L V S Q D N F G F D L A A V E A A A K 458
 AAGAGCTCATCCGGCAGGAGAACTGGAGCAGCTAGCCGCGCGCTTCGACCCGAAAGCGTCCATGCTGAGACCTGGCTGTCGGAGAACTCAACGCTGGTCCAGCAGGATAACTTTGGTTTCGATCTGGCGGCGTGTAGGCGGCGGCA 1500

>|< SEGMENT 4

K H E A I E T D I F A Y E E R V Q A V V A V C D E L E S E R Y H D V K R I L L R K D N V M R L W T Y 508
 AGAAGCAGAAAGCATCGAAACCGACATCTCGCCTACGAGGAGCGGTCAGGCGGTGGTGGCTTGGCATGAAGTGGAGCGTATCACAGTGAAGCGCATCTCTCGCAAGGACAACTTATCGCTTGTGGACCT 1650

L L E L L R A R R M R L E I S L Q L Q Q N F Q E H L Y I L D N M E E I K Q L L M T D D Y G K H L M G 558
 ATTTGTTGGAGCTTCTGAGAGCGCGCCGATCGCCTGGAGATCTGTTGCAGCTGCAGCAGAAGTCCAGGAGATGCTGATACCTCGCAACATGGAGGAGATCAAGCAGCTGCTGATGACCGACGATTATGGCAAGCATCTGATGG 1800

>|< SEG 5

V E D L L Q K H S L V E A D I N I L G E R V K V V V Q N S Q K F L S D D P E S Y K P C D P E I I V S 608
 CGGTGGAGGATCTGCTCGAAGCACTCGCTTGTGGAAGCCGATCAATATCTTGGCGAGCGCGTCAAGGTGGTGTTCAGAACTCGCAGAAATCTCTGAGCGACGACCCAGAGTCTGACAAACCGTGGATCCCGAGATCATTGTCT 1950

R V Q D L E D A Y A E L V R L A V E R R S R L E E S R K L W Q F Y W D T A D E E N W I K E K E Q I V 658
 CGCGCTCCAGCAACTGGAGATGCTTACCGCAGAGTGGTCCGTTGGCCGTGGAACGTCGCAAGCGGCTGGAGGAGAGCCGCAAACTCTGGCAGTCTACTGGGACACCCGCGAGGAGAACTGGATCAAGGAGAAGGAGCAGATCG 2100

S T D E V G H D L T T V N L M L S K H K A L E S E I T S H D P Q L Q N V A K V G S E L I T E G H F G 708
 TCTCCACCCAGGAGTGGTCCAGCAGCTGACCCAGCTCAATCTGATGCTCAGCAAGCACAAGGCGCTGGAGTGGAGATCAGCTGCGCAGATCCCGAGCTGCAAAAATGGGCAAGGTCGGTTCGGAGCTGATCAGCGAGGTCCTTTG 2250

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A D R I K D R L K E I L N K W D H L L D L T K Y R R Q R L E N A V E Y F Q L F A D A D D V D N W M L 758
 GTGCCGATCGCATCAAGGATCGATTGAAGGAGATCTCAACAAGTGGGACCACTTCTGGACCTCACAAGTACCAGCGCCGCGCTGGAGAAATGCGCTCGAGTACTTCCAGCTGTTGCCGATGCCGAGATGGGCAACTGGATGT 2400

D T L R I V S S E D V G R D E A N V Q S L L K K H K D V A D E L K N Y A E V I D A L 800
 TGGACCCCTGGCATTGTTCCAGCAGGAGTGTGGTGGCAGGAGTAACTGACAGTCCCTCTAAAGAAGCACAAGGAGCTGGCCGAGGAGTGAAGAATACGCCAAGTGTGATGCGCCTG 2528

Figure 5. Nucleotide sequence and deduced amino acid sequence for the 5' end of *Drosophila* beta spectrin. Nucleotide sequence was derived from the B15 and B32 cDNA clones. The boundaries of segments 1-5 are noted; the 3' boundary of segment 6 has not yet been determined.

carboxy-terminal half of alpha actinin. Thus there is a complementary sequence relation between alpha actinin and the alpha and beta spectrin subunits. The models in Fig. 6a are aligned with the x-axis of Fig. 6b for reference.

Segment 1 of *Drosophila* beta spectrin displays a high level of similarity with segment 1 of the alpha actinins (49-55% identity) and the dystrophins (47% identity) (Fig. 7, Table I). The NH₂-terminal 36 amino acids and segment 1 of beta spectrin showed no resemblance to the typical spectrin repetitive segment. As shown in the consensus row of Fig. 7, 27% of the positions across segment 1 have identical residues for all of the proteins listed. In addition, many positions also have identical residues in any two of the three protein types (Fig. 7, bold characters). At such positions, the residues in the third protein type are also frequently identical with each other. Finally, *Drosophila* beta spectrin is 77% identical to human beta spectrin (Winkelmann, J., J. Chang,

and B. Forget, personal communication) over the segment 1 region.

In segment 2, the sequence similarity between beta spectrin and alpha actinin continues at a lower level (33-41% identity, Table I). The similarity is dispersed throughout segment 2, and gaps were not required for optimal alignment. The region immediately downstream from dystrophin segment 1 does not show a better match to beta spectrin segment 2 than did shuffled versions of the same sequences. *Drosophila* beta spectrin shows 66% identity with human beta spectrin (Winkelmann, J., J. Chang, and B. Forget, personal communication) over segment 2.

Segment 2 of chicken alpha actinin bears limited features of the spectrin repeat consensus (Baron et al., 1987). A small region around tryptophan 290 of alpha actinin, and the corresponding tryptophan 313 of *Drosophila* beta spectrin, resemble the region around the "invariant" tryptophan found

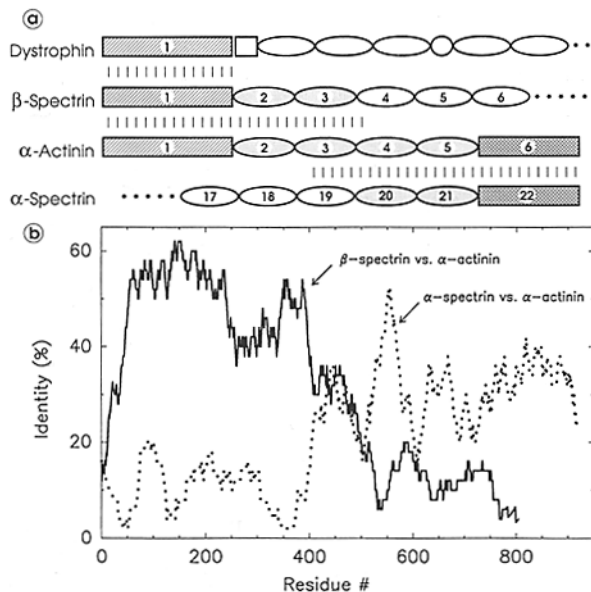


Figure 6. (a) Schematic models of dystrophin, beta spectrin, alpha actinin, and alpha spectrin showing segment designations and regions of similarity. Boxes represent nonrepetitive segments and ovals denote sequences that resemble the typical spectrin repetitive segment. The use of ovals in the dystrophin model is not intended to imply a close match with the spectrin repetitive segment motif. Stippling was used to distinguish alpha actinin-like segments whose match to the spectrin repetitive segment motif was improved by removal of eight residues within the segment (data not shown). Vertical bars roughly delineate the regions of similarity between the proteins represented by the models. The segments of each protein are numbered from left to right, and are not intended to indicate similarities between similarly numbered segments. Asterisks indicate regions of the molecules that are not shown. (b) Beta spectrin and alpha spectrin contain sequences homologous to the entire alpha actinin sequence. Beta spectrin sequence from residue 22 to the end of the available sequence (800 amino acids) was aligned with chicken alpha actinin sequence (Baron et al., 1987), beginning at the start codon using the University of Wisconsin Genetics Computer Group GAP program. *Drosophila* alpha spectrin sequence, equivalent to the length of alpha actinin and ending at the COOH terminus (residues 1,524–2,415; Dubreuil et al., 1989), was similarly aligned with the entire sequence of chicken alpha actinin. These alignments are illustrated schematically in a. A window of 50 residues in length was then moved along each set of aligned sequences one residue at a time, recording the number of identical matches within the window at each position. Gaps were counted as mismatches. Shuffled spectrin sequences plotted against alpha actinin gave averages of <12% identity when plotted in the same way, with peaks not exceeding 28%. (The similarity of *Drosophila* alpha spectrin to *Drosophila* alpha actinin is comparable to the similarity of *Drosophila* alpha spectrin to chicken alpha actinin.)

in erythroid spectrin (Speicher and Marchesi, 1984), but the match is otherwise poor, and the length from that residue to the next invariant tryptophan is larger than in typical spectrin repetitive segments. We suggest that this region is a divergent spectrin repetitive segment in both beta spectrin and in the alpha actinins. It may have originated from a spectrin repeat progenitor, but is no longer constrained by the structural or functional requirements that maintain the size and consensus sequence of the typical spectrin repetitive segments.

Segment 3 of beta spectrin and alpha actinin are a better match to the spectrin repetitive segment motif than the other

alpha actinin segments, particularly if 8 amino acids are deleted internally (data not shown, also see Dubreuil et al., 1989). The boundaries between segments 3, 4, 5, and 6 were placed according to their resemblance to the typical spectrin repeat, but we will reserve an analysis of beta spectrin repeats until the sequence of the entire molecule is available. In segment 3, the identity of *Drosophila* beta spectrin to the alpha actinins drops slightly (26–41% identity) and a one-residue gap is required in one sequence for each comparison to optimize the alignment. The similarity between beta spectrin and alpha actinin then drops to background levels \sim 30 residues into segment 4. The match between *Drosophila* beta spectrin and human beta spectrin in segment 3 is 75%.

Discussion

The Conserved Amino-terminal Domain of Beta Spectrin, Alpha Actinin, and Dystrophin

We have described a highly conserved domain at the amino-terminal end of beta spectrin that matches the conserved amino termini of alpha actinin and dystrophin. This shared domain represents a new defining characteristic for spectrin-related proteins and provides additional evidence that the spectrin subunits (including fodrin, which we consider to be a nonerythroid spectrin), alpha actinins, and dystrophins are members of a superfamily as proposed by Davison et al. (1989). Since spectrin was the first of these proteins to be described, and the characteristic 106-amino acid repetitive unit was first demonstrated in spectrin (Speicher and Marchesi, 1984), we propose to call this group of related proteins the spectrin superfamily.

The lack of sequence conservation between beta spectrin and dystrophin and between alpha actinin and dystrophin immediately downstream from segment 1 indicates that the downstream sequence is not important for the functions of segment 1 that are common to all three proteins (part of this downstream region was considered to be similar between alpha actinin and dystrophin by Hammonds [1987], but the level of match was very low and required several gaps, including one 43-residue gap, for alignment). The sequence similarity between chicken and human dystrophin also drops after segment 1 (Lemaire et al., 1988). These two are 84% identical (97% similar, no gaps) over segment 1, but only 59% identical (70% similar, with a 2-residue gap) over the following 100 amino acids.

Structural and Functional Analogies between Members of the Spectrin Superfamily

The conserved sequence domains of beta spectrin and alpha actinin suggest structural and functional analogies between the multimeric forms of these proteins. The alpha and beta spectrin subunits associate in an antiparallel fashion to form heterodimers, which in turn associate to form tetramers (Fig. 8). The tetramer is thought to be the most important functional state of spectrin and the binding sites at either end of the spectrin tetramer are responsible for its ability to cross-link F-actin (Cohen et al., 1980; Glenney et al., 1982). Alpha actinin forms a homodimer with the chains associated in an antiparallel orientation (Wallraff et al., 1986). Like the spectrin tetramer, the alpha actinin dimer also cross-links F-actin by virtue of actin binding sites at either end.

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1) Chick Dystrophin      (5) VLWYEEVEDDYER DV K      K I A QFAKCGRRRC EDLFN FRD RK LE C
2) Human Dystrophin    (1) MLWEEVEDCYER DV K      K V A QFSKFGKQH ENLFS LQD RR LD G
3) Fly Beta Spectrin   (36) FERSRIKALAEER SV K     K V SNLCR.VNCR ADLYV MRD KH IK V
4) Chick Alpha Actinin (18) EDWDRDLLDPAW KQ R     A C SNL.RKAGTQ ENIEE FRD LK ML V
5) Fly Alpha Actinin   (12) EEWEREGLLDPAW KQ K     A C SNL.RKAGTA DNIEE FRN LK ML V
6) Dicty Alpha Actinin (8) VSGNDKQLLNKAW IT K     A C SNL.RKLGSS EQIDT FTD IK AQ V
7) Consensus           E Q KTFT M N I D G L LLE

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1) LTGQKIA .EKGS..T V ALN NKA QILQRNN D VM SSDI G HKLT L N HUQVKDV
2) LTGQKLP .EKGS..T V ALN NKA RVLQRNN D VM STDI G HKLT L N HWQVKNV
3) LSGERLP PTKGK..M I CLE DKA QFLREQR H EM SHDI G ASLN L T RFAIQDI
4) ISGERLA PERGK..M V KIS NKA DFIASKG K VS AEEI G VKMT M T RFAIQDI
5) ISGETLP PDRGK..M F KIA NKA DFIASKG H VS AEEI G LKMT M T RFAIQDI
6) ISNDPVF VNKTPKLR I NIQ GLC KHIESHG K VG AEEL K LKMT M T RFAIQDI
7) K R N N V L V L I G V D N L G I W I I L

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1) MKNIMAGLQQTNSEKI S VRQS RN PQ NVI TS MS AFN LL S D FDWNAVASQQSPV
2) MKNIMAGLQQTNSEKI S VRQS RN PQ NVI TT MS ALN LI S D FDWNSVVCQQSAT
3) TIEEVDNKETSAKDA L CQNK AG HN NVR TT WR AFN II K D VQFEK.LSKTNAI
4) SVEE.....TSAKEG L YQRK AP KN NIQ NI MK GFC LI R E IDYK.LRKDDPL
5) SVEEM.....TAKEG L CQRK AP KN NVQ HL FK AFC LI R D IDYAK.LSKDNPL
6) SIEEL.....SAKEA L CQRK EG DR KVG HT FQ AFC LI K D INFDS.LNKDDKA
7) L L M T Y V N F S DGL A N HRP L

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1) QR DH NI ROH GIEKL PE V.ATAC KKSILM VTS (238)
2) QR EH NI RYQ GIEKL PE V.DTTY KKSILM ITS (234)
3) H N N N DV EDK GLAKL AE V.F.VEH EKSIIT VVT (268)
4) T N NT DV EKY DIPKM AE IVGTAR EKAINT VSS (245)
5) E N NT DV EKY DIPRM PD LINTPK ERAINT VCC (239)
6) G N QL DI EKE DIPKM VS HLDVVR ERSVNT VAQ (237)
7) L A F A L L D D P D Y

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Figure 7. Comparison of the segment 1 domains of beta spectrin, alpha actinin, and dystrophin. The sequences of human (Koenig et al., 1988) and chicken (Lemaire et al., 1988) dystrophins; *Drosophila* (*Fly*) beta spectrin; and chicken (Baron et al., 1987), *Drosophila* (thanks to E. Fyrberg for unpublished sequence data), and *Dictyostelium discoideum* (*Dicty*) (Noegel et al., 1987) alpha actinins were aligned iteratively against *Drosophila* beta spectrin using the University of Wisconsin Genetics Computer Group GAP program until no further gaps were assigned. Gaps introduced for optimal alignment are indicated by periods. The positions of the first and last residues of the aligned sequences in the parent protein are given in parentheses. Identical matches at a given position for every protein are represented by a character in the consensus line and blanks in the aligned sequences. Bold characters were used at any position where all of the residues for any two protein types are identical.

Table I. Pairwise Comparisons of the Sequences of Segments 1, 2, and 3

	Identity (similarity allowing for conserved substitutions)				
	Chicken alpha actinin	<i>Drosophila</i> alpha actinin	<i>Dictyostelium</i> alpha actinin	Chicken dystrophin	Human dystrophin
	%	%	%	%	%
Segment 1					
<i>Drosophila</i> beta spectrin	55 (74)	53 (74)	49 (66)	47 (65)	47 (65)
Chicken alpha actinin		84 (95)	61 (75)	46 (67)	40 (65)
<i>Drosophila</i> alpha actinin			62 (74)	38 (66)	36 (63)
<i>Dictyostelium</i> alpha actinin				34 (59)	34 (61)
Chicken dystrophin					84 (92)
Segment 2					
<i>Drosophila</i> beta spectrin	41 (60)	41 (59)	33 (55)		
Chicken alpha actinin		71 (86)	40 (62)		
<i>Drosophila</i> alpha actinin			34 (59)		
Segment 3					
<i>Drosophila</i> beta spectrin	41 (64)	39 (58)	26 (49)		
Chicken alpha actinin		67 (78)	21 (47)		
<i>Drosophila</i> alpha actinin			22 (48)		

Sequences were compared using the GAP program, allowing the insertion of pad characters to optimize pairwise alignments. The amino acid positions at the boundaries of segment 1 are shown in Fig. 7. The boundaries of segments 2 and 3 were determined according to their alignment with beta spectrin segments (Fig. 5). References for the sources of the sequence data are given in Fig. 7.

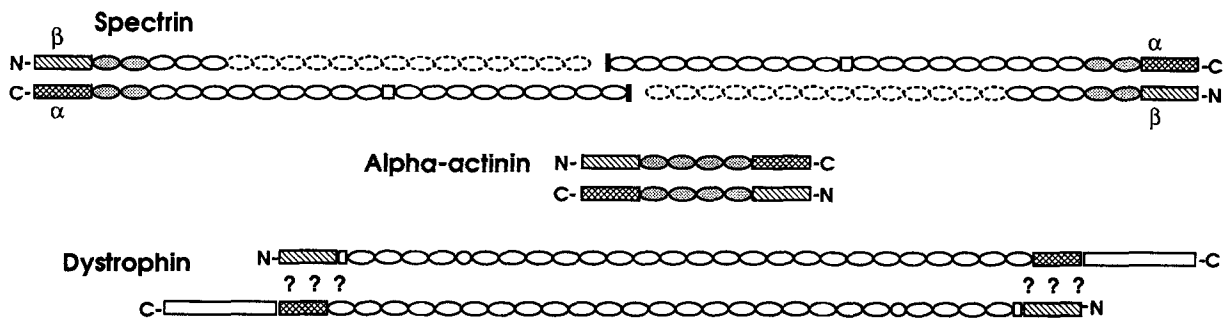


Figure 8. Schematic models of the spectrin tetramer, the alpha actinin dimer, and a putative dystrophin dimer. The segmental organization is illustrated by the same symbols and patterns as in Fig. 6 a. Dotted ovals indicate incomplete *Drosophila* beta spectrin sequence information. The length of each model is proportional to the sequence length. The crosshatched region toward the carboxy terminus of dystrophin was so designated because of the similarity of that region to the carboxy-terminal end of *D. discoideum* alpha actinin (Koenig et al., 1988).

As Fig. 8 illustrates, the multimeric structures of spectrin and alpha actinin are both characterized by the apposition of a conserved amino domain of one subunit (beta in the spectrin tetramer) with the conserved carboxy-terminal domain of the neighboring subunit (alpha in the spectrin tetramer) at either end of the molecule. The extensive similarity between the ends of these two different proteins implies that the spectrin tetramer and alpha actinin dimer use homologous sequences to form binding sites for evolutionarily conserved proteins such as actin, and possibly protein 4.1. It will be important to establish whether spectrin and alpha actinin do indeed interact with a common set of binding partners besides actin.

Interactions between the subunits may be important in regulating the binding functions of both the spectrin tetramer and the alpha actinin dimer. Both spectrin chains are required for full actin binding activity, and Coleman et al. (1987) have shown that the beta subunit is the determining factor in protein 4.1-stimulated spectrin-actin interaction. Ca^{++} modulates the actin cross-linking activity of non-muscle alpha actinin, and Noegel et al. (1987) have proposed a model whereby Ca^{++} binding affects a conformational change in the COOH terminus, perturbing the interaction of the NH_2 terminus of the neighboring subunit with F-actin (also see Wasenius et al., 1989). Ca^{++} has also been shown to decrease the association of sea urchin spectrin with actin filaments (Fishkind et al., 1987).

There are parallels with the structure of spectrin and alpha actinin in the dystrophin molecule, particularly if dystrophin monomers self-associate in an antiparallel homodimer as do the subunits of alpha actinin (Fig. 8; Koenig et al., 1988; Hoffman and Kunkel, 1989). The spectrin-based membrane skeleton of the human erythrocyte has been modeled as an ionic gel whose elastic properties allow the cell to withstand repeated deformations as it passes through narrow capillaries (Elgsaeter et al., 1986). Given the submembrane distribution of dystrophin (see Hoffman and Kunkel, 1989 for review) and its structural similarities to spectrin, it, like spectrin, may participate in an elastic meshwork that protects the integrity of the sarcolemma through the deformations imposed by muscular contraction.

Although our analysis has emphasized the similarities between spectrin, alpha actinin, and dystrophin, the sequence organization of these proteins differ from each other in both subtle and major ways. These differences may account for

specific functions and regulatory mechanisms in each type of protein.

Can Members of the Spectrin Superfamily Complement One Another?

The conservation of domains in the spectrin subunits, alpha actinin, and dystrophin through a great evolutionary distance implies crucial cellular functions for these proteins. Yet, two independent studies (1. Wallraff et al., 1986, and Schleicher et al., 1988; 2. Noegel and Witke, 1988) have shown that mutational inactivation of the *Dictyostelium* alpha actinin gene had little or no measurable effect on cellular function. Also, some muscular dystrophy patients were found to lack substantial portions of dystrophin, including most of the NH_2 terminus, but still manifest the relatively mild Becker phenotype (Koenig et al., 1988). The finding of very similar functional domains in different cytoskeletal proteins suggests that there is a degree of redundancy built into the cytoskeletal system (see Bray and Vasiliev, 1989), where, for some functions, members of the superfamily may complement a genetic defect in one another. It will be illuminating in this regard to examine the possibility of interactions between these proteins and to characterize additional proteins with homologous domains, if they exist.

Evolutionary Implications

The structural similarity of beta spectrin, alpha spectrin, and alpha actinin homologues in *Drosophila* and man shows that these genes existed in much the same form before the split between vertebrates and invertebrates. The extensive sequence similarity that we report between beta spectrin and alpha actinin extends through the amino-terminal half of alpha actinin, overlapping the region of similarity between alpha spectrin and alpha actinin (Fig. 6 b) (Wasenius et al., 1989; Dubreuil et al., 1989). This relationship of spectrin subunits with alpha actinin suggests that they are evolutionarily related through a simple set of genetic mechanisms. These mechanisms probably include unequal crossing over between segments (Smith, 1973), such that alpha actinin and spectrin arose one from the other, or from a common precursor. While other events (such as insertions or further unequal cross-over events) appear to complicate the relation of dystrophin to alpha actinin and the spectrin subunits, it is clear that the members of the spectrin superfamily arose from the

shuffling of a common set of domains very early in the evolution of the cell.

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Note added in proof: After this paper was accepted for publication, the complete sequence of *Dictyostelium* gelation factor was reported (Noegel, A. A., S. Rapp, F. Lottspeich, M. Schleicher, and M. Stewart. 1989. *J. Cell Biol.* 109:607-618). This protein has an amino-terminal sequence that is similar to the amino-terminal domains of beta spectrin, alpha actinin, and dystrophin, but does not share other attributes of the spectrin superfamily. In place of the spectrin-like alpha-helical repeats, it has repetitive segments whose sequence predicts a cross-beta conformation.

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